

ISSN 0390-6078

Volume 104

OCTOBER
2019 - S2



 **haematologica**

Journal of the Ferrata Storti Foundation

**47° Congress of the Italian Society of Hematology
Rome, Italy, October 7-9, 2019**

ABSTRACT BOOK

www.haematologica.org

haematologica

Looking for a definitive source
of information in hematology?

Haematologica is an Open Access
journal: all articles are completely
free of charge

Haematologica
is listed on *PubMed, PubMedCentral,*
DOAJ, Scopus and many other
online directories

5000 / amount of articles read daily

4300 / amount of PDFs downloaded daily

2.20 / gigabytes transferred daily

WWW.HAEMATOLOGICA.ORG

Editor-in-Chief

Luca Malcovati (Pavia)

Managing Director

Antonio Majocchi (Pavia)

Associate Editors

Omar I. Abdel-Wahab (New York), H el ene Cav e (Paris), Simon Mendez-Ferrer (Cambridge), Pavan Reddy (Ann Arbor), Andreas Rosenwald (Wuerzburg), Monika Engelhardt (Freiburg), Davide Rossi (Bellinzona), Jacob Rowe (Haifa, Jerusalem), Wyndham Wilson (Bethesda), Paul Kyrle (Vienna), Swee Lay Thein (Bethesda), Pieter Sonneveld (Rotterdam)

Assistant Editors

Anne Freckleton (English Editor), Cristiana Pascutto (Statistical Consultant), Rachel Stenner (English Editor), Kate O'Donohoe (English Editor), Ziggy Kennell (English Editor)

Editorial Board

Jeremy Abramson (Boston); Paolo Arosio (Brescia); Raphael Bejar (San Diego); Erik Berntorp (Malm o); Dominique Bonnet (London); Jean-Pierre Bourquin (Zurich); Suzanne Cannegieter (Leiden); Francisco Cervantes (Barcelona); Nicholas Chiorazzi (Manhasset); Oliver Cornely (K oln); Michel Delforge (Leuven); Ruud Delwel (Rotterdam); Meletios A. Dimopoulos (Athens); Inderjeet Dokal (London); Herv e Dombret (Paris); Peter Dreger (Hamburg); Martin Dreyling (M unchen); Kieron Dunleavy (Bethesda); Dimitar Efremov (Rome); Sabine Eichinger (Vienna); Jean Feuillard (Limoges); Carlo Gambacorti-Passerini (Monza); Guillermo Garcia Manero (Houston); Christian Geisler (Copenhagen); Piero Giordano (Leiden); Christian Gisselbrecht (Paris); Andreas Greinacher (Greifswald); Hildegard Greinix (Vienna); Paolo Gresele (Perugia); Thomas M. Habermann (Rochester); Claudia Haferlach (M unchen); Oliver Hantschel (Lausanne); Christine Harrison (Southampton); Brian Huntly (Cambridge); Ulrich Jaeger (Vienna); Elaine Jaffe (Bethesda); Arnon Kater (Amsterdam); Gregory Kato (Pittsburg); Christoph Klein (Munich); Steven Knapper (Cardiff); Seiji Kojima (Nagoya); John Koreth (Boston); Robert Kralovics (Vienna); Ralf K uppers (Essen); Ola Landgren (New York); Peter Lenting (Le Kremlin-Bicetre); Per Ljungman (Stockholm); Francesco Lo Coco (Rome); Henk M. Lokhorst (Utrecht); John Mascarenhas (New York); Maria-Victoria Mateos (Salamanca); Giampaolo Merlini (Pavia); Anna Rita Migliaccio (New York); Mohamad Mohty (Nantes); Martina Muckenthaler (Heidelberg); Ann Mullally (Boston); Stephen Mulligan (Sydney); German Ott (Stuttgart); Jakob Passweg (Basel); Melanie Percy (Ireland); Rob Pieters (Utrecht); Stefano Pileri (Milan); Miguel Piris (Madrid); Andreas Reiter (Mannheim); Jose-Maria Ribera (Barcelona); Stefano Rivella (New York); Francesco Rodeghiero (Vicenza); Richard Rosenquist (Uppsala); Simon Rule (Plymouth); Claudia Scholl (Heidelberg); Martin Schrappe (Kiel); Radek C. Skoda (Basel); G erard Soci e (Paris); Kostas Stamatopoulos (Thessaloniki); David P. Steensma (Rochester); Martin H. Steinberg (Boston); Ali Taher (Beirut); Evangelos Terpos (Athens); Takanori Teshima (Sapporo); Pieter Van Vlierberghe (Gent); Alessandro M. Vannucchi (Firenze); George Vassiliou (Cambridge); Edo Vellenga (Groningen); Umberto Vitolo (Torino); Guenter Weiss (Innsbruck).

Editorial Office

Simona Giri (Production & Marketing Manager), Lorella Ripari (Peer Review Manager), Paola Cariati (Senior Graphic Designer), Igor Ebuli Poletti (Senior Graphic Designer), Marta Fossati (Peer Review), Diana Serena Ravera (Peer Review)

Affiliated Scientific Societies

SIE (Italian Society of Hematology, www.siematologia.it)

SIES (Italian Society of Experimental Hematology, www.siesonline.it)

Information for readers, authors and subscribers

Haematologica (print edition, pISSN 0390-6078, eISSN 1592-8721) publishes peer-reviewed papers on all areas of experimental and clinical hematology. The journal is owned by a non-profit organization, the Ferrata Storti Foundation, and serves the scientific community following the recommendations of the World Association of Medical Editors (www.wame.org) and the International Committee of Medical Journal Editors (www.icmje.org).

Haematologica publishes editorials, research articles, review articles, guideline articles and letters. Manuscripts should be prepared according to our guidelines (www.haematologica.org/information-for-authors), and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, prepared by the International Committee of Medical Journal Editors (www.icmje.org).

Manuscripts should be submitted online at <http://www.haematologica.org/>.

Conflict of interests. According to the International Committee of Medical Journal Editors (<http://www.icmje.org/#conflicts>), “Public trust in the peer review process and the credibility of published articles depend in part on how well conflict of interest is handled during writing, peer review, and editorial decision making”. The ad hoc journal’s policy is reported in detail online (www.haematologica.org/content/policies).

Transfer of Copyright and Permission to Reproduce Parts of Published Papers. Authors will grant copyright of their articles to the Ferrata Storti Foundation. No formal permission will be required to reproduce parts (tables or illustrations) of published papers, provided the source is quoted appropriately and reproduction has no commercial intent. Reproductions with commercial intent will require written permission and payment of royalties.

Detailed information about subscriptions is available online at www.haematologica.org. Haematologica is an open access journal. Access to the online journal is free. Use of the Haematologica App (available on the App Store and on Google Play) is free.

For subscriptions to the printed issue of the journal, please contact: Haematologica Office, via Giuseppe Belli 4, 27100 Pavia, Italy (phone +39.0382.27129, fax +39.0382.394705, E-mail: info@haematologica.org).

Rates of the International edition for the year 2019 are as following:

	<i>Institutional</i>	<i>Personal</i>
<i>Print edition</i>	<i>Euro 700</i>	<i>Euro 170</i>

Advertisements. Contact the Advertising Manager, Haematologica Office, via Giuseppe Belli 4, 27100 Pavia, Italy (phone +39.0382.27129, fax +39.0382.394705, e-mail: marketing@haematologica.org).

Disclaimer. Whilst every effort is made by the publishers and the editorial board to see that no inaccurate or misleading data, opinion or statement appears in this journal, they wish to make it clear that the data and opinions appearing in the articles or advertisements herein are the responsibility of the contributor or advisor concerned. Accordingly, the publisher, the editorial board and their respective employees, officers and agents accept no liability whatsoever for the consequences of any inaccurate or misleading data, opinion or statement. Whilst all due care is taken to ensure that drug doses and other quantities are presented accurately, readers are advised that new methods and techniques involving drug usage, and described within this journal, should only be followed in conjunction with the drug manufacturer’s own published literature.

The origin of a name that reflects Europe's cultural roots.

Ancient Greek

αἷμα [haima] = blood
αἵματος [haimatos] = of blood
λόγος [logos] = reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter,
used as a noun) = hematological subjects

Modern English

The oldest hematology journal,
publishing the newest research results.
2018 JCR impact factor = 7.570

47° Congress of the Italian Society of Hematology
Rome, Italy, October 7-9, 2019

CONSIGLIO DIRETTIVO

Paolo Corradini, Presidente
Emanuele Angelucci, Vice Presidente
Sergio Siragusa, Vice Presidente
Mario Boccardo
Michele Cavo
Nicola Di Renzo
Gianluca Gaidano
Francesca Patriarca, Tesoriere
Giulia Perrone, Segretario
Giuseppe Rossi
Alessandro M. Vannucchi

COMITATO SCIENTIFICO

Pier Luigi Zinzani, Presidente
Anna Falanga
Gian Luca Forni
Mauro Krampera
Pellegrino Musto
Adriano Venditti
Umberto Vitolo

SEGRETERIA PERMANENTE SIE

Via Marconi, 36 - 40122 Bologna
Tel. 051 6390906 - Fax 051 4219534
E-mail: segreteriasie@ercongressi.it
www.siematologia.it

SEGRETERIA ORGANIZZATIVA

Studio E.R. Congressi
Via Marconi, 36 - 40122 Bologna
Tel. 051 4210559 - Fax 051 4210174
E-mail: ercongressi@ercongressi.it
www.ercongressi.it

ABSTRACT BOOK

47° Congress of the Italian Society of Hematology
Rome, Italy, October 7-9, 2019

Con il contributo non condizionante di

ABBVIE

AMGEN
CELGENE
GILEAD SCIENCES
JANSSEN ITALIA
PFIZER ITALIA
ROCHE

NOVARTIS

ALEXION
DAIICHI SANKYO ITALIA
INCYTE BIOSCIENCES
JAZZ HEALTHCARE ITALY
KYOWA KIRIN
SANDOZ
SANOFI GENZYME
SERVIER
TAKEDA

ASTRAZENECA
BLUEBIRD BIO
DIASORIN
ISTITUTO GENTILI
MUNDIPHARMA PHARMACEUTICALS
SHIRE

ATARA BIOTHERAPEUTICS
NEOVII BIOTECH GMBH
TILLOMED ITALIA
WERFEN

47° Congress of the Italian Society of Hematology

Rome, Italy, October 7-9, 2019

Best Abstracts

.....1

Oral Communications

session 1.	C-001-C-005	Non Hodgkin Lymphoma 1	7
session 2.	C-006-C-010	Myeloma and Monoclonal Gammopathies 1	9
session 3.	C-011-C-015	Acute Myeloid Leukemia 1	11
session 4.	C-016-C-018	Myeloproliferative Neoplasms 1	14
session 5.	C-019-C-025	Chronic Lymphatic Leukemia	17
session 6.	C-026-C-030	Allogeneic and Autologous Transplantation	19
session 7.	C-031-C-035	Chronic Myeloid Leukemia	22
session 8.	C-036-C-040	Anemia and Thalassemia	25
session 9.	C-041-C-045	Hodgkin Lymphoma	27
session 10.	C-046-C-050	Infections	29
session 11.	C-051-C-055	Hemostasis, thrombosis, thrombocytopenia and platelet diseases	32
session 12.	C-056-C-060	Acute Lymphatic Leukemia	34
session 13.	C-061-C-065	Chronic Lymphatic Leukemia 2	37
session 14.	C-066-C-070	Myeloproliferative Neoplasms 2	39
session 15.	C-071-C-075	Myelodysplastic Syndromes	42
session 16.	C-076-C-080	Allogeneic and Autologous Transplantation 2	44
session 17.	C-081-C-085	Non Hodgkin Lymphoma 2	46
session 18.	C-086-C-090	Myeloma and Monoclonal Gammopathies 2	49
session 19.	C-091-C-095	Acute Myeloid Leukemia 2	51
session 20.	C-096-C-100	Quality of Life, Pain Therapy and Home Care, Supportive Care	53

Posters

session 1.	P-001-P-011	Non-Oncological Hematology	56
session 2.	P-012-P-020	Non Hodgkin Lymphoma 1	60
session 3.	P-021-P-030	Myeloma and Monoclonal Gammopathies 1	64
session 4.	P-031-P-040	Myeloma and Monoclonal Gammopathies 2	68
session 5.	P-041-P-051	Myeloproliferative Neoplasms 1	72
session 6.	P-052-P-058	Hodgkin Lymphoma 1	74
session 7.	P-059-P-065	Allogeneic and Autologous Transplantation 1	80
session 8.	P-066-P-072	Allogeneic and Autologous Transplantation 2	84
session 9.	P-073-P-084	Acute Myeloid Leukemia 1	87
session 10.	P-085-P-094	Acute Lymphatic Leukemia	92
session 11.	P-095-P-103	Quality of Life, Pain Therapy and Home Care, Supportive Care 1	96
session 12.	P-104-P-117	Chronic Lymphatic Leukemias and Lymphoproliferative Syndromes	100
session 13.	P-118-P-129	Cytogenetics, Molecular Genetics and Myelodysplastic Syndromes	106
session 14.	P-130-P-138	Non Hodgkin Lymphoma 2	111
session 15.	P-139-P-148	Myeloma and Monoclonal Gammopathies 3	116
session 16.	P-149-P-158	Myeloproliferative Neoplasms 2	120
session 17.	P-159-P-168	Myeloproliferative Neoplasms 3	124
session 18.	P-169-P-176	Hodgkin Lymphoma 2	127
session 19.	P-177-P-184	Allogeneic and Autologous Transplantation 3	131
session 20.	P-185-P-196	Acute Myeloid Leukemia 2	134
session 21.	P-197-P-210	Chronic Myeloid Leukemia	140
session 22.	P-211-P-218	Quality of Life, Pain Therapy and Home Care, Supportive Care 2	147
session 23.	P-219-P-229	Infections	150

Published Only

sessions	PU-001-PU-053.	155
----------	----------------	-------	-----

Main Program

..... 175

Authors Index

..... a

47° Congress of the Italian Society of Hematology Rome, Italy, October 7-9, 2019

BEST ABSTRACTS

B001

LENTIGLOBIN GENE THERAPY IN PATIENTS WITH TRANSFUSION-DEPENDENT β -THALASSEMIA AND NON- β / β GENOTYPES: OUTCOMES FROM THE COMPLETED PHASE 1/2 NORTHSTAR AND ONGOING PHASE 3 NORTHSTAR-2 STUDIES

F. Locatelli, M. Walters, J. Kwiatkowski, S. Hongeng, J. Porter, J. Rasko, M. Sauer, A. Thrasher, I. Thuret, G. Schiller, H. Elliot, B. Deary, Y. Chen, G. Tao, R. Colvin, A. Thompson

IRCCS Bambino Gesù Children's Hospital, UCSF Benioff Children's Hospital, Children's Hospital of Philadelphia, Mahidol University Ramathibodi Hospital, University College London Hospitals, University of Sydney Royal Prince Alfred Hospital, Medizinische Hochschule Hannover, UCL Great Ormond Street Institute of Child Health, Hôpital de la Timone, David Geffen School of Medicine at UCLA, bluebird bio, bluebird bio, bluebird bio, bluebird bio, bluebird bio, Northwestern University Feinberg School of Medicine

LentiGlobin contains autologous CD34+ hematopoietic stem cells (HSCs) transduced ex vivo with the BB305 lentiviral vector (LVV) encoding β -globin with a T87Q substitution. LentiGlobin gene therapy has been evaluated in patients with transfusion-dependent β -thalassemia (TDT) and non- β / β genotypes in the Phase 1/2 Northstar study (NCT01745120) and Phase 3 Northstar-2 study (NCT02906202), in this trial using a refined manufacturing process).

HSCs were mobilized using G-CSF and plerixafor, collected through apheresis, and transduced with the BB305 LVV. Patients received single-agent, pharmacokinetic-guided, myeloablative busulfan and were then infused with transduced cells. Patients were followed for both safety and efficacy. Statistics are shown as median (min-max).

As of 14 September 2018, 10 and 16 patients with TDT and non- β / β genotypes have been treated in Northstar (follow-up: 36.0 [29.3-48.1] months; age: 16-34 yrs) and Northstar-2 (follow-up: 9.3 [0.7-20.4] months; age: 8-34 yrs), respectively. All patients with >2 months follow-up engrafted. Platelet engraftment was achieved at 39.5 (19-191) and 44.5 (20-84) days in Northstar and Northstar-2, respectively.

In Northstar, 8/10 patients with non- β / β genotypes achieved transfusion independence (TI; weighted average hemoglobin [Hb] \geq 9 g/dL without red blood cell [RBC] transfusions for \geq 12 months). Duration of TI was 38.0 (21.2-43.6) months; all responses are ongoing. Weighted average Hb during TI was 10.2 (9.3-13.2) g/dL. In patients who achieved TI, liver iron content decreased by 38% and 68% at Month 48 (n=2).

In Northstar-2, 10/11 patients with \geq 3 months follow-up stopped RBC transfusions with Hb of 11.1-13.3 g/dL comprising 7.7-10.6 g/dL HbAT87Q at last visit. The primary endpoint of TI was achieved by 2/3 patients and 5/6 patients with \geq 1-year follow-up had improved myeloid:erythroid ratios (0.18-0.45 to 0.77-1.1).

The most common non-hematologic grade \geq 3 adverse events post-infusion (\geq 3 patients with non- β / β genotypes in either study) were stomatitis, febrile neutropenia, irregular menstruation, epistaxis, pyrexia, and liver veno-occlusive disease (which resolved with defibrotide).

In summary, 80% of patients with non- β / β genotypes treated with LentiGlobin in Northstar achieved TI. Available data using a refined manufacturing process in the ongoing Northstar-2 study indicate that patients can achieve near-normal Hb. The safety profile is consistent with myeloablative busulfan conditioning.

B002

IBRUTINIB TREATMENT DOESN'T INDUCE TP53 CLONAL EVOLUTION IN CHRONIC LYMPHOCYTIC LEUKEMIA

L. Cafforio, L.V. Cappelli, I. Del Giudice, C. Ilari, S. Raponi, M.S. De Propriis, P. Mariglia, A. Piciocchi, V. Arena, M. Vignetti, F.R. Mauro, A. Guarini, R. Foà

Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; GIMEMA Data Center, GIMEMA Foundation; GIMEMA Data Center, GIMEMA Foundation; GIMEMA Data Center, GIMEMA Foundation; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome Department of Molecular Medicine, Sapienza University of Rome; Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome, Italy

Ibrutinib (I) is active in chronic lymphocytic leukemia (CLL) patients (pts) with TP53 defects. To explore the dynamics of TP53 mutations (TP53-m) under I treatment, 58 (37 IGHV unmutated, 21 mutated) pts underwent a longitudinal TP53 monitoring by deep sequencing. Forty-four treatment-naïve (TN) pts received I+rituximab (IR) front-line (GIMEMA LLC1114 trial-NCT02232386; median I exposure: 2.7 years; range: 1.2-3.7); 14 relapsed/refractory (R/R) pts received I single agent after 1.5 (range: 1-4) lines of chemoimmunotherapy (median I exposure: 2.5 years; range: 2.1-4). Samples were sequenced on a MiSeq Illumina (mean coverage 5000X). Variant allele frequency (VF) was corrected to cancer cell fraction (CCF) by the % of CD19+/CD5+ cells. Of 44 TN pts at baseline, 27 (61%) were TP53-wild-type (WT) and 17 (39%) carried 23 TP53-m (1.4 m/pt; range: 1-5): 17 (74%) were clonal (CL) (mean VF: 58.8%; range: 18-94) and 6 (26%) subclonal (SCL) (mean VF: 5.3%; range: 2.1-9.2). Overall, 7 (30.5%) CL TP53-m persisted stable on treatment, 9 (39%) CL decreased, 3 (13%) (1 CL+2 SCL) increased and 4 (17.5%) (1 CL+3 SCL) were lost. No TP53-m was acquired under I. The mutational patterns per pt are shown in the Figure. Of 14 R/R pts, prior to I 4 (29%) were WT and 10 (71%) carried 31 TP53-m (3.1 m/pt; range: 1-11): 11 (35.5%) were CL (mean VF: 31.9%; range: 10.5-78.8) and 20 (64.5%) SCL (mean VF: 2.9%; range: 0.9-6.8). After I treatment, 8 (26%) TP53-m (4 CL+4 SCL) persisted stable, 8 (26%) (4 CL+4 SCL) decreased, 4 (13%) (2 CL+ 2 SCL) increased, 11 (35%) (2 CL+9 SCL) were lost and 2 novel SCL emerged. No TP53-m arose in the 4 WT pts over time. Overall, in both TN and R/R pts most TP53-m remained stable or decreased (69.5% and 52%, respectively), some increased (13% and 13%) and some were lost (17.5% and 35%) (p=NS). Notably, while no novel mutation arose in TN pts, 2 occurred in R/R pts. Although the lymphocyte count significantly decreased during IR/I exposure (TN: 40.7 vs 11.5x10⁹/L, p=0.019; R/R: 39.7 vs 7.1x10⁹/L, p=0.0034), the mean CCF of the existing TP53-m remained stable on treatment (TN: 60.7% vs 42.7%; R/R: 20.5% vs 20.2%). In conclusion, I in any line of therapy decreases the TP53 CL and SCL complexity; the emergence/expansion of TP53-m is rare. In TP53 WT pts, I never induced novel TP53-m after >2 years exposure. The significant de-

crease of lymphocytosis with stable CCF proves the I effectiveness on both TP53-m and WT cells, regardless of previous therapies.

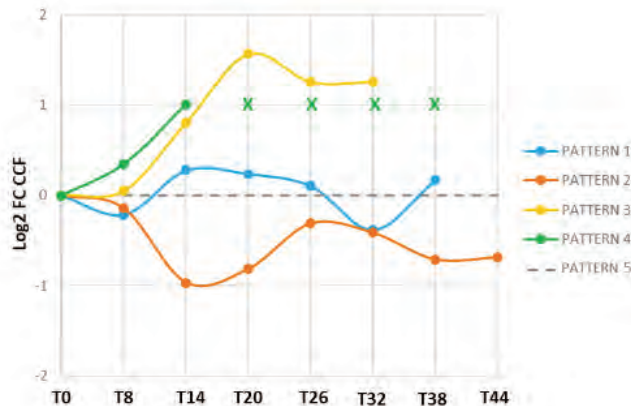


Figure. TN patients: log fold change (FC) of the cancer cell fraction (CCF) of TP53 mutations at each time point (T), reported in months, vs T0. Each color corresponds to a group of patients with a common pattern: 1) CLTP53-m present from T0 and persisting CL with a stable CCF (n=5); 2) CLTP53-m present from T0 and persisting CL with decreasing CCF (n=7); 3) CL or SCLTP53-m present from T0 with increasing CCF (n=2); 4) SCLTP53-m disappearing (n=1) (green stars correspond to timepoints where the mutations disappear); 5) absence of any detectable TP53-m over time (n=27). Two patients with a complex mutational architecture are not shown.

B003

GILTERITINIB SIGNIFICANTLY PROLONGS OVERALL SURVIVAL IN PATIENTS WITH FLT3-MUTATED RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA: RESULTS FROM THE PHASE 3 ADMIRAL TRIAL

A.E. Perl, G. Martinelli, J.E. Cortes, A. Neubauer, E. Berman, S. Paolini, P. Montesinos, M.R. Baer, R.A. Larson, C. Ustun, F. Fabiano, A. Di Stasi, R. Stuart, R. Olin, M. Kasner, F. Ciceri, W. Chou, N. Podoltsev, C. Recher, H. Yokoyama, N. Hosono, S. Yoon, J. Lee, T. Pardee, A.T. Fathi, C. Liu, X. Liu, E. Bahceci, M.J. Levis

Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IRST IRCCS, Meldola, Italy; University of Texas, MD Anderson Cancer Center, Houston, TX, USA; Universitätsklinikum Giessen und Marburg GmbH, Marburg, Germany; Memorial Sloan-Kettering Cancer Center, New York City, NY, USA; L. and A. Seràgnoli Institute of Hematology, Bologna University Medical School, Bologna, Italy; Hospital Universitari i Politècnic La Fe, Valencia & CIBERONC, Instituto Carlos III, Madrid, Spain; University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA; University of Chicago, Chicago, IL, USA; University of Minnesota, Minneapolis, MN, USA; Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy; University of Alabama at Birmingham, Birmingham, AL, USA; Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA; University of California San Francisco, San Francisco, CA, USA; Thomas Jefferson University, Philadelphia, PA, USA; IRCCS San Raffaele Scientific Institute, Milano, Italy; National Taiwan University, Taipei City, Taiwan; Yale University School of Medicine, New Haven, CT, USA; Institut Universitaire du Cancer Toulouse – Oncopole, Cedex, France; Sendai Medical Center, National Hospital Organization, Sendai, Japan; University of Fukui, Fukui, Japan; Seoul National University, Seoul, Republic of Korea; Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; Wake Forest Baptist Medical Center, Winston-Salem, NC, USA; Massachusetts General Hospital, Harvard Medical School, Boston, MA; Astellas Pharma Global Development, Northbrook, IL, USA; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA

Introduction: Gilteritinib is a potent/selective oral FLT3 inhibitor. Based upon interim analysis response rates from the ADMIRAL phase 3 study of gilteritinib vs salvage chemotherapy (SC) in patients (pts) with relapsed/refractory (R/R) FLT3-mutated (FLT3mut+) AML

(NCT02421939), gilteritinib was approved as single-agent therapy in this population. We present the final results of this pivotal trial.

Methods: Adults with confirmed FLT3mut+ AML (FLT3-ITD or FLT3-TKD D835/I836 mutations) refractory to induction chemotherapy, or in untreated first relapse, were randomized (2:1) to receive continuous 28-day cycles of 120-mg/day gilteritinib or prerandomization-selected SC: low-dose cytarabine (LoDAC), azacitidine (AZA), mitoxantrone/etoposide/cytarabine (MEC), or fludarabine/cytarabine/ granulocyte colony-stimulating factor/idarubicin (FLAG-IDA). Prior FLT3 inhibitor use, other than midostaurin or sorafenib, was excluded. Overall survival (OS) and the combined rate of complete remission/complete remission with partial hematologic recovery (CR/CRh) were co-primary endpoints. Safety/tolerability was also examined.

Results: A total of 371 pts were randomized: 247 to gilteritinib and 124 to SC (MEC, 25.7%; FLAG-IDA, 36.7%; LoDAC, 14.7%; AZA, 22.9%). Median age was 62 years (range, 19-85). Baseline FLT3 mutations were: FLT3-ITD, 88.4%; FLT3-TKD, 8.4%; both FLT3-ITD and FLT3-TKD, 1.9%; unconfirmed, 1.3%. Overall, 39.4% of pts had refractory AML and 60.6% had relapsed AML. Patients assigned to gilteritinib had significantly longer OS (9.3 months) than SC (5.6 months; hazard ratio for death=0.637; p=0.0007); 1-year survival rates were 37.1% and 16.7%, respectively. The CR/CRh rates for gilteritinib and SC were 34.0% and 15.3%, respectively (nominal p=0.0001); CR rates were 21.1% and 10.5%. Common adverse events (AEs) in all randomized pts were febrile neutropenia (43.7%), anemia (43.4%), and pyrexia (38.6%). Common grade ≥3 AEs related to gilteritinib were anemia (19.5%), febrile neutropenia (15.4%), thrombocytopenia (12.2%), and decreased platelet count (12.2%). Exposure-adjusted serious treatment-emergent AEs were less common with gilteritinib (7.1/patient-year) than SC (9.2/patient-year).

Conclusions: In pts with R/R FLT3mut+ AML, gilteritinib demonstrated superior efficacy compared with SC and had a favorable safety profile. These results change the treatment paradigm for R/R FLT3mut+ AML and establish gilteritinib as the new standard of care.

B004

CARFILZOMIB, LENALIDOMIDE, DEXAMETHASONE (KRd) WITH OR WITHOUT TRANSPLANTATION IN NEWLY DIAGNOSED MYELOMA PATIENTS: SUBGROUP ANALYSIS IN THE FORTE TRIAL

F. Gay, C. Cerrato, M.T. Petrucci, R. Zambello, E. Rivolti, S. Ballanti, P. Omedè, M. Gentile, A. Bernardini, F. Narni, A. Capra, G. De Sabbata, A. Gozzetti, P. Galièni, R. Rizzi, N. Pescosta, M. Cea, S. Molica, A. Cafro, C. Musolino, A. Spadano, V. Montefusco, P. Musto, M. Cavo, M. Boccadoro

GIMEMA/ European Myeloma Network, Italy

Background: Four 28-day induction cycles of KRd followed by autologous stem-cell transplantation (ASCT) and 4 KRd consolidation (KRd_ASCT_KRd), and with 12 KRd cycles (KRd12) induced high and comparable response rate and minimal residual disease (MRD) negativity in newly diagnosed myeloma (NDMM) patients. Both regimens were superior to carfilzomib-cyclophosphamide-dexamethasone (KCd) induction followed by ASCT and KCd consolidation (KCd-ASCT-KCd) (Gay F ASH 2018). We evaluated the benefit of KRd_ASCT_KRd vs KRd12 in specific patient subgroups.

Methods: 474 NDMM patients ≤65 years were randomized to KRd_ASCT_KRd or KRd12 or KCd_ASCT_KCd. We compared rate of ≥VGPR, ≥CR, sCR, MRD negativity (centralized, second generation flow cytometry, sensitivity 10⁻⁵) after consolidation with KRd_ASCT_KRd vs KRd12 in patients with Revised International Staging System (R-ISS) 1 and R-ISS 2/3. High-risk patients may sometimes respond rapidly, but relapse early. Therefore, we also analyzed early relapse rate (<18 months from randomization) in the two arms. We conducted a multivariate logistic regression analysis to assess factors predictive of early relapse.

Results: Median follow-up was 25 months. On intention-to-treat analysis, KRd_ASCT_KRd and KRd12 had similar rates of ≥VGPR, ≥CR, sCR, MRD negativity in the overall population (Table 1A). Similarly, MRD negativity and response rates in the two arms were comparable in patients with R-ISS Stage 1 and with R-ISS Stage 2/3 (Table 1B). Of note, MRD negativity rate in high-risk patients was ~50%. In the overall population, early relapses were significantly lower with

KRd_ASCT_KRd vs KRd12 (12 patients [8%] vs 26 patients [17%]; $p=0.015$), mainly related to a significantly lower rate of early relapse in high-risk patients (R-ISS Stage 2/3) (11 patients [12%] vs 22 patients [23%]; $p=0.05$, respectively). Very few patients with R-ISS Stage 1 relapsed, with no difference between KRd_ASCT_KRd and KRd12 (0 vs 2 patients). In multivariate regression analysis, patients receiving KRd_ASCT_KRd had a reduced risk of early relapse vs those treated with KRd12 (OR 0.42; $p=0.021$); R-ISS Stage 2 (OR 3.6; $p=0.001$) and R-ISS Stage 3 (OR 4.85; $p=0.003$) increased the risk of early relapse vs R-ISS 1.

Conclusions: KRd-ASCT-KRd and KRd12 were equally effective in inducing high-quality responses, and ~50% of high-risk patients achieved MRD negativity. In addition, ASCT was beneficial in high-risk patients, reducing the risk of early relapse.

Table 1.

	Table 1A: Overall population		Table 1B: Subgroup analysis			
	KRd_ASCT_KRd	KRd12	R-ISS 1		R-ISS 2/3	
	N=158	N=157	KRd_ASCT_KRd N=48	KRd12 N=39	KRd_ASCT_KRd N=92	KRd12 N=94
≥CR	44%	43%	46%	49%	39%	38%
≥VGPR	60%	61%	60%	64%	56%	57%
MRD negative	89%	87%	92%	79%	86%	86%
MRD positive	58%	54%	69%	62%	51%	47%

B005

SECOND PRIMARY MALIGNANCY IN MYELOFIBROSIS PATIENTS TREATED WITH RUXOLITINIB

N. Polverelli, E. Elli, E. Abruzzese, G.A. Palumbo, G. Benevolo, M. Breccia, M. Tiribelli, M. Bonifacio, A. Tieghi, B. Martino, N. Sgherza, M. D'Adda, M. Bergamaschi, M. Crugnola, F. Cavazzini, C. Bosi, G. Binotto, A. Isidori, D. Bartoletti, G. Auteri, R. Latagliata, L. Gandolfi, L. Scaffidi, D. Cattaneo, K. Codeluppi, M. Trawinska, D. Griguolo, A. Cuneo, M. Krampera, G. Semenzato, F. Di Raimondo, R.M. Lemoli, M. Cavo, N. Vianelli, D. Russo, A. Iurlo, F. Palandri

Unit of Blood Diseases and Stem Cells Transplantation, Department of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili of Brescia, Brescia; Hematology Division, San Gerardo Hospital, ASST Monza, Monza; Division of Hematology, Ospedale S. Eugenio, Roma; Department of Scienze Mediche, Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia", University of Catania, Catania; Division of Hematology, Città della Salute e della Scienza Hospital, Torino; Division of Cellular Biotechnologies and Hematology, University Sapienza, Roma; Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Udine; Department of Medicine, Section of Hematology, University of Verona, Verona; Department of Hematology, Azienda USL - IRCCS di Reggio Emilia, Reggio Emilia; Division of Hematology, Azienda Ospedaliera "Bianchi Melacrino Morelli", Reggio Calabria; Division of Hematology, Casa Sollievo Sofferenza, San Giovanni Rotondo; Division of Hematology, ASST Spedali Civili of Brescia, Brescia; Clinic of Hematology, Department of Internal Medicine (DiMI), IRCCS AOU San Martino-IST, Genova; Division of Hematology, Azienda Ospedaliero-Universitaria di Parma, Parma; Division of Hematology, University of Ferrara, Ferrara; Division of Hematology, AUSL di Piacenza, Piacenza; Unit of Hematology and Clinical Immunology, University of Padova, Padova; Hematology and Stem Cell Transplant Center Marche Nord Hospital, Pesaro; Institute of Hematology "L. and A. Seràgnoli", Sant'Orsola-Malpighi University Hospital, Bologna; Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Ruxolitinib (RUX), the first JAK1/JAK2 inhibitor approved for myelofibrosis (MF) therapy, has been recently associated with the occurrence of second primary malignancies (SPMs), mainly lymphoid neoplasms and non-melanoma skin cancers (NMSCs). Herein, we analyzed incidence and risk factors of SPMs in 589 MF pts treated with RUX in 20 European Hematology Centers. Cumulative incidence of SPMs was calculated according to Fine and Gray model with death without SPM as competitive event. Uni and multivariate analysis were carried out by Fine-Gray proportion hazard regression for competing risks. The char-

acteristics of the MF cohort were the following: median age 65y (15-88); male gender 58%; PMF 51%; JAK2, CALR and MPL mutation 83%, 11% and 2% of 530 evaluable pts; median RUX exposure 36 mos (1-86), prior cytoreduction use in 362 pts (HU 98%), previous neoplasms in 40 pts (43 events).

Seventy pts (12%) developed 74 SPMs after RUX start, with a cumulative incidence of 2.3%, 4.5% and 14.3% at 1, 2 and 5y, respectively. Incidence rate of SPMs was 3.91 100 pts/y. Clinical features were comparable between pts with or without SPMs. NMSCs were the most common SPMs accounting for 38 events in 34 pts, other neoplasms involved urological district (13), lung (9), GI tract (6), hematopoietic tissue (2, 1 CML and 1 Langerhans cell histiocytosis), others (6). In 17 out of 213 deceased pts (8%), SPM was the ultimate cause of death. SPM diagnosis did not affect OS, even after excluding NMSC, with projected survival of 81 and 84% at 2y for pts with or without SPM ($p=0.96$). By univariate analysis, age at RUX start >55y ($p=0.035$), CALR mutation ($p=0.023$), HU time-exposure ($p=0.003$) and male sex ($p<0.001$) were associated with increased incidence of SPMs; in multivariate analysis, CALR mutation, HU use and sex maintained statistical significance (HR 3.78, CI95% 1.61-8.87 $p=0.002$, HR 1.07, CI95% 1.02-1.13 $p=0.01$ and HR 2.63, CI95% 1.08-6.43, $p=0.034$ respectively). After exclusion of NMSCs, HU lost any statistical significance (HR 1.01, CI95% 0.94-1.08); on the contrary, mutational status and sex confirmed their prognostic value (HR 2.99, CI95% 1.28-7.02 $p=0.011$ and HR 2.67, CI95% 1.17-6.09 $p=0.019$, respectively). Notably, RUX time-exposure, and starting/cumulative dose were not associated with SPMs. In conclusion, mutational status and male sex represent the major predictive factors for SPMs occurrence in RUX-treated pts. HU time-exposure was strongly associated with NMSCs.

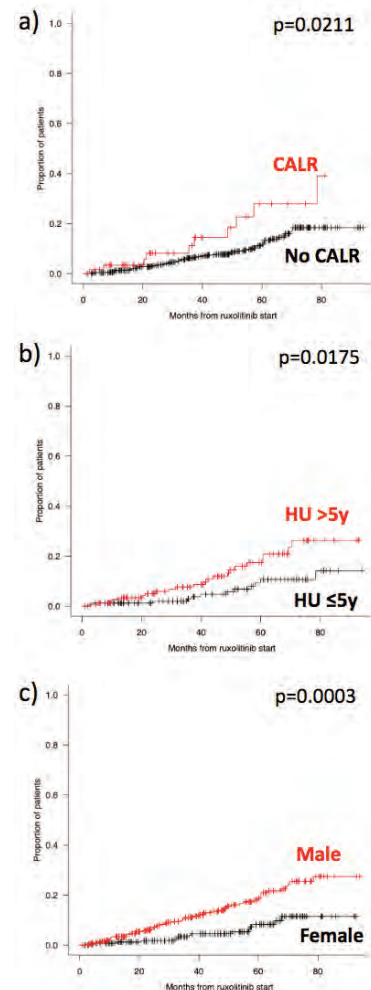


Figure 1. Cumulative incidence of SPMs according presence of CALR mutation (a), HU exposure >5y (b) and male sex (c).

B006**ABSTRACT WITHDRAWN****B007****CD19 REDIRECTED CAR NK CELLS ARE EQUALLY EFFECTIVE BUT LESS TOXIC THAN CAR T CELLS IN ACUTE LYMPHOBLASTIC LEUKEMIA**

S. Caruso¹, C. Quintarelli^{1,2}, S. Sivori³, S. Carlomagno⁴, M. Falco⁵, I. Boffa¹, D. Orlando¹, M. Guercio¹, Z. Abbaszadeh¹, M. Sinibaldi¹, S. Di Cecca¹, A. Camera¹, B. Cembrola¹, K. Bovetti¹, A. Pitisci¹, M. Andreani¹, L. Vinti¹, F. Del Bufalo¹, M. Algeri¹, G. Li Pira¹, A. Moseley⁶, B. De Angelis¹, L. Moretta⁷, F. Locatelli^{1,8}

¹Department of Onco-Haematology and Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS; ²Federico II University of Naples, Department of Clinical Medicine and Surgery; ³University of Genoa, Department of Experimental Medicine (DIMES) and Centre of Excellence for Biomedical Research (CEBR); ⁴University of Genoa, Department of Experimental Medicine (DIMES); ⁵Istituto G Gaslini, Laboratorio di Immunologia Clinica e Sperimentale, Dipartimento di Ricerca e Diagnostica; ⁶Sandhill Therapeutics; ⁷Department of Immunology, Bambino Gesù Children's Hospital, IRCCS; ⁸Department of Pediatrics, Sapienza, University of Rome, Italy

Based on the clinical success observed in B-cell lymphoproliferative disorders with CD19-directed chimeric antigen receptor engineered T cells (CAR-T), we hypothesized that combining the CAR specificity with the innate allo-reactivity of KIR-mismatched NK (Natural Killer) cells might provide a powerful tool for adoptive cell therapy. The use of a third-party bank of CAR-NK cells offers the advantage of an immediate availability in the allogeneic setting. We developed a feeder-free culture resulting in a 3.2-log expansion of CAR-NK cells after 20 days of culture. Expanded NK cells preserve a high percentage of CD56+CD57- cells (85±13%), associated with high proliferative capability. NK cells transduction was 38%±15% and it was stable up to 60 days after *in vitro* culture CAR-NK cells display significant anti-leukemia activity towards CD19+ leukemia and lymphoma cell lines (LCL 721.221, DAUDI and BV173) and primary blasts obtained from Bcp-ALL patients. Co-culture experiments, using a 1:1 E/T ratio, showed that, while the antitumor activity was already remarkable for NK cells (60±30%, 71±33% and 54±23% of residual LCL 721.221, DAUDI and BV173 cells, respectively; p<0.05 vs T cells), it reached the highest level when CAR-NK cells were used as effectors (7±9%, 16±30% and 22±16% of residual LCL 721.221, DAUDI and BV173 cells, respectively; p<0.05 vs NK cells). Importantly, INF-α production was significantly lower upon CAR-NK activation compared to CAR-T cells (DAUDI 384±194 ng/ml vs 1860±678 ng/ml, p=0.002). CAR-NK cells exert high degree of tumor control on primary Bcp-ALL blasts (2.1±2% of residual blasts vs 5.4±1.6%; p=0.04). In an *in vivo* leukaemia xenograft mice model, we evaluated whether CAR-NK cells are associated with a lower toxicity profile compared to CAR-T cells. While the *in vivo* antileukemia activity was superimposable between CAR-T and CAR-NK cells, mice treated with two i.v. infusions of 10x10⁶ CAR-CD19 NK cells had a 100% overall survival at 50 days compared to 20% of mice receiving 10x10⁶ CAR-CD19 T cells; p=0.01, which succumbed due to a xenograft reaction. These *in vitro* and *in vivo* data demonstrate the feasibility of clinical scale feeder-free expansion of NK cells or CAR-NK cells for treatment of CD19+ ALL. We suggest that *ex-vivo* expanded, feeder-free NK cells could be universally applied for 'off-the-shelf' immuno-gene-therapy, and that their innate allo-reactivity can be safely harnessed to potentiate allogeneic cell therapy.

B008**A PHASE 2 OPEN-LABEL PROOF OF CONCEPT STUDY OF THE ORAL, SMALL MOLECULE FACTOR D INHIBITOR, ACH-4471, IN UNTREATED PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)**

A. Risitano, A. Kulasekararaj, J.W. Lee, R. Notaro, R.A. Brodsky, M. Huang, M. Geffner, P. Browett

Università Federico II di Napoli, King's College Hospital-NHS Foundation Trust, London, Catholic University of Korea, Seoul, Azienda Ospedaliera Universitaria Careggi, Firenze, Johns Hopkins University School of Medicine, Baltimore, Achillion Pharmaceuticals, New Haven and Blue Bell, University of Auckland

Background: Factor D (fD) is a highly specific serine protease that cleaves factor B as a rate-limiting step of the alternative pathway (AP) of complement. Thus, fD is a promising target in diseases of excess AP activation, such as PNH.

Aims: We hypothesize that ACH-4471, a novel, oral, small molecule fD inhibitor, represents a potential PNH treatment option.

Methods: Data are presented for this Phase 2, dose-finding, proof of concept trial in PNH patients not receiving anti-complement treatment. Ten adult PNH patients were included with anemia (Hgb < 12 g/dL and adequate reticulocytosis), PNH Type III RBC/granulocyte clone size > 10%, LDH > 1.5X ULN, platelets > 50,000 µL. ACH-4471 Starting doses ranged from 100-150 mg TID, with dose escalation based on response to a maximum of 200 mg TID. The primary efficacy parameter was the change in LDH vs baseline at Day 28. Secondary efficacy parameters were Hgb relative to baseline at Days 28 and 84 and LDH vs baseline at Day 84. Safety, tolerability, PK/PD and patient reported outcomes (PRO) were also measured. After 12 weeks of treatment, patients entered a separate long-term extension study.

Results: Ten patients received ACH-4471. Two discontinued; one for personal reasons unrelated to safety and the other, for a serious adverse event of elevated ALT/AST coincident with breakthrough hemolysis, which resolved without sequelae. The remaining 8 patients completed treatment and entered a long-term extension study. Baseline mean LDH for these 8 patients was 5.6X ULN and was 1.5X ULN and 2.15X ULN at Days 28 and 84, respectively. Baseline mean Hgb was 9.7 g/dL with a mean increase of 1.2 g/dL and 1.8 g/dL at Day 28 and Day 84, respectively. Mean reticulocyte counts and total bilirubin also decreased to the normal range with treatment. No C3 deposition on PNH RBC was observed. Mean baseline FACIT-FATIGUE score at baseline was 37 and increased by 10 and 13 points at Day 28 Day 84, respectively.

Conclusions: Proof of concept has been demonstrated with ACH-4471, with meaningful improvements in LDH, Hgb and PRO. These improvements were observed in monotherapy, confirming that upstream complement inhibition at the level of fD can prevent MAC-mediated intravascular hemolysis and C3-mediated extravascular hemolysis in the absence of terminal pathway blockade (i.e., anti-C5 agents). Further improvements in efficacy is expected with next-generation, oral fD inhibitors, with increased potency and twice daily administration.

B009

A BAL-DRIVEN ANTIMICROBIAL TREATMENT IMPROVES CLINICAL OUTCOME IN HEMATOLOGIC MALIGNANCIES PATIENTS WITH LUNG INFILTRATES DETECTION: A PROSPECTIVE MULTICENTER STUDY OF THE SEIFEM GROUP

F. Marchesi, C. Cattaneo, M. Criscuolo, M. Delia, M. Dargenio, M.I. Del Principe, A. Spadea, N.S. Fracchiolla, L. Melillo, K. Perruccio, C. Alati, D. Russo, M. Garzia, P. Zappasodi, M.G. Cefalo, D. Armiento, S. Cesaro, N. Decembrino, A. Mengarelli, A. Busca, L. Pagano

Hematology and Stem Cell Transplant Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; Hematology Division, ASST-Spedali Civili di Brescia, Italy; Dipartimento di Scienze radiologiche, Radioterapiche ed Ematologiche, Fondazione Policlinico Universitario A. Gemelli - IRCCS, Rome, Italy; Hematology and Bone Marrow Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Italy; Hematology and Stem Cell Transplantation Unit, 'Vito Fazzi' Hospital Lecce, Italy; Hematology, Department of Biomedicine e Prevention, Tor Vergata University, Rome, Italy; Department of Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; Division of Hematology, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; Pediatric Hematology Oncology, Azienda Ospedaliera Universitaria, Ospedale Santa Maria della Misericordia, Perugia, Italy; Hematology Unit, Bianchi-Melacrino-Morelli Hospital, Reggio Calabria, Italy; Bone Marrow Transplant Unit, University of Brescia and ASST-Spedali Civili, Brescia, Italy; Hematology, San Camillo-Forlanini Hospital, Rome, Italy; Division of Hematology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Italy; Hematology, San Eugenio Hospital, Rome, Italy; Hematology, Campus Bio-Medico University Hospital, Rome, Italy; Pediatric Hematology Oncology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; Pediatric Hematology Oncology, IRCCS Policlinico San Matteo, University of Pavia, Italy; Stem Cell Transplant Center, AOU Città' della Salute e Della Scienza, Turin, Italy; Institute of Hematology, Fondazione Policlinico A. Gemelli - IRCCS, Università Cattolica Sacro Cuore, Rome, Italy

BAL is useful for characterization of lung infiltrates (LI) in patients (pts) with hematologic malignancies (HM), but prospective studies evaluating its efficacy in response to antimicrobial treatment (tx) and survival are lacking. We conducted a prospective observational study in 18 hematology institutes participating to SEIFEM group from Jan-1 to Sept-30, 2018. Adult and pediatric severely immunocompromised HM pts with LI detection were enrolled according to the following criteria: 1) presence of a host risk factor for IFD (EORTC/MSG 2008); 2) absence of any microbiological and/or serological documentation at diagnostic work-up (including serum galactomannan, GM); 3) fever or respiratory distress not responding to broad-spectrum antimicrobial tx.

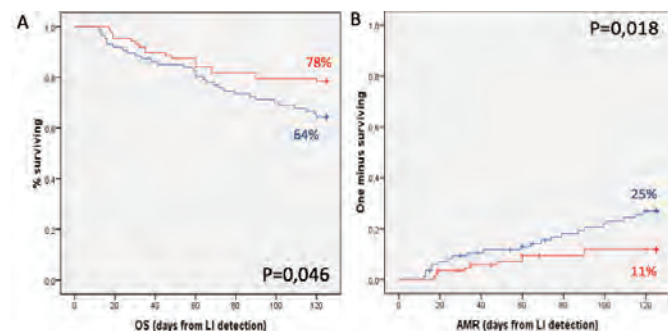


Figure 1. Overall survival (A) and attributable mortality rate (B) by comparing a BAL-driven antimicrobial treatment (red line) with an empirical approach (blue line).

According to investigators, the pts fulfilling these criteria underwent BAL. Out of 3.055 pts admitted on wards in the study period, 434 (14%) had LI and 177 (6%) were enrolled and evaluable for analysis. Median age was of 60 years (1-83). Most pts had a AML/MDS (81), followed by lymphoma (41), ALL (27), and other HM (36). Median ANC count was of $460/\text{mm}^3$, and 106 patients (60%) had experienced a prolonged and severe neutropenia ($\text{ANC} < 100/\text{mm}^3$ for a median of 12

days). BAL was feasible in 145 cases (82%) in a median time of 4 days from LI detection, whereas in the remaining cases BAL was not performed for clinical decision. A putative causal agent was detected in 111 cases (76%): in 41 cases BAL was positive for GM, in 36 for bacteria, in 5 for Pneumocystis and in the remaining cases for viruses. In 87 cases (61%) the results of BAL allowed to guide the antimicrobial tx. We observed a significant better outcome of LI at day +30 from its detection in the pts in which a BAL-driven antimicrobial tx was possible (improve/resolution rate: 71% vs 60%; $p=0,04$). Moreover, we observed a significant better outcome in terms of 120d-OS (78% vs 64%; $p=0,046$) and 120d-AM (11% vs 25%; $p=0,018$) for pts in which a BAL-driven antimicrobial tx was possible (Figure 1). The multivariate analysis showed that only younger age and a BAL-driven antimicrobial tx were significantly related to lower 120d-AM. We did not observe any severe AE, but only 5 cases of grade 1-2 AE occurred after the procedure. In conclusion, in our on-study patient population: 1) BAL allows to detect a putative agent of LI in about 75% of cases; 2) BAL is feasible in almost all cases; 3) a BAL-driven antimicrobial tx allows to improve clinical outcome and survival.

B010

RANDOMIZED STUDY FOR THE TREATMENT OF PRIMARY IMMUNE THROMBOCYTOPENIC PURPURA (PITP) IN NEWLY DIAGNOSED UNTREATED ADULT PATIENTS. COMPARISON OF STANDARD DOSE PREDNISONE VERSUS HIGH-DOSE DEXAMETHASONE. PRELIMINARY RESULTS. GIMEMA PROTOCOL ITP0207

M.G. Mazzucconi, F. Paoloni, C. Santoro, A. Sau, N. Vianelli, I.D. Vincelli, M. Ruggeri, E. Rivolti, L. Gugliotta, G. Gaidano, M. Napolitano, N. Cascavilla, C. Carbone, V. Pavone, V. De Stefano, G. Avvisati, M. Iodice, E. Baldacci, P. Fazi, F. Rodeghiero; on behalf of the GIMEMA Working Party "Anemie e Piastrinopenie"

Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I; Data Center-GIMEMA Foundation - Franco Mandelli Onlus; Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I; Azienda USL Pescara, Unità Operativa Ematologia Clinica; AOU di Bologna, Policlinico S. Orsola Malpighi, UOC Ematologia; Dipartimento di Emato-Oncologia Azienda Ospedaliera Bianchi Melacrino Morelli; AULSS 8 Berica - Ospedale di Vicenza - UOC Ematologia; AUSL di Reggio Emilia - Arcispedale Santa Maria Nuova, IRCCS - SC Ematologia; AUSL di Reggio Emilia - Arcispedale Santa Maria Nuova, IRCCS - SC Ematologia; S.C.D.U. Ematologia, DIM ECS e Dipartimento Oncologico - Università del Piemonte Orientale Amedeo Avogadro; AOU Policlinico P. Giaccone - UO Ematologia; Istituto di Ematologia, IRCCS Ospedale Casa Sollievo della Sofferenza; Azienda Ospedaliera Spedali Civili Brescia, U.O. Ematologia; Unità Operativa Ematologia, Azienda Ospedaliera, Pia Fondazione di Culto e Religione Card. G. Panico; Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Area Ematologica; Policlinico Universitario Campus Bio Medico - UOC Ematologia e Trapianto di Cellule Staminali; Data Center-GIMEMA Foundation - Franco Mandelli Onlus; Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I, Data Center-GIMEMA Foundation - Franco Mandelli Onlus AULSS 8 Berica - Ospedale di Vicenza - UOC Ematologia

Background: Corticosteroids are the mostly used first-line treatment in adult patients (pts) with pITP, but the best initial approach to this disease is still matter of debate. Comparison between standard doses of prednisone/prednisolone (PDN) and pulsed high-dose dexamethasone (HD-DXM) seems to be advisable.

Aim: To evaluate therapy intensification with HD-DXM in newly diagnosed untreated adult pITP pts for response improvement at 6 months after initial response, in comparison with standard PDN doses. Study design Open label, randomized, multicentre phase III study: ARM A, PDN 1 mg/Kg/d P.O. for 4 consecutive weeks, then tapering within 14 days; ARM B, DXM, 40 mg/d P.O. for 4 consecutive days, every 14 days, for 3 consecutive courses. Initial response ARM A, at d42 from PDN start; ARM B, at d46 from HD-DXM start. Final response At d180 from initial response for both arms. Response criteria: complete response (CR), platelet (plt) count $\geq 100 \times 10^9/\text{L}$. Partial response, (PR), plt count $> 50 < 100 \times 10^9/\text{L}$. Minimal response (MR), plt count $> 20 < 50 \times 10^9/\text{L}$. No response (NR), plt count $\leq 20 \times 10^9/\text{L}$ or $> 20 \times 10^9/\text{L}$ and $< 50 \times 10^9/\text{L}$ plus bleeding. Patients Untreated adult pITP pts ($> 18 < 80\text{y}$), plt count $< 20 \times 10^9/\text{L}$ or $> 20 \times 10^9/\text{L}$ and $< 50 \times 10^9/\text{L}$ plus bleeding. Results 113 eligi-

ble pts were enrolled, 59 in ARM A, 54 in ARM B, M 43, F 70, median age 45y (18.2-79.5), median plt count $8.5 \times 10^9/L$ (1-51). Initial response ARM A, CR 35/56 (62.50%), PR 7/56 (12.50%), MR 2/56 (3.57%), (OR, 78.57%) NR 12/56 (21.43%). ARM B, CR 31/49 (63.27%), PR 11/49 (22.45%), MR 4/49 (8.16%) (OR, 93.88%), NR 3/49 (6.12%): difference between the 2 ARMS concerning global responses is statistically significant ($p=0.0284$). Final response ARM A, 13 pts not evaluable, CR 22 (51.16%), PR 3 (6.98%), MR 1 (2.33%) (OR, 60.47%), NR 12 (27.91%), loss of response 5 (11.63%); ARM B, 10 not evaluable, CR 18 (46.15%), PR 4 (10.26%), MR 1 (2.56%) (OR, 58.97%), NR 3 (7.69%), loss of response 13 (33.33%). Median follow-up was 46.0 mos (IQR 16.9-80.4). Considering the 49 responding pts, at 48 mos, 80.71% (95%CI: 68.65-94.89) was alive in response, difference between two arms was not statistically significant: 89.84% (95%CI: 77.34-100) in ARM A vs 70.41% (95%CI: 51.22-96.79) in ARM B ($p=0.1069$). Conclusions Initial response is significantly better in ARM B, but this result is not confirmed at day 180 ($p=0.8907$) and during follow-up. HD-DXM, indeed, seems to be able to induce a prompt initial response, reducing bleeding risk.

B011

STANDARDIZATION OF 18F-FDG PET/CT ACCORDING TO DEAUVILLE CRITERIA FOR MRD EVALUATION IN NEWLY DIAGNOSED TRANSPLANT ELIGIBLE MULTIPLE MYELOMA (MM) PATIENTS: JOINED ANALYSIS OF TWO PROSPECTIVE RANDOMIZED PHASE III TRIALS

E. Zamagni, C. Nanni, L. Dozza, T. Carlier, P. Tacchetti, A. Versari, S. Chauvie, A. Gallamini, M. Attal, B. Gamberi, D. Caillot, F. Patriarca, M. Macro, M. Boccadoro, L. Garderet, S. Fanti, A. Perrot, F. Gay, P. Sonneveld, L. Karlin, C. Bodet-Milin, P. Moreau, F. Kraeber-Bodéré, M. Cavo

Seragnoli Institute of Hematology, Bologna University School of Medicine; Nuclear Medicine, AOU Policlinico S.Orsola-Malpighi, Bologna, Seragnoli Institute of Hematology, Bologna University School of Medicine, Nuclear Medicine department, Nantes University Hospital and CRCINA INSERM UMR 1232, France; Seragnoli Institute of Hematology, Bologna University School of Medicine, Nuclear Medicine, S. Maria Nuova Hospital, IRCCS Reggio Emilia, Italy; MedicalPhysics Unit, Santa Croce e Carle Hospital, Cuneo, Italy; Research and Innovation Department A Lacassagne Cancer Center, Nice, France; Hematology Department, University Hospital, Toulouse France; Hematology Unit, Azienda Ospedaliera ASMN, IRCCS, Reggio Emilia, Italy; Hematology Department, University Hospital, Dijon France; Hematologic Clinic, Udine University, Italy; Hematology Department, University Hospital, Caen, France; Myeloma Unit, Division of Hematology, University of Torino, Italy; Hematology Department, University Hospital Saint-Antoine, Dijon, France; Nuclear Medicine, AOU Policlinico S.Orsola-Malpighi, Bologna, Italy; Hematology Department, University Hospital, Nancy, France; Myeloma Unit, Division of Hematology, University of Torino, Italy; Hematology Department Rotterdam The Netherlands; Hematology Department, University Hospital, Lyon, France; Nuclear Medicine department, Nantes University Hospital and CRCINA INSERM UMR 1232, France; Hematology Department, University Hospital, Nantes, France; Nuclear Medicine department, Nantes University Hospital and CRCINA INSERM UMR 1232, France; Seragnoli Institute of Hematology, Bologna University School of Medicine, Italy

18F-FDG-PET/CT is currently considered as the standard technique to define minimal residual disease (MRD) status outside the bone marrow (BM) in multiple myeloma (MM) patients. In this regard, standardization and definition of cut-offs for positivity/negativity is of highly importance.

Aim of the present study was to standardize PET/CT evaluation by centralized imaging and revision and to define criteria for PET negativity after therapy, in a joined analysis of a sub-group of patients (pts) with newly diagnosed transplant-eligible MM, enrolled in 2 independent European randomized phase III trials (EMN02/HO95 and IFM2009) (Cavo M *et al.*, Blood 2017 abs; Attal M *et al.*, NEJM 2017).

236 patients were enrolled and followed for a median of 62.9 months (mos). By study design, PET/CT scans were performed locally at baseline (B) and prior to the start of maintenance (PM), uploaded in a central website and re-interpreted a-posteriori in a blinded independent central review process, by a panel of expert nuclear medicine physicians. The five-point Deauville scores (DS) (1-5) were applied to the following parameters: bone marrow (BM), focal lesions (FLs), extramedullary disease (EMD). At B, 80% of the pts had FLs, with a median (m) SUVmax of 5.

mBM SUVmax was 3. FLs DS (FS) and BM DS (BMS) > 3 (higher than the liver) were present in 79.8% and 35.5% of the pts, respectively. EMD was present in 11% of the pts. PM, mFLs and BM SUVmax were 3.6 and 2.3, respectively, with 53.5% and 71.2% of the pts obtaining a FS and BMS ≤ 2 and 79% and 91.4% ≤ 3 , respectively. In univariate analysis, at Landmark time PM, attaining a FS and BMS ≤ 3 was the strongest predictor for prolonged PFS (FS ≤ 3 vs >3: m40 vs 26.6 mos, HR 0.6, $p=0.0019$; BMS ≤ 3 vs >3: m39.8 vs 26.6 mos, HR 0.47, $p=0.024$, respectively) and OS (FS ≤ 3 vs >3: estimate at 63 mos 73% vs 63.6% mos, HR 0.51, $p=0.028$; BMS ≤ 3 vs >3: estimate at 75.5% vs 49.7%, HR 0.28, $p=0.002$, respectively) and could be identified as the most representative cut-off values for PET negativity. In Cox multivariable analysis, FS and BMS ≤ 3 at PM were independent predictors of prolonged PFS (HR 0.58 and 0.41, respectively) and OS (HR 0.36 and 0.24, respectively).

In conclusion, reduction of FDG uptake lower than the liver after therapy, both in the FLs and in the BM, was an independent predictor for improved PFS and OS and can be proposed as the standardized criteria to define PET negativity, confirming the value of Deauville scores in MM.

B012

R-CHOP PRECEDED BY BLOOD-BRAIN BARRIER PERMEABILIZATION WITH ENGINEERED TUMOR NECROSIS FACTOR (TNF) IN RELAPSED OR REFRACTORY PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE CNS (RPCNSL): FINAL RESULTS OF THE INGRID TRIAL

T. Calimeri, G.M. Conte, M. Ponzoni, F. Fallanca, D. Cattaneo, E. Scarano, F. Curnis, M. Sassone, M. Foppoli, F. Gagliardi, A. Chiara, S. Perrone, C. Cecchetti, P. Lopedote, F. Ciceri, N. Anzalone, A. Corti, A.J.M. Ferreri

IRCCS San Raffaele Scientific Institute, Università Vita-Salute San Raffaele, ASST Fatebenefratelli Sacco University Hospital, Italy

PCNSLs are usually treated with high-dose methotrexate-based combinations that require hospitalization and extensive expertise. R-CHOP could overcome these difficulties, but CNS availability of related drugs is poor. TNF induces blood-brain barrier (BBB) permeabilization and enhances CNS access of drugs. Coupling TNF with NGR, a peptide that targets CD13+ tumor vessels, improves its biological effects. Here, we report results of activity, safety and BBB permeabilization of NGR-hTNF combined with R-CHOP in pts with rPCNSL enrolled in a phase II trial (NCT03536039). HIV-neg adults with PCNSL failed after methotrexate-based chemo and measurable disease were enrolled and treated with 6 courses of R-CHOP21 preceded by NGR-hTNF (0.8 g/m²). Primary endpoint was overall response rate (ORR). Treatment would be declared active with ≥ 12 responses. Secondary endpoints were changes induced by NGR-hTNF, both in vessel permeability assessed by DCE-MRI and 99mTc-DTPA-SPECT, and in anticancer drug levels in CSF and plasma, and CD13 expression on diagnostic tumor samples. 28 pts (median age 58 yo, range 26-78; 14 males) were enrolled; 21 (75%) pts had intermediate-high IELSG score. Pts were heavily pretreated: 25 had received ASCT, WBRT or both; 15 had refractory disease. NGR-hTNF/RCHOP was active: the predetermined activity threshold (≥ 12 responses) was achieved, with confirmed tumor response in 21 pts (75%; 95%CI= 59-91), which was complete in 11. At a median follow-up of 12 (4-20) months, 8 pts remain relapse free and 9 are alive. Treatment was well tolerated; toxicities were quickly solved without dose reductions or interruptions. G4 toxicities were neutropenia (44% of courses), thrombocytopenia (20%), anemia (2%), and FN (1%). 15 SAE were recorded in 13 pts: seizures (3), DVT (2), infections (5), syncope (2), constipation, FN, and LVEF reduction. There were 7 g1-2 TNF infusion reactions. DCE-MRI and SPECT studies showed an increase of vascular permeability after NGR-hTNF infusion in tumor and perilesional areas. Specificity of this effect was suggested both by CD13 expression in all tumor samples, and absence of changes in CSF/plasma drug levels after NGR-hTNF infusion. NGR-hTNF/RCHOP is active and safe in pts with rPCNSL. CD13, the target of TNF, was expressed in tumor tissue and, consistently, NGR-hTNF enhanced vascular permeability specifically in tumor and perilesional areas. This innovative approach deserves to be addressed as first-line treatment in PCNSL pts.

ORAL COMMUNICATIONS

Non Hodgkin Lymphoma 1

C001

OUTCOMES IN FIRST RELAPSED-REFRACTORY YOUNGER PATIENTS WITH MANTLE CELL LYMPHOMA: RESULTS FROM THE MANTLE-FIRST STUDY

C. Visco, A. Di Rocco, M. Tisi, L. Morello, A. Evangelista, V.R. Zilioli, C. Rusconi, S. Hohaus, R. Sciarra, A. Re, C. Tecchio, A. Chiappella, A.M. Niebla, R. McCulloch, G. Gini, T. Perrone, L. Nassi, E. Pennese, P.M. Stefani, M.C. Cox, V. Bozzoli, A. Fabbri, V. Polli, S. Ferrero, I.A. De Celis, A. Sica, L. Arcaini, M. Balzarotti, S. Rule, U. Vitolo

Department of Medicine, Section of Hematology, University of Verona; Sapienza University, Cellular Biotechnologies and Hematology; Cell Therapy and Hematology, San Bortolo Hospital; Hematology, Humanitas Clinical and Research Center; Clinical Epidemiology, Città della Salute e della Scienza and CPO Piemonte; Hematology, ASST Grande Ospedale Metropolitano Niguarda; Institute of Hematology, Policlinico Gemelli Foundation, Catholic University of the Sacred Heart; Haematology Oncology, Fondazione IRCCS Policlinico San Matteo; Hematology, Spedali Civili, Brescia; Hematology, Città della salute e della scienza university hospital; Hematology, Vall d'Hebron Institut de Recerca; Haematology, University of Plymouth and Derriford Hospital; Hematology, Department of Clinical and Molecular Sciences, Marche Polytechnic University; Hematology, University of Bari; Hematology, Azienda Ospedaliero-Universitaria Maggiore della Carità; Hematology, UOSD "Centro Diagnosi e Terapia dei Linfomi", PO Santo Spirito; Hematology, Treviso Hospital; Hematology, AOU Sant'Andrea; Hematology, P.O. V. Fazzi; Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena; Hematology, Ospedale degli Infermi; Molecular Biotechnologies and Health Sciences, University of Torino/AOU Città della Salute e della Scienza di Torino; Hematology, AUSL/IRCCS Santa Maria Nuova Hospital; Hematology, Policlinico di Napoli, Italy

Introduction: Patients with mantle cell lymphoma (MCL) that fail induction treatment represent a difficult-to-treat population with poor outcome. There is a paucity of large-scale treatment data in relapsed or refractory (r/r) MCL.

Methods: MANTLE-FIRST, an international, retrospective study, evaluated outcomes in patients with MCL which had progressive disease or first relapse when treated upfront with high dose cytarabine (HDAC), followed by autologous stem cell transplantation when appropriate. Overall survival (OS), and progression-free survival (PFS) were estimated from the time of salvage therapy. The previously defined threshold of 24 months was used to define patients as early- or late-POD.

Results: Overall, 258 patients with r/r MCL were included, with a median follow-up from time of relapse or progression of 38 months (range 1-122). Second line regimens consisted of rituximab bendamustine (R-B, 22%), R-B and cytarabine (R-BAC, 29%), Ibrutinib (18%), or other regimens (31%). Overall response was 79% in ibrutinib and R-BAC treated patients, as compared to R-B (62%), or other regimens (30%). Complete response were significantly higher with R-BAC (63%) than with R-B (43%, $p=0.02$), ibrutinib (34%, $p=0.002$), or other regimens (22%, $p<0.0001$). Median OS and PFS for all patients were 34 ± 5 and 13 ± 8 months, respectively. PFS curve according to second line therapy is shown in Figure 1. For patients with early-POD ($n=126$), outcomes were poor, irrespectively of second line therapy (median OS 10 ± 1 months), unless for those reaching allogeneic transplant ($n=27$, median OS 63 ± 24 months). Ibrutinib was associated with longer PFS and OS (10 ± 1 and 30 ± 13 , respectively), compared to immunochemotherapy (6 ± 2 and 12 ± 2 months, $p=0.09$ and $p=0.08$). For late-POD ($n=132$), median OS was 74 ± 9 months, and PFS was 34 ± 5 . Ibrutinib and R-BAC (not reached) and R-B (73 ± 21) had similar significant OS advantage, compared to other regimens (41 ± 11 , $p=0.03$). At multivariate analysis, factors significantly associated to a reduction of the risk of death were CR after second line regimens (HR 0,19), allogeneic transplant (HR 0,40), ibrutinib second line therapy (HR 0,47), and late-POD (HR 0,49).

Conclusions: MANTLE-FIRST is the largest patient-level pooled analysis of HDAC treated patients with first r/r MCL. Our analysis shows that Ibrutinib is the best performer in early-POD patients, R-BAC is as-

sociated with higher CR rate, while allogeneic transplant remains the only curative option.

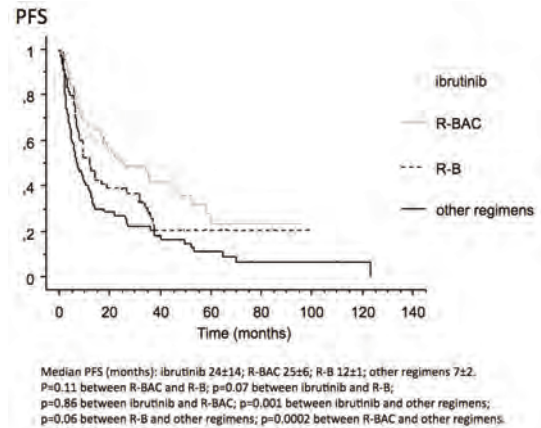


Figure 1.

C002

ABSTRACT WITHDRAWN.

C003

LENALIDOMIDE/RITUXIMAB (R2) VERSUS RITUXIMAB/PLACEBO EFFICACY BY POD24 STATUS AND TIME TO NEXT TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY INDOLENT NHL (AUGMENT)

A. Pinto, J. Gribben, M. Trneny, K. Izutsu, N.H. Fowler, X. Hong, H. Zhang, F. Offner, A. Scheliga, G. Nowakowski, F. Re, L.M. Fogliatto, P. Scheinberg, I. Flinn, C. Moreira, M. Czuczman, S. Kalamvakas, P. Fustier, C. Wu, J.P. Leonard

Istituto Nazionale Tumori Fondazione G. Pascale IRCCS, Centre for Haematology Oncology Barts Cancer Institute, Charles University General Hospital, National Cancer Center Hospital, The University of Texas MD Anderson Cancer Center, Fudan University Shanghai Cancer Center, Tianjin Medical University Cancer Institute and Hospital, UZ Gent, INCA Instituto Nacional De Câncer, Mayo Clinic, Azienda Ospedaliero Universitaria di Parma, Hospital de Clinicas de Porto Alegre, Hospital A Beneficência Portuguesa de São Paulo, Sarah Cannon Research Institute/Tennessee Oncology, Instituto Português de Oncologia Do Porto Francisco Gentil Epe, Celgene Corporation, Celgene Corporation, Meyer Cancer Center Weill Cornell Medicine and New York Presbyterian Hospital

Relapse is common in indolent lymphoma, and relapse ≤ 2 y from initial chemoimmunotherapy (ie, POD24) in follicular lymphoma (FL) patient (pts) has been associated with worse prognosis and survival. Objectives here were twofold: To examine the impact of POD24 status in relapsed/refractory (R/R) FL pts and to analyze time to next treatment (TTNT) in R/R FL and marginal zone lymphoma (MZL) pts receiving lenalidomide+rituximab (R2) vs rituximab/placebo (R/placebo). The AUGMENT phase III study evaluated pts with R/R FL gr 1-3a and MZL after ≥ 1 prior systemic therapy (not rituximab refractory). Randomization was 1:1 to R2 (lenalidomide PO 20 mg/d, d1-21/28 X12 cycles [c]+rituximab [R] IV 375 mg/m²/wk, c1, d1, 8, 15, 22 and c2-5, d1) and R/placebo (same schedule). The primary endpoint was PFS by 2007 IWG. POD24 was defined post-hoc as PD/relapse ≤ 2 y from initial antilymphoma treatment, which included immuno and/or chemotherapy. Secondary/exploratory analyses were time to next antilymphoma/chemotherapy treatment (TTNLT/TTNCT), response to next treatment, and PFS2. PFS2 was time from randomization to first PD/death (any cause) after next or start of 3rd antilymphoma treatment. As of 22 June 2018, 178 pts were randomized to R2 (FL, $n=147$; MZL, $n=31$) and 180 to R/placebo (FL, $n=148$; MZL, $n=32$). Median PFS was superior for R2 vs R/placebo (39.4 vs 14.1 mo; HR=0.46; $p<0.0001$). Of the FL pts, 56 (38%) R2 and 57 (39%) R/placebo pts were POD24. Median PFS was improved in FL pts receiving R2 vs R/placebo, irrespectively of POD24 status (HR=0.41 [95% CI, 0.24-0.68] with POD24 and HR=0.43 [95% CI, 0.28-0.65] with no POD24). Best responses were similar in

each arm in FL pts with or without POD24. Treatment with R2 (vs R/placebo) reduced the risk of relapse/progression by 59% in FL pts with POD24, and improved both ORR and CR. Similar outcomes were shown for FL pts who relapsed $\leq 2y$ from diagnosis. Median TTNLT, TTNCT, and PFS2 were not reached for R2, and significantly longer than R/placebo (HR=0.54, 0.50, and 0.52, respectively). For R2 (n=49) and R/placebo (n=80) pts receiving next antilymphoma therapy, response was generally higher with R2 (57% ORR; 31% CR) vs R/placebo (36% ORR; 16% CR). R2 demonstrated superior efficacy (vs R/placebo) in FL pts, including those with POD24 who are historically associated with worse outcomes. Additionally, these analyses suggest that R2 (vs R/placebo) prolonged TTNT and was associated with longer PFS2, enabling greater response to next therapy.

C004

TREATMENT OF RELAPSED/REFRACTORY PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA PATIENTS WITH CHECK- POINT INHIBITORS: THE EXPERIENCE OF ISTITUTO SERAGNOLI

B. Casadei, C. Pellegrini, V. Stefoni, G. Lolli, M. Carella, A. Broccoli, L. Nanni, M. Marangon, A. Morigi, N. Fabbri, G. Gabrielli, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia L. e A. Seràgnoli, Università di Bologna, Italy

Primary mediastinal B-cell lymphoma (PMBCL) patients (pts) with unsatisfactory response to the first line regimen usually display a poor prognosis, as the disease is often refractory to chemotherapy. The amplification of the chromosome 9p24.1 has been recognised as a genetic hallmark of PMBCL, leading to the survival and proliferation advantage gained by malignant cells as well as their ability to escape a physiologically mounted immune response. In this context, the blockade of programmed death-1 (PD-1)/ programmed death ligand 1 and 2 axis by pembrolizumab seems to be safe, providing a substantial clinical benefit in heavily pre-treated pts. We report our experience with 27 relapsed/refractory PMBCL (rrPMBCL) pts treated with anti-PD1 antibody (pembrolizumab or nivolumab) alone or in combination with brentuximab vedotin (BV) (Table 1).

others at the flat dose of 200 mg every 3 weeks. All pts was refractory to the last treatment with a median number of previous therapies of 3 (range 2-8). Eleven pts experienced drug-related adverse events (AEs), mostly grade 1-2, none discontinued treatment due to AEs. After a median of 14 cycles (range 19-52), 6 pts obtained a complete response (CR) and one a partial response (PR) with an overall response rate (ORR) of 38.8%. With a median follow-up of 12 months (range 22-38 mo) who obtained a CR is still in CR, none of them received an ASCT as consolidation therapy and 2/6 pts are in response for more than 2 years. Patient in PR underwent to ASCT, converting the PR into a CR. All responders were still alive at data analysis. In the last 2 years, 9 rrPMBCL pts were also treated with nivolumab (240 mg flat dose) in combination with BV (1.8 mg/Kg) every 3 weeks (Table 1). Eight pts had a drug related AEs, 5 experienced a peripheral neuropathy (mostly grade 2-3) due to BV, leading to a discontinuation of the drug in 3 patients after 12, 16 and 20 cycles. The ORR was 55.5% with 3 pts in CR and 2 in PR after a median of 14 cycles (range 3-26), all of them are still on treatment except for one patient who died in PR due to a sepsis. From our experience, the use of PD-1 inhibitors alone or in combination with BV seems to be feasible and promising, leading to overcome the chemoresistance of rrPMBCL.

C005

BENDAMUSTINE E RITUXIMAB COME TRATTAMENTO NEI PAZIENTI CON LINFOMA DELLA ZONA MARGINALE: ESPERIENZA NELLA PRATICA CLINICA QUOTIDIANA

V. Stefoni, B. Casadei, C. Pellegrini, G. Lolli, M. Carella, A. Broccoli, L. Nanni, M. Marangon, A. Morigi, N. Fabbri, G. Gabrielli, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia L. e A. Seràgnoli, Università di Bologna, Italy

Rituximab plus bendamustine is an effective and manageable treatment option for patients (pts) affected by indolent non Hodgkin lymphoma including marginal zone lymphomas (MZL). First line therapy of patients with MZL isn't to this day well established and various regimen with chemoimmunotherapy can be used. The aim of this retrospective and monocentric study is to analyze the effectiveness and safety of the use of BR regimen in MZL pts in first line or with relapsed/refractory disease in daily clinical practice. The treatment schedule was rituximab at the dose of 375 mg/m² on day 1 of the cycle and bendamustine at the dose of 90 mg/m² on day 2 and 3, every 28 days for a maximum of 6 cycles. We analyzed 125 pts: 82 had BR as first line approach, whereas 43 had BR as subsequent line of therapy. One thousand and eight pts underwent 6 cycles of BR regimen, 13 had early discontinuation due to toxicity, 3 due to progression disease (PD) and 1 stopped treatment as per his own decision after achieving a complete response (CR) at interim restaging. Final responses were: 70 CR (56%), 40 partial response (PR, of which 2 very good PR), 6 stable disease and 9 PD, leading to an overall response rate (ORR) of 88%. CR rate was 52.4% in previously untreated and 62.8% in pre-treated pts, respectively. ORR was 87.8% in previously untreated and 88.4% in pre-treated pts, respectively. Forty-four pts (35.2%) had at least one grade III-IV hematological toxicity, mainly neutropenia. Less frequent were extra-hematological adverse events: only 15 patients had grade III-IV events. Nevertheless, all toxicities quickly resolved and globally only 13 events led to treatment interruption. At a median follow up of 30 months, median progression free survival and median disease free survival were not reached. Sixty-four patients (42 previously untreated and 22 pre-treated pts, respectively) are in continuous CR with a median duration of response of 23 months. At the latest available follow up, only 6 patients were deceased due to PD. No differences in response rate or in survivals were observed between patients who did or did not undergo BR in first line. The BR regimen effective in MZL patients inducing prolonged disease control at any point in the therapeutic algorithm. Side effects can be frequently severe but manageable without repercussions for the success of the therapy.

Table 1. Patient's characteristics and features treatment related.

PATIENT'S CHARACTERISTICS		
	Pembrolizumab	Nivolumab + BV
N	18	9
M:F	6:12	3:6
Median Age (range)	27 (19-60)	42 (19-54)
Stage III/IV, n	6	2
Bulky disease, n	12	8
Median number of previous therapy (range)	3 (2-8)	3 (2-4)
Previous ASCT, n	5 (3 were refractory)	3 (2 were refractory)
Previous RT, n	8	2
Previous BV, n	4 (all refractory)	0
Refractory to the first line of therapy, n	10	8
Refractory to the last line of therapy, n	18	9
TOXICITY		
	Pembrolizumab	Nivolumab + BV
Haematological toxicity any grade, n	4/18	5/9
Haematological toxicity grade 3-4, n	3/4	5/5
Extra-haematological toxicity any grade, n	9/18	7/9
Extra-haematological toxicity grade 1-2, n	7/9	5/7
Extra-haematological toxicity grade 3-4, n	2/9	6/7
Serious Adverse Events, n	0	3/7 (1 resolved)
RESPONSE		
ORR, n (%)	7/18 (38.8%)	5/9 (55.5%)
CR, n	6	3
PR, n	1	2
PD, n	11	4
Median time to best response, mo (range)	2.8 (1-8.6)	3 (1.2-13)
Median cycle for all population, n (range)	5 (1-52)	10 (1-26)*

*4 patients are still in treatment. ASCT: autologous stem cell transplantation, RT: radiotherapy, BV: brentuximab vedotin, ORR: overall response rate, CR: complete response; PR: partial response, PD: progression disease, mo: months.

From April 2014 to April 2017, 18 rrPMBCL pts with a median age of 27 years old (range 19-60) received a median of 5 cycles (range 1-52) of pembrolizumab, two at the dose of 10 mg/Kg every 2 weeks and the

Myeloma and Monoclonal Gammopathies 1

C006

DOUBLE vs SINGLE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW UP (10-YEARS) ANALYSIS OF RANDOMIZED PHASE 3 STUDIES

S. Rocchi, H. Goldschmidt, L. Rosinol, L. Pantani, S. Zweegman, H.J. Salwender, J.J. Lahuerta, H.M. Lokhorst, M.T. Petrucci, I. Blau, A. Oriol, N. Testoni, K. Weisel, R. Rios, F. Patriarca, J. Blanchard, L. Dozza, M-V. Mateos, M. Galli, J.F. San-Miguel, M. Boccadoro, J. Blade, P. Sonneveld, M. Cavo

Seràgnoli Institute of Hematology Bologna University School of Medicine, Heidelberg University, Hematopoietic Stem Cell Transplantation Unit Institute of Hematology and Oncology IDIBAPS Josep Carreras Research Institute Hospital Clinic i Provincial, Seràgnoli Institute of Hematology Bologna University School of Medicine, VUMC, Department of Hematology and Oncology Asklepios Hospital Hamburg Altona, Department of Hematology Hospital Universitario 12 de Octubre, Department of Hematology VU University Medical Center, Department of Cellular Biotechnology and hematology Sapienza University of Rome, Department of Hematology Oncology and Tumorimmunology Charité University Berlin, Institut Català d'Oncologia Institut Josep Carreras Hospital Germans Trias i Pujol, Seràgnoli Institute of Hematology Bologna University School of Medicine, Department of Internal Medicine II Universitätsklinikum Tubingen, Hospital Universitario Virgen de las Nieves de Granada, Hematology and Bone Marrow Transplant Unit University of Udine, Hospital Universitario Ramón y Cajal, Seràgnoli Institute of Hematology Bologna University School of Medicine, Department of Hematology University Hospital of Salamanca (HUS/IBSAL), Hematology and Bone Marrow Transplant Unit ASST Papa Giovanni XXIII, Centro Investigación Médica Aplicada (CIMA) Clínica Universidad de Navarra, Department of Molecular Biotechnologies and Health Sciences Hematology Division University of Torino, Hematology Department IDIBAPS Hospital Clínica de Barcelona-Servicio de Onco-Hematología, Department of Hematology Erasmus Medical Center, Seràgnoli Institute of Hematology Bologna University School of Medicine

Introduction: The comparison of double vs single autotransplantation (ASCT-2 vs ASCT-1) for newly diagnosed multiple myeloma patients (pts) is highly debated. To address this controversial issue, we performed a long-term follow up analysis of pt-level data from three phase 3 trials [GIMEMA MMY-3006, PETHEMA/GEM, HOVON-65/GMMG-HD4] of bortezomib(V)-based induction therapy before ASCT, followed by consolidation/maintenance with V. According to study design, pts were assigned to receive either ASCT-1 or ASCT-2, thus allowing a comparison between these treatments.

Methods: The intention-to-treat population included 909 pts, of whom 501 assigned to ASCT-1 and 408 to ASCT-2 at study entry.

Results: With a median follow up of 117 months (mos) (IQR 91-126), assignment to ASCT-2 resulted in superior PFS (median: 47 vs 38 mos, $p=0.0008$) and 10-yr OS probability (58% vs 47%, $p=0.0002$). PFS and OS benefits with ASCT-2 vs ASCT-1 were retained also across prespecified subgroups, including pts with high-risk cytogenetics (cyto): median PFS, 36 vs 20 mos ($p=0.032$); 10-yr OS rates, 51% vs 34% ($p=0.004$). In a multivariate Cox regression analysis, principal independent predictors for prolonged PFS and OS included ASCT-2 (HR 0.81, $p=0.048$ and HR 0.75, $p=0.036$, respectively), ISS stage I+II (HR 0.62, $p<0.001$ and HR 0.66, $p=0.010$), standard risk cyto (HR 0.57, $p<0.001$ and HR 0.55, $p<0.001$) and complete remission as best response (best CR) (HR 0.53, $p<0.001$ and HR 0.55, $p<0.001$). ISS II+III, high-risk cyto and failure to achieve best CR were used to build a score index that stratified pts into 3 subgroups at low-(LR), intermediate-(IR) and high-risk (HR) with 0, 1 or 2-3 adverse variables, respectively. Median PFS for these subgroups was 87, 53 and 27 mos ($p<0.001$), while the corresponding 10-ys OS rates were 78%, 53% and 32% ($p<0.001$). No OS benefit was demonstrated with ASCT-2 vs ASCT-1 in LR group (53% vs 28%, $p=0.093$). Conversely, ASCT-2 significantly prolonged both PFS (32 vs 20 mos, $p=0.012$) and OS (43% vs 20%, $p=0.001$) of HR pts. Notably, the greatest benefit from ASCT-2 vs ASCT-1 was seen in the ultra-HR subgroup (3 adverse variables) with two-fold increased PFS (35 vs 14 mos, $p=0.008$) and 56% reduction in the risk of death.

Conclusions: Results of this pooled analysis of phase 3 trials incorpo-

rating V-based triplets into ASCT, confirmed the superiority of ASCT-2 over ASCT-1 in terms of extended PFS and OS, in particular in HR pts.

C007

RARE, BUT COMPLEX CHROMOSOMAL REARRANGEMENTS, CAUSED EITHER BY SINGLE-STEP OR BY STEPWISE CATASTROPHIC GENOMIC EVENTS, SIGNIFICANTLY IMPACT THE PROGNOSIS OF MULTIPLE MYELOMA PATIENTS

V. Solli, A. Poletti, M. Martello, L. Pantani, E. Borsi, R. Termini, E. Zamagni, P. Tacchetti, S. Rocchi, K. Mancuso, L. Cifarelli, L. Agboyi, M. Cavo, C. Terragna

Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale (DIMES)-Istituto di Ematologia L.A.Seràgnoli, Istituto di Ematologia L.A.Seràgnoli, Azienda Ospedaliera Sant'Orsola-Malpighi, Bologna, Italy

Background: Multiple Myeloma (MM) is a genetically complex disease, characterized by the recurrence of several chromosomal aberrations, which impair the disease prognosis. Besides these, the use of genome wide technologies has recently highlighted the existence of heterogeneous chaotic genomic events, generically defined "chromoanagenesis", including chromothripsis (caused by single-step genomic events) and stepwise events (consequence of multiple, small and sequential genomic events, occurring throughout subsequent cell cycles). The prognostic impact of chromoanagenesis has not been yet fully elucidated. Aims of the present study were (1) to set up a reliable bioinformatic method, to distinguish, characterize and dynamically define the different chromoanagenesis events (CEs); (2) to correlate their presence to the disease prognosis.

Patients and Methods: 512 newly diagnosed MM patients (pts), whose genomic data were available, have been included in the present study.

Results: An algorithm, able to characterize CEs, was set up and tested on genomic data of all pts. Criteria able to discriminate among the 2 different CEs were defined, by taking into account both previously reported guidelines for CEs identification and the MM-specific, highly heterogeneous genomic context. Overall, 77 pts (15%) were shown to carry at least one CE: 49/77 (64%) and 28/77 (36%) carried either chromothripsis or stepwise events, respectively; both events were scattered across the whole genome, with any locus-specific bias. Pts with chromothripsis were more likely to carry both IgH translocations and chr17p del, whereas pts with progressive catastrophic events were mostly hyperdyploid and carried chr1q amp. The onset of CEs has been shown to impact on pts' progression-free and overall survival (PFS, OS), with HR of 1.322($p=0.05$) and 1.731($p=0.002$), respectively. In particular, the stepwise events had a greater impact on PFS (HR 1.61, $p=0.034$) and on OS (HR 2.063, $p=0.006$). Stepwise events retained an independent prognostic factor when evaluated in multivariate model including also chr17p del, t(4;14) and ASCT randomization.

Conclusions: The occurrence of genomic catastrophic events significantly impact on both OS and PFS of MM pts. Despite the co-segregation with low/intermediate-risk genomic aberrations, stepwise events seem to have a more adverse prognostic impact on survival, as compared to chromothripsis.

Acknowledgements: AIRC_IG2014-15839, RF-2016-02362532

C008

CLONAL EVOLUTION IN MULTIPLE MYELOMA PATIENTS RECEIVING MAINTENANCE THERAPY

C. Terragna¹, V. Solli², A. Poletti², M. Martello², E. Borsi², L. Pantani², R. Termini¹, P. Tacchetti¹, S. Rocchi², K. Mancuso², I. Rizzello², L. Cifarelli¹, L. Agboyi², L. Dozza², I. Caratozzolo², E. Zamagni², M. Cavo²

¹Istituto di Ematologia L.A.Seràgnoli, Policlinico Sant'Orsola-Malpighi; ²Università degli Studi di Bologna - DIMES, Italy

Multiple Myeloma (MM) disease progression is often coupled to radical changes of the genomic sub-clonal architecture. Although it has been assumed that therapy bears a major role as selective driver to induce genomic changes, the identification of specific therapy-driven modifications has been so far prevented by the scarce number of homogeneously-treated cohorts of pts with paired samples (diagnosis, D and relapse, R). In this study we estimated the role of maintenance therapy in eliciting genomic changes in a cohort of MM pts up-front treated with PI-based regimens. Whole genome Copy Number Alterations (CNAs) landscape was obtained by analyzing SNPs array with dedicated R packages in 43 pts, whose BM-CD138+ cell fractions were collected both at D and at first R. 25 pts received maintenance therapy (19 LEN, 6 PIs) and 18 did not. To define pts evolution patterns, an analysis algorithm was first developed to assess the CNAs changes between D and R. Results were then bi-clustered, hence revealing common subgroups of genes and pts showing similar behaviors, under the same therapeutic selective pressure: overall, 20 and 23 pts relapsed with branching and stable pattern, respectively. We then focused on CN changes of specific genomic regions, whose prognostic role has been already established in MM, i.e. CN losses of TP53 on chromosome (chr) 17p, Rb1 on chr13q, WWOX on chr16p, cdkn1c and FAM46C on chr1p; CN gain of CKS1B on chr1q. When present at D, these CNAs tended to persist throughout pts' clinical follow up, regardless of whether pts received or not maintenance. The emersion of any of these CNAs at R was more likely in pts receiving maintenance (40% vs 17% of cases), even if maintenance seemed to favor a better disease control both in pts carrying durably any of these CNAs and in pts where any of them emerged at R. Pts with branching evolution pattern of any of the above mentioned CNAs particularly benefitted of maintenance therapy, with OS post first R significantly longer than pts who did not received maintenance therapy (50 vs 14 months, p=0.004). Genomic changes caused by the selective pressure of maintenance therapy and provoking the emersion of branched clones at R are likely to require time to take place. This might explain the extended survival observed in pts who received maintenance and relapsed with branching evolution patterns, as compared to that of pts whose genomic landscape tended to remain stable.

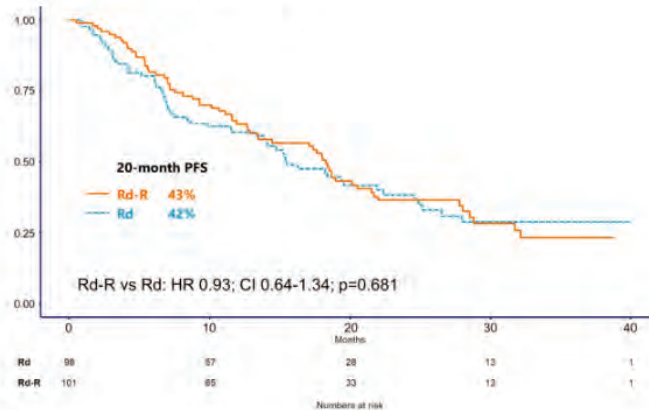
Thanks to: AIRC_IG2014-15839, RF-2016-02362532

nance: 10 mg/day [21 days] until progression) or continuous Rd (R: 25 mg/day for 21 days; d: 20 mg, days 1,8,15,22 until progression), as adopted in patients >75 years in the FIRS trial (Hulin JCO 2016). The primary endpoint was event-free survival (EFS), considering progression and safety.

Results: Of 199 evaluable patients, 98 were randomized to Rd-R and 101 to continuous Rd. Median age was 75 and 76 years (p=0.06); 47% in Rd-R vs 57% in continuous Rd were intermediate-fit for age (≥76 years), 53% vs 43% due to an impairment in CCI, ADL or IADL (p=ns). The median follow-up was 25 months. Best response rates were comparable between Rd-R and continuous Rd (≥PR: 73% vs 63%; ≥VGPR: 43% vs 35%). EFS was 9.3 vs 6.6 months (HR 0.72, 95% CI 0.52-0.99, p=0.04). No difference in progression-free survival (PFS) and overall survival (OS) was observed. 20-month PFS was 43% vs 42% (HR 0.93, 95% CI 0.64-1.34, p=0.681), 20 month-OS was 84% versus 79% (HR 0.73, 95% CI 0.40-1.33, p=0.306; Figure 1). Rate of ≥1 non-hematologic G3-4 AE was 31% vs 39%. The most frequent G3-4 AEs were neutropenia (17% vs 14%), infections (9% vs 11%) and skin rash (3% vs 7%). Lenalidomide was reduced in 33% of Rd-R and 43% of continuous Rd patients, and discontinued in 19% vs 23% of patients, respectively. Lenalidomide median relative dose intensity was 100% in Rd-R and 90% in continuous Rd.

Conclusion: Switching to maintenance with lenalidomide only – thus with no steroids - after 9 cycles of Rd was feasible, with no negative impact but with outcome comparable to standard continuous Rd. This is the first prospective randomized phase III trial for intermediate-fit NDMM patients evaluating a frailty-adjusted approach to balance efficacy and safety.

Progression-free survival



Overall survival

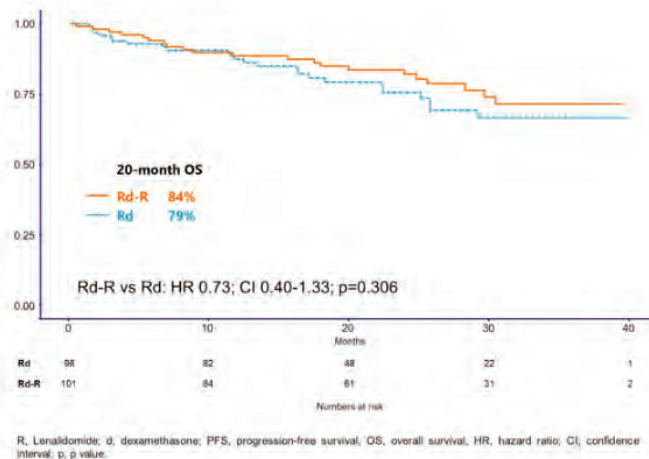


Figure 1.

C009

TREATMENT WITH DOSE/SCHEDULE-ADJUSTED RD-R vs CONTINUOUS RD IN ELDERLY INTERMEDIATE-FIT NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: RESULTS OF RV-MM-PI-0752 PHASE III RANDOMIZED STUDY

A. Larocca, M. Salvini, G. Gaidano, N. Cascavilla, G. Benevolo, M. Galli, V. Montefusco, T. Caravita, F. Monaco, A. Capra, N. Giuliani, G. Gazzera, S. Pulini, S. Ronconi, N. Pescosta, A.M. Liberati, F. Patriarca, C. Cellini, P. Tosi, M. Offidani, M. Cavo, M. Boccadoro, S. Bringhen

GIMEMA/ European Myeloma Network, Italy

Background: Continuous lenalidomide-dexamethasone (Rd) is effective and safe in elderly newly diagnosed myeloma (NDMM) patients. Yet, the outcome of patients >75 years is inferior to younger ones, and not only advanced age but also severe adverse events (AEs) may negatively affect duration of treatment and survival. We investigated the efficacy and feasibility of dose/schedule-adjusted Rd followed by lenalidomide maintenance (Rd-R) vs standard continuous Rd in intermediate-fit NDMM patients.

Methods: Intermediate-fit NDMM patients (Age 76-80 years or ADL≤4 or IADL≤5 or CCI≥2), with a frailty score= (<http://www.myelomafrailtyscorecalculator.net/>) were randomized to 9 28-day cycles of Rd-R (R: 25 mg/day for 21 days; d: 20 mg, days 1,8,15,22; R mainte-

C010

TREATMENT WITH DARATUMUMAB IN PATIENTS WITH MULTIPLE MYELOMA ASSOCIATED AL AMYLOIDOSIS

F. Fazio¹, M. Basset², P. Milani², A. Foli², M. Nuvolone², R. Foà¹, M.T. Petrucci¹, G. Merlini², G. Palladini²

¹Dipartimento di Medicina Traslazionale e di precisione, Sezione di Ematologia, Università Sapienza di Roma; ²Centro per lo Studio e la Cura delle Amiloidosi Sistemiche, Fondazione IRCCS Policlinico S. Matteo, Università di Pavia, Italy

Introduction: Daratumumab is a novel agent in the treatment of multiple myeloma (MM). Three clinical trials are evaluating daratumumab in primary AL amyloidosis. After this drug was marketed for MM, it became routinely accessible also to patients with myeloma-associated AL amyloidosis (maAL). We evaluated the effectiveness of daratumumab in a consecutive series of patients with maAL.

Materials and Methods: The prospectively maintained database of the Pavia Amyloid Center was searched for patients with relapsed/refractory maAL treated with daratumumab from September 2016 to February 2019. Forty-six patients were included.

Results: Median age was 62 years (range: 34-79 years) and 27 (59%) patients were males. The heart was involved in 33 patients (72%) and the kidney in 31 (70%). Median bone marrow plasma cell infiltrate was 20% (range: 11-50%). Median time from diagnosis to treatment initiation was 39 months (range: 3-127 months). Thirty-six (78%) patients were refractory to the previous line of therapy. Median number of prior treatments was 3 (range: 1-9). All but one patients were exposed to bortezomib, 24 (52%) to alkylating agents, 32 (70%) received an immunomodulatory agent [11 (24%) thalidomide, 22 (48%) lenalidomide and 12 (26%) pomalidomide] and 12 (26%) underwent autologous stem cell transplant. Thirty-two (70%) patients received daratumumab monotherapy. The remaining patients received daratumumab in association with bortezomib in 8 (17%) and with lenalidomide in 6 (13%) cases. Severe adverse events were observed in 13 (28%) patients, pneumonia in four cases, depressive syndrome in two and bronchitis, pleural effusion, neutropenia, fever, atrial fibrillation, hyperkalemia and hepatotoxicity in one case each. Median follow-up of living patients was 11 months. Only one patient died due to progressive disease. After 8 infusions 39 patients were evaluable for hematologic response (HR) (with AL amyloidosis criteria). Thirty-four patients (87%) achieved a HR: complete response in 7 (18%), very good partial response in 23 (59%) and partial response in 4 (10%). Cardiac response was observed in 5 of 23 evaluable patients (22%) and renal response in 14 of 24 patients (58%).

Discussion and Conclusions: Daratumumab is a rapidly effective agent in patients with AL amyloidosis and MM. Data from ongoing clinical trial are eagerly awaited.

Acute Myeloid Leukemia 1

C011

VERY LONG-TERM RESULTS OF ALL-TRANS RETINOIC ACID AND ARSENIC TRIOXIDE IN NON-HIGH RISK ACUTE PROMYELOCYTIC LEUKEMIA: LATEST UPDATE OF THE ITALIAN-GERMAN APL0406 RANDOMIZED TRIAL.

L. Cicconi¹, U. Platzbecker², G. Avvisati³, F. Paoloni⁴, C. Thiede⁵, M. Vignetti⁴, P. Fazi⁴, F. Ferrara⁵, M. Divona⁶, F. Albano⁷, F. Effcace⁴, M. Sborgia⁸, E. Di Bona⁹, M. Breccia¹⁰, E. Borlenghi¹¹, R. Cairoli¹², A. Rambaldi¹³, L. Melillo¹⁴, G. La Nasa¹⁵, W. Fiedler¹⁶, P. Brossart¹⁷, B. Hertenstein¹⁸, H.R. Salih¹⁹, O. Annibaldi³, M. Wattad²⁰, M. Lubbert²¹, C.H. Brandts²², M. Hanel²³, C. Rollig⁵, N. Schmitz²⁴, H. Link²⁵, C. Frairia²⁶, C. Fozza²⁷, A.M. D'Arco²⁸, N. Di Renzo²⁹, A. Cortelezzi³⁰, F. Fabbiano³¹, K. Dohner³², A. Ganser³³, H. Dohner³², S. Amadori⁴, F. Mandelli^{4†}, M.T. Voso¹, G. Ehninger³⁵, R.F. Schlenk³⁴, F. Lo Coco^{1†}

¹Department of Biomedicine and Prevention, University Tor Vergata, Rome, Italy; ²Universitätsklinikum Carl Gustav Carus der Technischen Universität, Dresden, Germany; ³Unità Operativa Complessa (UOC) di Ematologia e Trapianto di cellule staminali, Università Campus Bio-Medico, Rome, Italy; ⁴GIMEMA Data Center, Rome, Italy; ⁵Universitätsklinikum Carl Gustav Carus der Technischen Universität, Dresden, Germany; ⁶Dipartimento di Ematologia e Trapianto di Cellule Staminali, Ospedale Cardarelli, Napoli, Italy; ⁷Laboratorio di Oncoematologia, Policlinico Tor Vergata, Rome, Italy; ⁸Dipartimento di Ematologia, Università di Bari, Bari, Italy; ⁹U.O. di Ematologia Clinica, Pescara; ¹⁰Dipartimento di Terapie cellulari ed Ematologia, Unità Operativa di Ematologia, Ospedale S. Bortolo, Vicenza, Italy; ¹¹Dipartimento di Biotecnologie Cellulari ed Ematologia, Università La Sapienza, Rome, Italy; ¹²Dipartimento di Ematologia, Spedali Civili, Brescia, Italy; ¹³Grande Ospedale Metropolitano Niguarda, SC Ematologia, Milan, Italy; ¹⁴Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; ¹⁵Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy; ¹⁶Centro Trapianti Midollo Osseo, Ospedale R. Binaghi, Università di Cagliari, Cagliari, Italy; Azienda Ospedaliera Pugliese Ciaccio, Catanzaro; ¹⁷University Hospital Hamburg-Eppendorf, Hamburg, Germany; ¹⁸University Hospital Bonn, Department of Oncology, Hematology, Immuno-Oncology and Rheumatology, CIO Bon, Germany; ¹⁹Klinikum Bremen Mitte, Bremen, Germany; ²⁰University Hospital Tübingen, Germany; ²¹Kliniken Essen Süd, Essen, Germany; ²²University Medical Center, Freiburg, Germany; ²³Goethe University Frankfurt, Frankfurt, Germany; ²⁴Klinikum Chemnitz gGmbH, Chemnitz, Germany; ²⁵Asklepios Klinik St Georg Hamburg, Hamburg, Germany; ²⁶Klinik für Innere Medizin I, Westpfalz-Klinikum, Kaiserslautern, Germany; ²⁷Città della Salute e della Scienza, Hematology, Torino, Italy; ²⁸University of Sassari, Sassari, Italy; ²⁹U.O. Medicina Interna e Onco-Ematologica P.O. "Umberto I," Nocera Inferiore, Nocera Inferiore, Italy; ³⁰Ospedale Vito Fazzi, Lecce, Italy; ³¹Foundation Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy; ³²Divisione di Ematologia e Unità di Trapianti di Midollo Osseo, Ospedale Riuniti Villa Sofia-Cervello, Palermo, Italy; ³³University Hospital Ulm, Ulm, Germany; ³⁴Hannover Medical School, Hannover, Germany; ³⁵NCT Trial Center, National Center for Tumor Diseases Heidelberg, Germany

All-trans retinoic acid (ATRA) and arsenic trioxide (ATO) combination therapy has been recently established as the new standard of care for non-high risk acute promyelocytic leukemia (APL). The Italian-German APL0406 trial has first shown that this chemotherapy-free approach is at least not inferior to the ATRA-chemotherapy regimen. We hereby provide a very long-term update on the outcome of the 276 patients enrolled in the extended series of the APL0406 trial. The APL0406 study is a prospective, randomized, phase III, non-inferiority trial conducted by the Italian cooperative group GIMEMA and by the German groups AMLSG and SAL. The study enrolled 276 patients aged 18-70 years with newly diagnosed, genetically proven low-intermediate risk APL between Oct 2007 and Jan 2013. Patients were randomly assigned to receive ATRA-ATO for induction and consolidation or ATRA-idarubicin induction followed by 3 cycles of consolidation with ATRA-chemotherapy and maintenance therapy. The primary objective of the study was EFS; secondary endpoints included OS, DFS and CIR. The present analysis was performed in March 2018 following an intent-to-treat (ITT) principle. With a median follow-up of 66.4 months (range: 0.9-116.7), the 6-year EFS for the 263 patients evaluable in the ITT

analysis was 96.6% (95%CI: 93.4-99.9) and 77.4% (95%CI: 70.2-85.4) in the in the ATRA-ATO and ATRA-chemotherapy groups, respectively (p<0.0001). The 6-year DFS, CIR and OS rates for the ATRA-ATO and ATRA-chemotherapy groups were 96.6% vs 79.8% (p<0.0001), 1.7 vs 15.5% (p=0.00015) and 98.3% vs 89.8% (p=0.004), respectively. This updated analysis of the APL0406 study shows that the advantage of ATRA-ATO over ATRA-CHT increases with time in terms of both efficacy and safety.

C012

THIRD GENERATION SEQUENCING OF NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA: IMPLICATIONS FOR PROGNOSIS

N. Bartalucci¹, F. Mannelli¹, E. Contini¹, S. Romagnoli¹, G. Gianfaldoni², B. Scappini², E. Pelo², F. Pancani², A. Magi², A. Bosi², P. Guglielmelli¹, A.M. Vannucchi¹

¹CRIMM-Centro Ricerca e Innovazione Malattie Mieloproliferative, Università di Firenze; ²AOUC Azienda Ospedaliero Universitaria Careggi, Unità di Ematologia, Firenze, Italy

Karyotype and molecular genetics are the most relevant prognostic factors in acute myeloid leukemia (AML). In normal karyotype (nk) AML, prognostically meaningful recurrent gene mutations may be found, but effective prognostic assessment in this category still remains unsatisfactory. To tackle this issue, we performed long-reads genome sequencing by the Oxford Nanopore technology (ONT) to identify copy number variations (CNV) in samples from 54 nk AML pts. ONT sequencing is not affected by the classical sources of bias such as GC-content and mappability. Data were analysed by the novel package Nano-GLADIATOR (Bartalucci N *et al.*, Bioinformatics, 2019). Of the pts, median age 49y, 27 (50%) were NPM1mut, 16 (30%) FLT3ITDmut and 11 (20%) CEBPAbi-allelic mut; overall, 42 pts (78%) entered CR and median survival was 47.4 months. The median number of CNV detected was 4 (range, 1-16), represented by gain or loss of genomic material in 38.3% and 61.7% of cases, respectively. The detected CNV involved 21 different chromosomes with no significant recurrent alterations. As expected, there was no large alteration involving >50% of one chromosome arm. Considering all CNV length, expressed as base pairs (bp), we found that 63.9% were alterations spanning <1 Mega bases (Mb), while 1.6% and 34.5% were 1Mb<CNV<30Mb and larger than 30Mb, respectively. The median number of bp involved in CNV was 6.35x10⁶ (range 4.88-159.36). Considering the allelic burden (VAF) of CNV, ROC analysis identified a VAF value of 50% as the best to stratify pts in 2 groups showing a significantly different median Event Free Survival (EFS) of 7.2 months and not-reached, respectively (p=0.021) (Figure 1).

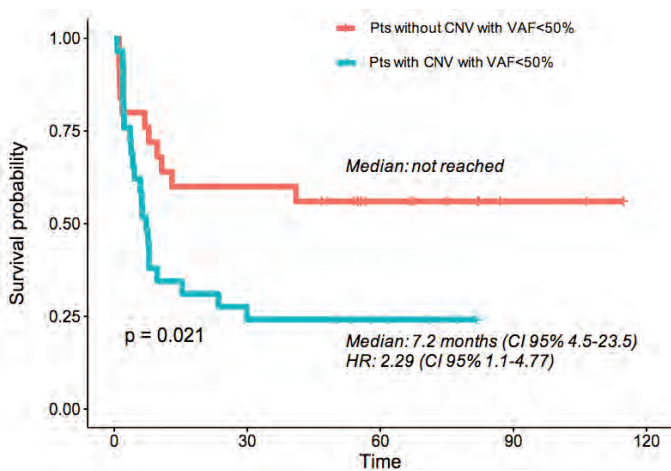


Figure 1. Survival probability according to VAF stratification.

Focusing on CNV involving <1Mb only, we found that pts harboring ≥1CNV with VAF<50% had an EFS of 6 months, while the population with no CNV with VAF<50% did not reach the median EFS value (p=0.009). These variables remained significant (p=0.046) also in mul-

tivariate analysis including age and mutational status for NMP1, FLT3 and CEBPA genes. In summary, by using ONT we were able to identify an array of genome alterations went undetected with standard karyotyping. We found that VAF of CNV regions was correlated with EFS, suggesting that lower VAF, as a measure of clonal heterogeneity, may increase AML chemoresistance reducing EFS. These data, that will be updated at the meeting, indicate a potentially novel parameter for effective prognostic stratification of nk AML pts. Supported by AIRC and GIMEMA.

C013

IN ADULT ACUTE MYELOID LEUKEMIA (AML) PERIPHERAL BLOOD MEASURABLE RESIDUAL DISEASE (MRD) BY FLOW CYTOMETRY (FC) IS FEASIBLE AND IS PROGNOSTICALLY RELEVANT

L. Maurillo, F. Buccisano, A. Piciocchi, M.I. Del Principe, A. Candoni, L. Melillo, V. Calafiore, R. Cairoli, P. De Fabritiis, G. Storti, P. Salutati, F. Lanza, G. Martinelli, M. Luppi, P. Mazza, M.P. Martelli, A. Cuneo, E. Albano, F. Fabbiano, A. Tafuri, A. Chierichini, A. Tieghi, N.S. Fracchiolla, D. Capelli, R. Foà, C. Alati, E. La Sala, M.T. Voso, P. Fazi, M. Vignetti, M. Irno Consalvo, T. Ottone, S. Lavorgna, W. Arcese, F. Lo Coco, S. Amadori, A. Venditti

Fondazione Policlinico Tor Vergata, Università Tor Vergata, Fondazione GIMEMA, Università Tor Vergata, Divisione di Ematologia Università di Udine, Fondazione IRCCS Casa Sollievo della Sofferenza, AOU Policlinico Vittorio Emanuele, ASST Grande Ospedale Metropolitano Niguarda, Ospedale S. Eugenio, AORN G Moscati, Ospedale civile Pescara, Divisione Ematologia Ravenna, Ematologia e Oncologia Medica Seragnoli, Ematologia Università di Modena, Ematologia Ospedale di Taranto, Ematologia Università di Perugia, Ematologia Università di Ferrara, Ematologia Università di Bari, Ospedali riuniti Villa Sofia-Cervello, Ospedale S. Andrea, Ospedale S. Giovanni-Addolorata, Arcispedale Santa Maria Nuova, IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano, Ospedale Le Torrette Ancona, Ematologia Università La Sapienza, Ematologia G.O.M Bianchi Melacrino Morelli, Fondazione GiMEMA, Università Tor Vergata, Fondazione GIMEMA, Ematologia Università La Sapienza, Università Tor Vergata, Università Tor Vergata, Università Tor Vergata, Università Tor Vergata, Università Tor Vergata, Università Tor Vergata, Fondazione GIMEMA, Università Tor Vergata, Roma

The usefulness of peripheral blood (PB) as an alternative source to bone marrow (BM) for MRD detection in AML has been tested in few studies with small series of patients. The present study explored whether MRD detection in PB is feasible and has a prognostic relevance. A total of 362 adult AML patients enrolled to GIMEMA AML12, AML13 and AML1310 protocols were monitored for MRD after the achievement of complete remission. Using FC, we compared the levels of MRD in 362 and 339 pairs of PB and BM after induction and consolidation, respectively. Median age was 49 years (range 18-73); 126 (32%), 117 (30%) and 150 (38%) patients received as post consolidation therapy allogeneic stem cell transplantation (SCT) autologous stem cell transplantation (AuSCT) or no transplantation, respectively. After induction and consolidation, the findings in BM and PB were significantly concordant (Pearson correlation r=0.40 and 0.74, respectively, p<0.001 for both comparisons). As a second step, the analysis was carried out including only 154 younger patients treated homogeneously in the AML1310 protocol. Median age was 50 years (range 18-60), and 52 (34%), 49 (32%) and 53 (34%) patients underwent SCT, AuSCT and no SCT, respectively. The levels of PB residual leukemic cells (RLC) were tested by ROC analysis and the optimal threshold was set at 0.002% after consolidation. Therefore, patients with PB RLC values below and equal or exceeding the cut-off of 0.002% were classified as PB MRD- and PB MRD+, respectively. No significant difference in OS was observed between the PB MRD+ and PB MRD-groups; the median duration of DFS was not reached among the patients with a PB MRD-status, whereas it was 22 months among those in the PB MRD+ group (p=0.025). In multivariate analysis the variables independently associated with shorter DFS were PB MRD+ status, ELN high risk, no SCT as post consolidation therapy (p=0.02, 0.001, <0.0001, respectively). By combining PB MRD status with BM MRD status (BM threshold set at 0.035%) we were able to identify three categories of patient: BM+PB+ (78), BM-PB+ (34), BM-PB- (34) whose DFS at 2 years was 52%, 42% and 74%, respectively (p=0.031; Figure 1). In conclusions: (1) MRD lev-

els in PB of AML patients are measurable and correlated to those measured in BM (2) PB MRD determination after consolidation therapy has a prognostic role; (3) combined assessment of MRD in BM and PB enhances sub-stratification of patients in further categories of risk.

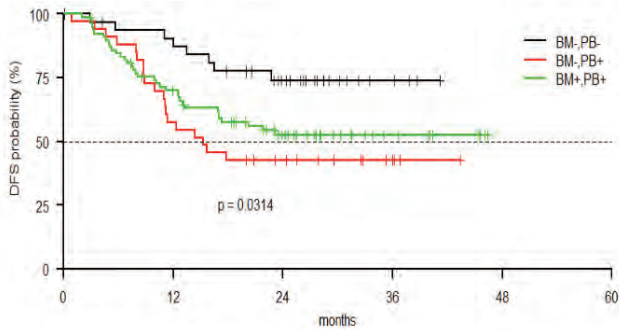


Figure 1.

C014

MUTATIONAL PROFILE OF LEUKEMIC STEM CELLS IN FLT3-ITD POSITIVE AML

T. Ottone, V. Alfonso, S. Travaglini, D.F. Angelini, G. Guerrero, S. Lavorgna, M. Divona, L. Iaccarino, M. Irno Consalvo, L. Cicconi, M. De Bardi, B. Neri, V. Martini, L. Fianchi, L. Battistini, L. Maurillo, F. Buccisano, A. Venditti, W. Arcese, F. Lo Coco, M.T. Voso

Department of Biomedicine and Prevention, University of Rome Tor Vergata, Fondazione Santa Lucia, Laboratorio di Neuro-Oncoematologia, Hematology Unit, Sant' Eugenio Hospital, Hematology Unit, F. Spaziani Hospital, Department of Hematology, Università Cattolica del Sacro Cuore

Introduction: Acute Myeloid Leukemia (AML) is characterized by heterogeneous genetic and immunophenotypic abnormalities, associated with clinical outcome. Although available treatments induce complete remission in ~80% of AML, some patients will eventually relapse, due to the persistence of leukemia stem cells (LSCs), which correspond to the CD34/CD123/CD99/CD25+ subset in FLT3-ITD+ AML. Aim. The aim of this study was to deeply characterize the molecular profile of LSCs to shed light on the heterogeneity and subclonal architecture of FLT3-ITD+ AML.

Materials and Methods: Out of 150 patients diagnosed with AML, 39 were mutated for FLT3-ITD. Using the Cytoflex High-Speed Cell Sorter, the CD34/CD123/CD99/CD25+ LSCs and CD34+/CD123/CD99/CD25- cell subsets were sequentially sorted in 12 FLT3-ITD+ patients. Targeted NGS sequencing was then performed on 32 samples collected at diagnosis and relapse, using the Oncomine™ Myeloid Research Assay panel on the Ion Torrent™ S5 sequencer.

Results: A significantly higher FLT3-ITD mutation load was observed within the LSC compartment, as compared to MNC ($p=0.006$). Conversely, CD34+/CD123/CD99/CD25- precursors displayed low or absent FLT3-ITD burden ($p=0.002$). We then performed targeted NGS mutation analysis on MNCs and several purified cell fractions from 12 FLT3-ITD mutated patients. The most frequently mutated genes were NRAS (40% of cases), RUNX1, TET2 (30%), DNMT3A, BCOR, IDH2, NPM1, FLT3-TKD, and KRAS (20%). The mutational profile of MNCs was then compared to that of FLT3-ITD+ purified LSC and/or to the CD34+ precursors in 7 patients. In 4 cases, the molecular profile was similar in MNC and LSC, while in 3 cases additional mutations in BCOR, EZH2, NRAS and KRAS were found in MNC, indicating that the more mature blast population may acquire additional transforming events. During the disease course, clonal evolution occurred in 3 pts, with loss or acquisition of mutations at relapse. In one patient, we then sorted several cell populations at relapse, 8 months after initial diagnosis. Expansion of FLT3-ITD+ blasts occurred at relapse, while the number of LSC remained stable and barely detectable. These data confirm that LSC may represent the treatment-resistant FLT3-ITD reservoir, driver of disease relapse.

Conclusions: We hypothesize that unravelling the genetic profiles of LSCs driving therapy resistance and disease progression may allow to identify combinations of therapeutic targets to finally eradicate LSCs in AML.

C015

PROGNOSTIC VALUE OF CLONAL HEMATOPOIESIS-MUTATIONS DETECTED AT DIAGNOSIS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE

C. Caprioli, O. Spinelli, S. Salmoiraghi, T. Interemesoli, P. Zanghi, R. Cavagna, A. Michelato, K. Buklijas, L. Elidi, F. Delaini, E. Oldani, G. Gianfaldoni, A. Bosi, F. Marmont, E. Audisio, D. Ferrero, E. Teruzzi, L. De Paoli, G. Rossi, E. Borlenghi, I. Cavattoni, M. Tajana, A.M. Scattolin, D. Mattei, P. Corradini, L. Campiotti, F. Ciceri, M. Bernardi, E. Todisco, A. Cortelezzi, S. Cortelazzo, B. Falini, C. Pavoni, R. Bassan, A. Rambaldi, F. Lussana

ASST Papa Giovanni XXIII, AOU Careggi, AOU Città della Salute e della Scienza, AO San Gerardo, AO SS. Antonio e Biagio e Cesare Arrigo, ASST Spedali Civili, Ospedale San Maurizio, ASST Ospedale di Cremona, Ospedale dell'Angelo e SS. Giovanni e Paolo, AO S. Croce e Carle, Fondazione IRCCS Istituto Nazionale dei Tumori, Università dell'Insubria, IRCCS Ospedale San Raffaele, Istituto Europeo di Oncologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Clinica Humanitas-Gavazzeni, Università di Perugia, Dipartimento di Onco-Ematologia Università degli Studi di Milano, Italy

Background: Mutations in DNA and chromatin regulation genes have been associated with clonal hematopoiesis (CH) in older healthy subjects, but are also involved in leukemogenesis as early events. In AML patients (pts), the prognostic role of pre-treatment CH-mutations as a group remains to be elucidated.

Aims: To investigate the distribution and clinical impact of CH-mutations detected at diagnosis in normal karyotype (NK) AML pts within the prospective NILG trial 02/06 [ClinicalTrials.gov Identifier: NCT00495287].

Methods: For 213 out of 270 NK AML pts enrolled into the trial, a molecular profile was obtained at diagnosis with standard approach and targeted NGS. CH-mutations were defined as DNMT3A, TET2, ASXL1, IDH1 and IDH2. All pts were treated with intensive chemotherapy eventually followed by alloHSCT.

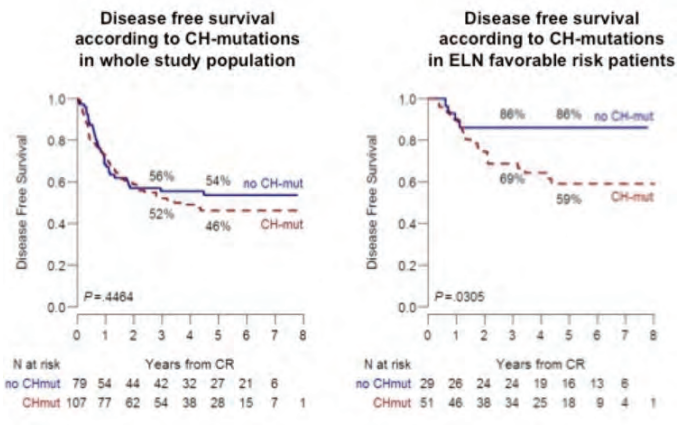


Figure 1.

Results: At least one CH-mutation was reported for 61.5% of pts, while 38.5% had no CH-mutations. Pts carrying CH-mutations were older than pts without CH-mutations ($p=0.0003$). The most recurrent CH-mutation was DNMT3A (83/131), followed by TET2 (32/131), IDH2 (26/131), IDH1 (20/131) and ASXL1 (18/131). A single CH-mutation was observed in 65% of pts, while 33.5% had a double mutation and 1.5% a triple mutation. Overall, CH-mutations were detected across all ELN risk groups. NPM1-mutated pts more likely carried a CH-mutation ($p<0.0001$), while the opposite was reported for pts with a biallelic CEBPa mutation ($p<0.0001$). Taken together, CH-mutations did not affect the 5-years disease free survival (DFS) and overall survival (OS) (46% vs 54%, $p=0.45$; and 50% vs 55%, $p=0.21$). Within the ELN risk categories, the presence of CH-mutations negatively affected the 5-years DFS (86% vs 59%, $p=0.03$) and OS (86% vs 71%, $p=0.09$) only for pts in the favorable risk group. Among CH-mutations, TET2 emerged as an independent risk factor for the achievement of complete remission (CR) [OR 0.25 (95% CI 0.08-0.7), $p=0.008$], but not for OS [OR 1.56 (95% CI 0.89-2.75), $p=0.12$]. AlloHSCT was performed in 186 pts in first CR and provided a significant benefit in terms of OS

($p=0.001$), irrespective of the presence of CH-mutations reported in 107 pts ($p=0.87$).

Conclusions: CH-mutations are frequently detected at diagnosis in NK AML pts across all ELN risk groups, but more likely in NPM1 mutated pts. The detection of CH-mutations might refine the risk of relapse in pts within the ELN favorable risk group, while their role is limited in pts with more aggressive disease profile.

Myeloproliferative Neoplasms 1

C016

HEREDITARY ERYTHROCYTOSIS AND THROMBOCYTOSIS: EXPERIENCE OF CENTER OF RESEARCH AND INNOVATION OF MYELOPROLIFERATIVE NEOPLASMS (CRIMM)

A. Pacilli¹, C. Mannarelli¹, G. Rotunno¹, F. Giona², M. Santopietro², A. Pancrazzi¹, F. Gesullo¹, S. Fiaccabrino¹, F. Mannelli¹, A.M. Vannucchi¹, P. Guglielmelli¹

¹CRIMM-Centro Ricerca e Innovazione delle Malattie Mieloproliferative Azienda Ospedaliera-Universitaria Careggi Florence-IT, Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, DENOTHE Excellence Center Florence; ²Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Università Sapienza, Roma, Italy

Congenital erythrocytosis (CEry) includes cases with low Epo levels and variants in the Epo receptor gene (EPOR) or cases with increased Epo levels, high-oxygen affinity hemoglobin variants, BPGM deficiency or altered oxygen-sensing pathway. Congenital thrombocytosis (CThr) can be associated with overproduction of Tpo, altered function of its receptor (MPL) and JAK2 mutations other than V617F. We used a custom NGS panel to sequence pts referred to our center (CRIMM; Center Research and Innovation of Myeloproliferative Neoplasms) for Ery and Thr, triple-negative (TN) for JAK2V617F, MPLW515 and CALR mut, isolated or familial; genes included were BPGM, HBA1/2, HBB, EPOR, HIF2A, PHD2, SH2B3, VHL, JAK2, THPO, MPL, GSN, GATA1, WAS, RUNX1, SRC. From July 2016 to March 2019, we collected 108 subjects with suspected CEry (including 10 families with ≥ 2 members) or CThr and no evidence of other causes. Among Ery cases, four pts (3.7%) resulted JAK2V617F or exon 12 positive at low variant allele fraction (VAF) ($<2\%$), one was MPLW515S positive (VAF:19%), and were diagnosed as Polycythemia vera (PV) and essential thrombocythemia (ET), respectively, and excluded from further analysis. Of the 82 cases with Ery (75.9%), 23 (21.3%) displayed a bone marrow consistent with chronic myeloproliferative neoplasms (MPN), including 2 primary myelofibrosis, 8 ET and 13 PV. The MPN cases displayed a median age of 37.2 years and 22% of these patients were younger than 20 years. Thirty-five pts (44.3%) displayed at least one heterozygous variant associated with CEry: 11 (31.4%) had mutation in PHD2, 7 (20%) HIF2A, 8 (22.8%) HBB or HBA, 3 (9.4%) EPOR, 6 (18.8%) gelsolin (GSN). In 5/10 families we found the presence of a common variant in all members (2 HIF2A (I533V or F374Y), 2 HBB (V21M or E102D), 1 EPOR (W439X)). Regarding pts with CThr, we found 2 heterozygous variants in GSN, 1 homozygous variant in WAS but also mutations in genes usually associated with CEry, including PHD2 ($n=2$), HBB ($n=1$), SH2B3 ($n=1$). These data indicate that in pts with unexplained Ery, the oxygen sensing pathway and the high-oxygen affinity haemoglobin variants are the most affected genes. Furthermore, we show that use of custom gene panel may help to identify a causative gene in more than one third of cases in or outside a familial setting.

C017

DELETION OF CALR GENE BY CRISPR/CAS9 GENOME-EDITING MIMICS THE EFFECTS OF MUTANT CALRETICULIN IN HEMATOPOIETIC CELLS

L. Calabresi¹, M. Balliu¹, N. Bartalucci¹, S. Romagnoli¹, L. Maggi², F. Annunziato², P. Guglielmelli¹, A.M. Vannucchi¹

¹CRIMM-Centro Ricerca e Innovazione delle Malattie Mieloproliferative, ²Dipartimento di Medicina Sperimentale e Clinica, Università di Firenze, Italy

Calreticulin (CALR) mutation in myeloproliferative neoplasm (MPN) can be due to a 52 bp deletion (T1) or a 5 bp insertion (T2) in exon 9, that cause a frameshift resulting in novel C-terminal sequence common to all mutant CALR proteins. It has been hypothesized that mutant CALR associates with and activates the thrombopoietin receptor MPL and downstream JAK/STATs as well MAPK and PI3K signaling (Elf *et al.*, Blood 2018). However full characterization of mechanisms pertaining to mutant CALR remains to be pursued. By using CRISPR/Cas9 editing, we generated CALR knock-out (KO) starting from HL60, K562, UT7 and donors' CD34+ cells and T1 variant from K562 and UT7 cells. We found, as previously reported in CALR mut MPN pts (Theocharides

et al., Blood 2016), significant reduction of MPO levels (9-fold lower, $p < 0.01$) in HL60 KO compared to wild-type (WT) cells. Under treatment with ionomycin, K562 KO and T1 cells presented increased ER Ca²⁺ release and impaired capability in restoring Ca²⁺ homeostasis compared to WT cells, resembling Mks from CALR mut pts (Pietra et al., Leukemia 2016). A greater proportion of UT7 KO and T1 cells spontaneously expressed CD41/61 at d7 of culture (50-51% and 41-49%, respectively, for KO and T1 cells with GM-CSF or TPO versus 26-25% of parental cells; $p < 0.01$). Moreover, UT7 KO and T1 cells were able to grow without GM-CSF, that was otherwise necessary for WT cells. We found a constitutively increased phosphorylation of STAT3 (pSTAT3) in KO and T1 cells. STAT3 activation can occur through the actions of several growth factors. Among these, IL-6, which activates STAT3 through the gp130/JAK pathway, resulted overexpressed in KO and T1 cells compared to WT cells, both at mRNA (respectively 2- and 3.5-fold higher) and protein level (1.5- and 2-fold higher) ($p < 0.01$). Blockade of gp130 or IL-6 receptor led to a decrease of pSTAT3 and inhibition of growth in UT7 KO and T1 cells, unlike WT cells. Similar findings were noted in KO CD34+ cells that generated 10-fold more CFU-Mk colonies than control cells ($p < 0.05$). IL-6 mRNA levels were 6-fold higher in KO CD34+ cells and blockade of the IL-6 receptor reduced Mk colony formation of KO CD34+ cells (50%, $p < 0.05$), with no effect in WT cells. Overall, our data suggest that mutant CALR loses some physiological functions that are phenocopied by the absence of CALR protein. We speculate that blockade of IL-6/gp130/JAK signaling pathway could represent a new therapeutic target in CALR mutated MPNs.

C018

TARGETED NEXT-GENERATION SEQUENCING IDENTIFIES NOVEL GENE VARIANTS IN JAK2V617F NEGATIVE PATIENTS WITH ERYTHROCYTOSIS AND JAK2 GGCC_46/1 HAPLOTYPE

L. Anelli, P. Orsini, A. Minervini, A. Zagaria, N. Coccaro, E. Parciante, C.F. Minervini, C. Cumbo, G. Tota, L. Impera, A. Ricco, M.R. Conserva, G. Specchia, F. Albano

Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, University of Bari, Italy

The aetiology of erythrocytosis is complex; it can be classified as primary or secondary and acquired or congenital. To date, several studies have been performed on large patient cohorts to identify any clinical or laboratory feature helping to distinguish familial and congenital erythrocytosis or primary acquired forms such as polycythemia vera (PV). However, the differential diagnosis of erythrocytosis remains complex. The aim of this study was to analyze a group of 15 patients with suspected diagnosis of PV, negative for classic JAK2 gene mutations but positive for JAK2 GGCC_{46/1} haplotype, a germline combination of polymorphisms known to be associated with the onset of myeloproliferative neoplasms. A targeted next generation sequencing (NGS) approach by Ion Torrent sequencing was employed using a customized panel including 26 myeloid genes. Selected variants were investigated for a potential pathogenetic role using the SIFT and Polyphen scores and the Catalogue of Somatic Mutations in Cancer database. Overall, 24 genetic variants in 13 myeloid genes were identified in all 15 patients within the examined cohort. According to their frequency in the healthy population and to a germline or somatic nature in our patient series, these variants were divided into three different groups: polymorphisms, rare germline variants, and rare somatic variants. Overall, eight polymorphisms attributed a benign clinical significance, and having a frequency of 10-40% in healthy individuals, were detected at higher frequency in our patients in the following genes: JAK2 (rs2230724, rs2274649, rs2230722) ANKRD26 (rs7897309), GATA2 (rs2335052), TET2 (rs34402524 and rs2454206), and CALR (rs1049481). At least one of rare germline and somatic variants was detected in 12 of the 15 cases (80%), the most frequently mutated genes being TET2 (5/15, 33%), KIT (4/15, 27%), MPL and DNMT3A (3/15, 20%). Non canonical JAK2 K1055R and MPL V114M variants, mapping in JAK2 exon 23 and MPL exon3, respectively, were identified in 3 patients, one patient showing both. In conclusion, in this NGS study the large number of rare germline variants together with polymorphisms in the JAK2, ANKRD26, GATA2, TET2, and CALR genes is in accordance with the co-occurrence of the

JAK2 GGCC_{46/1} germline haplotype, that seems to predispose to an increased risk of developing erythrocytosis. However, the pathogenic contribution of each identified new gene variant warrants further investigations.

C019

MCP-1 -2518 A/G SINGLE NUCLEOTIDE POLYMORPHISM IS A JAK2V617F MUTATION SUSCEPTIBILITY MARKER AND CORRELATES TO DISEASE PROGRESSION IN PRIMARY MYELOFIBROSIS

E. Masselli¹, C. Carubbi¹, G. Pozzi¹, A. Percesepe², R. Campanelli, L. Villani³, G. Gobbi¹, V. Rosti³, M. Massa³, G. Barosi³, M. Vitale¹

¹Dipartimento di Medicina e Chirurgia, Università di Parma; ²Genetica Medica, Azienda Ospedaliero-Universitaria di Parma; ³Centro per lo Studio e la Cura della Mielofibrosi, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

The mutational landscape of Ph-negative myeloproliferative neoplasm is extremely complex and host genetic variations are emerging as essential players. We recently demonstrated that a single nucleotide polymorphism (SNP) of the Monocyte Chemoattractant Protein-1 (the -2518 A/G SNP of MCP-1) is an inherited host genetic factor associated with secondary myelofibrosis (sMF) and a biomarker of disease severity in MF (Masselli et al., Leukemia 2018). Moving from these findings, we assessed frequency and phenotypic correlations of this SNP in a cohort of 201 Caucasian primary MF (PMF) and 249 Caucasian control healthy subjects (CTRL). One-hundred and nineteen out of 201 PMF were males (59.2%). Median age was 52y (18-80y). N.103 patients (51.2%) were pre-fibrotic PMF. Driver mutations occurred as follows: 123 (61.2%) harbored a JAK2V617F mutation, 47 (23.4%) had CALR mutations, 20 (9.9%) were triple negative and n.8 (4%) had MPL mutations.

Table 1.

	MCP-1 A/G SNP genotype				P value (G/G vs. A/A+A/G)
	All	A/A	A/G	G/G	
Hb, g/L, mean (range)	n.201 12.6 (4.9-18.4)	n.115 12.6 (5.9-18.4)	n.73 12.7 (4.9-17.6)	n.13 11.8 (6.4-18)	0.33
Pts with Hb <10 g/dL, N (%)	n.201 42 (20.9)	n.115 25 (21.7)	n.73 11 (15.1)	n.13 6 (46.1)	0.02
WBC*, x10 ⁹ /L, mean (range)	n.201 8.9 (1.9-33)	n.115 9.0 (1.9-27)	n.73 8.9 (2.1-31)	n.13 8.4 (2.6-21.3)	0.66
Pts with WBC >12x10 ⁹ /L, N (%)	n.201 38 (18.9)	n.115 23 (20)	n.73 13 (17.8)	n.13 2 (15.4)	0.74
PLT, x10 ⁹ /L, mean (range)	n.201 455 (24-2323)	n.115 486 (25-2323)	n.73 424 (24-1114)	n.13 349 (17-700)	0.19
Pts with PLT <150 x10 ⁹ /L, N (%)	n.201 34 (16.9)	n.115 16 (13.9%)	n.73 4 (9.2%)	n.13 4 (30.8%)	0.16
Spleen index**, cm ³ , mean (range)	n.198 141.6 (90-850)	n.113 144.8 (90-850)	n.72 31.1 (89-399)	n.13 172.4 (90-486)	0.22
Pts with spleen index** >150 cm ³	n.198 43 (21.2)	n.113 22 (19.5)	n.72 15 (20.8)	n.13 6 (46.2)	0.02
LDH, mU/mL, x ULN, mean (range)	n.136 1.49 (0.19-4.49)	n.81 1.52 (0.50-4.49)	n.46 1.31 (0.19-2.83)	n.9 2.25 (0.71-4.46)	0.011
hs-CRP, ng/mL, mean (range)	n.160 0.64 (0.01-12.6)	n.97 0.54 (0.01-12.6)	n.52 0.79 (0.02-7.97)	n.11 0.88 (0.09-4.38)	0.6
Pts with hs-CRP >0.3 ng/mL, N (%)	n.160 52 (32.5)	n.97 39 (39.8)	n.52 15 (28.8)	n.11 7 (63.3)	0.025

* WBC was corrected for the number of circulating erythroblasts

** Spleen index is the product of the longitudinal by the transverse spleen axis, the latter defined as the maximal width of the organ

Statistically significant associations are enlighten in bold

Three patients had both JAK2V617F and CALR mutation. After a median follow-up of 84.7 months, 37 patients (18.4%) died, 38 (18%) experienced blast transformation (BT) and 23 patients (11.4%) received allogeneic hematopoietic stem cell transplantation (HSCT). Median age of CTRL was 64y (28-85y), and 132/249 (53%) were males. Both genotypic and allelic frequency of the SNP did not differ between PMF and CTRL (OR 1.06; 95% CI 0.78-1.44; $p = 0.69$ and OR 1.04; 95% CI 0.72-1.52; $p = 0.82$). However, the distribution of the G allele was significantly higher in JAK2V617F+ patients as compared to non-mutated (26.4% vs 21.8%; OR 1.99; 95% CI 1.22-3.24; $p = 0.005$). PMF carrying a homozygous genotype (G/G), hereby referred as the MCP-1 high-risk group, showed a higher frequency of severe anemia, massive splenomegaly and higher LDH plasma level at the time of diagnosis. Moreover, PMF typified by higher levels of high-sensitivity C-reactive protein, a well-established pro-inflammatory marker, were enriched in the G/G genotype (Table 1). To assess whether the presence of the G allele might

affect disease progression, we analyzed the risk of incurring into death for any cause, BT and HSCT, according to the genotype. In the composite outcome model, the MCP-1 high-risk group showed a significantly shorter time to event (HR 2.56; 95% CI 1.19-5.55; p=0.016). In conclusion, homozygosity for the MCP-1 SNP appears to identify PMF at high risk of a more severe disease presentation, a pro-inflammatory state and a higher chance to progression.

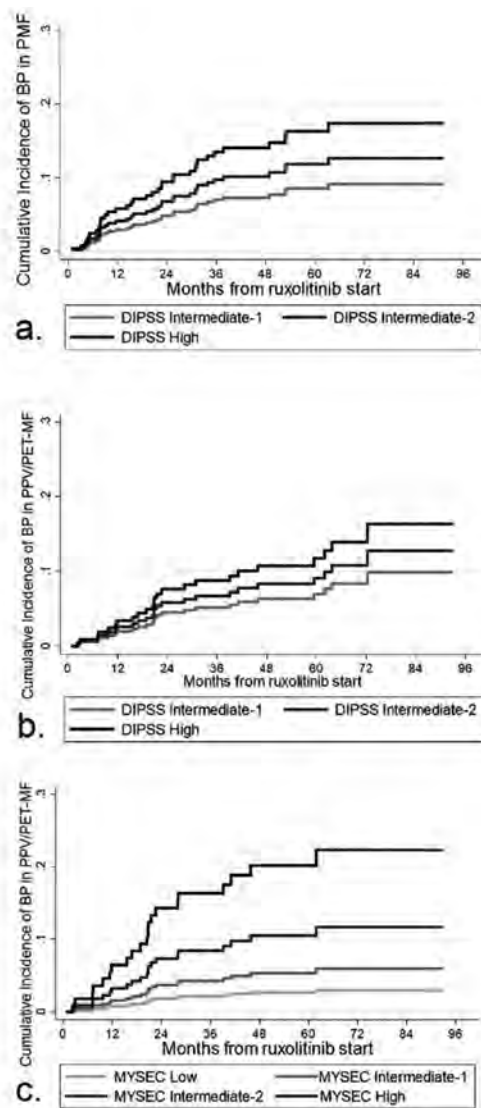
C020
RISK FACTORS FOR PROGRESSION TO BLAST PHASE IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB: AN ITALIAN STUDY ON 589 PATIENTS

F. Palandri, M. Breccia, N. Polverelli, M. Tiribelli, G. Benevolo, A. Tieghi, G. Binotto, M. Bonifacio, B. Martino, A. Iurlo, E.M. Elli, E. Abruzzese, M. Bergamaschi, N. Sgherza, F. Cavazzini, M. Crugnola, C. Bosi, A. Isidori, G. Auteri, E. Sutto, R. Latagliata, D. Griguolo, K. Codeluppi, D. Cattaneo, M. Trawinska, D. Bartoletti, M. Krampera, G. Semenzato, R.M. Lemoli, A. Cuneo, F. Di Raimondo, M. Cavo, N. Vianelli, G.A. Palumbo

Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Division of Cellular Biotechnologies and Hematology University Sapienza, Unit of Blood Diseases and Stem Cells Transplantation Department of Clinical and Experimental Sciences University of Brescia ASST Spedali Civili of Brescia, Division of Hematology and BMT Azienda Sanitaria Universitaria Integrata di Udine, Division of Hematology Città della Salute e della Scienza Hospital, Department of Hematology Azienda USL - IRCCS di Reggio Emilia, Unit of Hematology and Clinical Immunology University of Padova, Department of Medicine Section of Hematology University of Verona, Division of Hematology Azienda Ospedaliera 'Bianchi Melacrinò Morelli', Hematology Division Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Hematology Division San Gerardo Hospital, ASST Monza, Division of Hematology Ospedale S. Eugenio, Clinic of Hematology Department of Internal Medicine (DiMI) IRCCS AOU San Martino-IST, Division of Hematology University of Ferrara, Division of Hematology Azienda Ospedaliero-Universitaria di Parma, Division of Hematology AUSL di Piacenza, Hematology and Stem Cell Transplant Center Marche Nord Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Division of Cellular Biotechnologies and Hematology University Sapienza, Division of Hematology and BMT Azienda Sanitaria Universitaria Integrata di Udine, Department of Hematology Azienda USL - IRCCS di Reggio Emilia, Hematology Division Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Division of Hematology Ospedale S. Eugenio, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Department of Medicine Section of Hematology University of Verona, Unit of Hematology and Clinical Immunology University of Padova, Clinic of Hematology Department of Internal Medicine (DiMI) IRCCS AOU San Martino-IST, Division of Hematology University of Ferrara, Department of Scienze Mediche Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia" University of Catania, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Department of Scienze Mediche Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia" University of Catania

In the pre-ruxolitinib (RUX) era, several risk factors for progression to blast phase (BP) have been proposed in myelofibrosis (MF) patients (pts), namely peripheral blasts, thrombocytopenia, unfavorable cytogenetics, and high risk category. However, predictors of BP during ruxolitinib (RUX) therapy are unknown. To evaluate incidence and risk factors of BP in RUX-treated MF pts, we retrospectively collected clinical/laboratory data of 589 pts who received RUX in 20 European Hematology Centers. A time-to-event (BP) analysis was conducted with Fine & Gray model with death/time of stem cell transplant as competing risks. Cumulative Incidence Function among risk categories for DIPSS and MYSEC-PM was calculated applying the Gray's model. Of 589 MF pts [PMF, n.303, 51.5%; post-Polycythemia Vera MF (PPV-MF), n.164, 27.8%]; post-Essential thrombocythemia MF (PET-MF), n.122], 65 developed BP. In 61 pts, BP caused RUX withdrawal after a median time of 1.2 yrs (0.6-6.8); in 4 pts BP occurred after RUX stop (median time: 2.6 yrs). BP incidence rate was 3.6 x100 pt-yrs and was compara-

ble in PMF and PPV/PET-MF (p=0.1). In multivariable analysis, the probability of BP evolution for the entire cohort was significantly associated with PLT <150x10⁹/l (HR[95% CI]: 2.03[1.10-3.74], p=0.02), spleen length ≥10 cm (HR[95% CI]: 2.56[1.21-5.42], p=0.01), and blasts ≥3% (HR[95% CI]: 2.06 [1.02-1.14], p=0.04). Analysing PMF separately, univariate analysis failed to detect parameters associated with BP evolution. Conversely, in PPV/PET-MF, predictors for BP were PLT <150x10⁹/l (HR[95% CI]: 3.72[1.35-10.24], p=0.01) and blasts ≥3% (HR[95% CI]: 3.05[1.12-8.29], p=0.03); previous interferon (IFN) significantly reduced the risk of BP (HR[95% CI]: 0.72 [0.61-0.83], p<0.001). High DIPSS risk significantly predicted BP evolution in PMF (p=0.04, HR [95% CI]: 2.6 [1.1-6.5]) but not in PPV/PET-MF (p=0.35). In this latter cohort, only the MYSEC-PM score was associated with BP (p=0.01) (Fig.1). Estimated HRs, in reference to the lower score category, were: 1.18 (CI 95%: 1.10-1.29) for intermediate-1, 2.80 (CI 95%: 1.51-20.34) for intermediate-2, and 5.52 (CI 95%: 2.04-19.63) for high risk. HR for high risk pts, comparing to all lower risk groups, was 2.86 (CI 95%: 1.23-6.61). Overall, 11% of RUX-treated pts developed BP. The risk of BP was increased by thrombocytopenia/peripheral blasts at RUX start, and decreased by IFN use. DIPSS and MYSEC-PM predicted BP in PMF and PPV/PET-MF, respectively.



Cumulative incidence of blast phase transformation (BP) according to DIPSS risk score in PMF (a), DIPSS risk score in PPV/PET-MF (b) and MYSEC-PM risk score in PPV/PET-MF (c).

Figure 1.

Chronic Lymphatic Leukemia

C021

STAT3 MUTATIONS IMPACT ON OVERALL SURVIVAL IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA: A SINGLE CENTER EXPERIENCE OF 205 PATIENTS

G. Barilà, A. Teramo, G. Calabretto, C. Vicenzetto, V.R. Gasparini, L. Pavan, M. Leoncin, S. Vedovato, M. Facco, G. Semenzato, R. Zambello

Department of Medicine (DIMED), Hematology and Clinical Immunology section, Padua University School of Medicine, Padua, Italy

Large Granular Lymphocyte Leukemia (LGLL) is a rare and chronic lymphoproliferative disorder characterized by the expansion of LGLs. Patient's affected by LGLL, either CD3+ T-LGLL (Tab or Tgd depending on TCR rearrangement) and CD3- Chronic Lymphoproliferative disorder of NK cells (CLPD-NK), can be asymptomatic or develop cytopenia, mostly neutropenia. Somatic STAT3 and STAT5b mutations were recently discovered in approximately 40% of patients. The aim of this study is to analyse clinical and biological features of a large cohort of LGLL patients to identify prognostic markers affecting patients' outcome. From 1992 to 2018, clinical and biological data of 205 LGLL patients were collected. STAT3 exon 21 and STAT5b exon 16-18 mutations analysis was performed by Sanger sequencing. By phenotype, 129/205 (62.9%) patients were Tab LGLL (84/205, 41%, CD8+ LGLL, and 45/205, 21.9%, CD4+ LGLL), 23/205 (11.2%) Tgd LGLL and 36/205 (17.6%) CLPD-NK. Moreover, 17 patients (8.3%) were characterized by a biphenotypic variant.

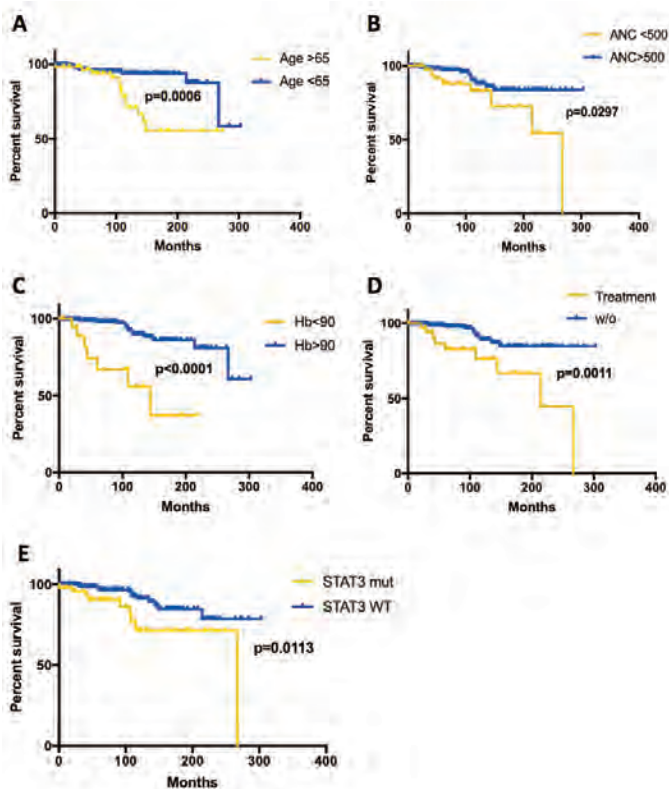


Figure 1.

Neutropenia ($ANC < 1,500/mm^3$) was the main relevant feature (78/205, 38%), with severe neutropenia ($ANC < 500/mm^3$) present in 21% of patients. Anemia ($Hb < 120 g/L$, 27/205, 13.2%), severe anemia ($Hb < 90 g/L$, 20/205, 9.8%) and thrombocytopenia ($PLTs < 100,000/mm^3$, 12/205, 4.8%) were less recurring. DNA samples of 166 and 152 patients were available for STAT3 and STAT5b mutations analysis, respectively. STAT3 mutations were detected in 47 patients (28.3%) and were associated with $ANC < 1,500/mm^3$ ($p < 0.0001$), $ANC < 500/mm^3$

($p < 0.0001$), $Hb < 90 g/L$ ($p = 0.0079$), thrombocytopenia ($p = 0.0316$), and treatment requirement ($p < 0.0001$). On the opposite, STAT5b mutations were found in 15 asymptomatic patients (9.8%) mostly in CD4+ LGLL (12/35, 34.3%). With a median follow up of 8 years, major features associated to reduced OS were age > 65 years ($p = 0.0006$), $ANC < 500/mm^3$ ($p = 0.0297$), $Hb < 90 g/L$, ($p < 0.0001$), treatment requirement ($p = 0.0011$) and STAT3 mutated status ($p = 0.0113$) while no differences were found between STAT5b mutated and wild-type patients ($p = 0.3514$). By multivariate analysis, age > 65 ($p = 0.0008$) and STAT3 mutated status ($p = 0.0113$) were independently associated to reduced OS. In conclusion, we identified clinical and biological features associated to reduced OS in LGLL patients and, for the first time, we demonstrated the dismal impact of STAT3 mutations in patients' survival, suggesting that this biological feature should be considered as a potential target of therapy.

C022

RETROSPECTIVE MULTICENTER 20 YEAR FOLLOW-UP IN HAIRY CELL LEUKEMIA

M. Criscuolo¹, A. Pulsoni², E. Tiacci³, E. Galli⁴, M.E. Nizzoli⁵, L. Trentin⁶, A. Frustaci⁷, M. Deodato⁷, M. Motta⁸, A. Spolzino⁹, M. Cantonetti¹⁰, M. Offidani¹¹, O. Annibali¹², A. Visentin⁶, A. Piciocchi¹³, B. Falini¹⁴, P.L. Zinzani¹⁵, L. Pagano⁴

¹Dipartimento Scienze Radiologiche Radioterapiche ed Ematologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS; ²Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome; ³Institute of Hematology and Center for Hemato-Oncology Research (C.R.E.O.), University and Hospital of Perugia; ⁴Dipartimento Scienze Radiologiche Radioterapiche ed Ematologiche, Fondazione Policlinico A. Gemelli IRCCS-Università Cattolica del Sacro Cuore; ⁵Department of Molecular Medicine, University of Pavia; ⁶Division of Hematology, Department of Medicine, University of Padua; ⁷Struttura complessa di Ematologia, ASST Grande Ospedale Metropolitano Niguarda; ⁸Haematology, ASST Spedali Civili di Brescia; ⁹Haematology, University of Parma; ¹⁰Hematology Unit, Tor Vergata University Hospital; ¹¹Clinica di Ematologia, AOU Ospedali Riuniti di Ancona; ¹²Hematology and Stem Cell Transplantation Unit, University Campus Bio-medico; ¹³Italian Group for Adult Hematologic Diseases - GIMEMA Foundation; ¹⁴Section of Hematology and Clinical Immunology, Department of Medicine, University of Perugia; ¹⁵Institute of Hematology L. e A. Seràgnoli, University of Bologna, Italy

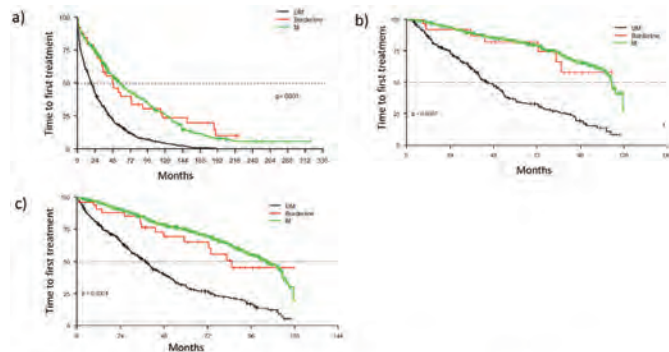
Hairy cell leukemia (HCL) is a rare lymphoproliferative disease with specific morphologic and molecular features and excellent prognosis. Although 72-98% complete remission (CR) after one or rarely two cycle of cladribine (2CDA) was reported, relapse occurs during long term follow-up. The risk of infection is not trivial and lasts until recovery of bone marrow function. These are preliminary results of a multicenter study, recruiting patients with HCL diagnosed between June 1996 and August 2018 in 20 Italian Hematologic centers, aimed at evaluating efficacy, safety and long term remission of 2CDA monotherapy as first line therapy. We recruited 243 patients: M/F ratio was 195/48, with a median age of 53 years (range 30-88). 27 patients (10.7%) presented with an infection, that was a pneumonia in 73% of cases. Concomitant diseases were: chronic pulmonary disease in 14 patients (5.8%), diabetes or chronic hepatic disease in 15 each (6.2%), cardiac disease in 8 (3.3%). 2CDA was administered intravenously in 164 patients (67.5%) and subcutaneously in 79 (32.5%). The overall response was 83.4%: CR in 117 patients (48%) and partial response (PR) in 86 (35.4%). 47 patients (54.6%) received a second course of therapy for PR or less: 28 received 2CDA alone or with rituximab, 9 pentostatin, 5 rituximab alone, 4 interferon α , 1 vemurafenib; 21 (43.7%) improved their response to CR. Infections were reported in 103 patients (42.4%): 75 (72.8%) were fever of undeterminate origin. Allergy was the most frequent adverse event (35, 14.4%): grade 3-4 was reported in 1 patients. Other adverse events occurred in 18 patients (7.4%), mostly cutaneous rash and liver enzymes rise. Duration of follow-up was 5 years or less in 116 patients: 6 died after a median of 18.6 months, due to infection in 3 cases, and 2 relapsed after a median of 43.8 months. 72 patients had a follow-up of 5-10 years: 1 patients died of infection and 7 relapsed after a median of 89.9 months. 55 patients had a follow-up longer than 10 years: all were alive and 7 relapsed after a median follow-up of 155.7 months. Overall mortality was 2.9%. As preliminary report, a second neoplasm was reported in 9 patients (3.7%): 3 lymphoproliferative disease, 2 skin cancer, 2 colon neoplasm, 1 breast can-

cer, 1 prostate cancer. 2CDA is effective in treating HCL, with an overall response rate of 83.4% after one course of treatment. Adverse events were rare and easily managed. Infections is the first cause of death.

Table 1.

Sex	n	range, %
M	195	80.2
F	48	19.8
Age (median)	53	30-88
Onset of disease		
Pneumonia	19	7.8
COPD	14	5.8
Diabetes	15	6.2
Hepatic disease	15	6.2
Cardiac disease	8	3.3
Therapy		
iv	164	67.5
sc	79	32.5
Response		
CR	117	48
PR	86	35.4
Second course		
Cladribine +/- Rituximab	28	59.6
Pentostatine	9	19.1
Rituximab	5	10.6
Interferon α	4	8.5
Vemurafenib	1	2.2
Relapse		
	11	
Adverse events		
Infections	28	11.5
FUO	75	30.9
Allergy	35	14.4
Second neoplasms	9	3.7
Other	18	7.4
Status		
Alive	236	97.1
Dead	7	2.9
OS, median	63	2.1-269.4

759 untreated CLL patients (cohort 1) sequenced at our Institution. Major stereotyped subset analysis was performed by the ARResT/AssignSubsets tool. Time-to-first treatment (TFT) was the clinical endpoint. Two independent series (n=308, n=451; cohort 2) of newly diagnosed CLL patients were used to validate the data. Cohort 1 included 385 (51%) UM-CLL, 36 (5%) BL-CLL and 338 (44%) M-CLL, with a higher frequency of UM-CLL due to the enrichment of cases enrolled in first-line clinical trials. Subset #2 CLL were 15 (2%) and were, as expected, characterized by a poor outcome. BL-CLL (97-97.9% IGHV identity) showed a TFT similar to that of M-CLL and significantly longer than that of UM-CLL (Figure 1a), despite the expected enrichment of subset #2 cases (5/36, 14%) within BL-CLL. The TFT of BL-CLL remained comparable to that of M-CLL also when the analysis was restricted to the 327 CLL patients at diagnosis of cohort 1 (125 UM-CLL, 19 BL-CLL and 183 M-CLL), including 200 Binet stage A. These findings were then validated in the independent cohort 2: 238 (31%) UM-CLL, 28 (4%) BL-CLL, 493 (65%) M-CLL; 709 Binet stage A; subset #2: 11/758, 1.4%. The TFT of BL was again similar to that of M-CLL (Figure 1b). When all newly diagnosed patients from cohorts 1 and 2 were pooled together, 363 UM-CLL, 47 BL-CLL and 676 M-CLL were identified. TFT is shown in Fig. 1c. BL-CLL at diagnosis (47/1086, 4.3%) showed a biologic profile comparable to that of M-CLL, with a similar frequency of unfavorable prognostic factors (CD38+: 17%; del11q/del17p+: 10%; NOTCH1 mutated: 4%; TP53 mutated: 8%). These results held true also considering only the 909 Binet stage A patients (data not shown). Our data suggest that the biology and prognosis of BL-CLL patients are similar to those of M-CLL and better than those of UM-CLL, thus supporting the 98% cut-off for clinical purposes. The issue of BL-CLL prognosis remains open to further investigations on larger series of patients.



Time to first treatment (TFT) analysis.
a) Cohort 1 (n=759): unmutated (UM) (n=385) vs borderline (BL) (n=36) vs mutated (M)-CLL (n=338);
b) cohort 2 (n=759): UM (n=238) vs BL (n=28) vs M-CLL (n=493);
c) newly diagnosed CLL from cohort 1 and 2 (n=1086): UM (n=363) vs BL (n=47) vs M-CLL (n=676)

Figure 1.

C023

CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH “BORDERLINE” PERCENTAGE OF IGHV MUTATIONS SHOW A FAVORABLE CLINICAL OUTCOME

S. Raponi, C. Ilari, I. Della Starza, I. Del Giudice, L.V. Cappelli, L. Caforrio, A. Piciocchi, G. Giuliani, S. Soddu, M. Cavalli, L.A. De Novi, P. Mariglia, F.R. Mauro, M. Vignetti, M. Gentile, G. Cutrona, R. Moia, D. Rossi, G. Gaidano, F. Morabito, A. Guarini, R. Foà

Hematology, Department of Translational and Precision Medicine, Sapienza University of Rome; GIMEMA Data Center, GIMEMA Foundation; Hematology Unit, Department of Hemato-Oncology, Ospedale Annunziata; Biotechnology Research Unit; Molecular Pathology Unit, IRCCS Ospedale Policlinico San Martino; Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont; Department of Hematology, Oncology Institute of Southern Switzerland and Institute of Oncology Research; Hematology Department and Bone Marrow Transplant Unit, Cancer Care Center, Augusta Victoria Hospital; Department of Molecular Medicine, Sapienza University of Rome

The different outcome of chronic lymphocytic leukemia (CLL) patients with unmutated (UM, $\geq 98\%$) or mutated (M, $< 98\%$) immunoglobulin heavy chain variable region genes (IGHV) is well known. The prognosis of cases with a % of IGHV identity close to 98% remains instead controversial. We therefore investigated the prognosis and biologic features of “borderline” (BL) IGHV CLL, defined as those with a 97-97.9% IGHV identity, according to the 2017 European Research Initiative on CLL (ERIC) recommendations. We retrospectively analyzed

C024

EFFICACY AND SAFETY OF FRONT-LINE TREATMENT WITH IBRUTINIB AND RITUXIMAB IN UNFIT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). FIRST REPORT OF THE GIMEMA LLC1114 STUDY

F.R. Mauro¹, S. Molica², F. Paoloni³, G. Reda⁴, L. Trentin⁵, M. Marchetti⁶, D. Pietrasanta⁷, M. Coscia⁸, R. Marasca⁹, P. Sportoletti¹⁰, G. Gaidano¹¹, L. Orsucci¹², C. Stelitano¹³, N. Di Renzo¹⁴, M. Liberati¹⁵, D. Gottardi¹⁶, F. Re¹⁷, F. Ilariucci¹⁸, P.L. Zinzani¹⁹, D. Mannina²⁰, A. Gozzetti²¹, A.L. Molinari²², M. Gentile²³, A. Chiarenza²⁴, L. Laurenti²⁵, M. Varettoni²⁶, A. Ibatucci²⁷, R. Murru²⁸, M. Tani²⁹, M.S. De Propriis¹, I. Del Giudice¹, I. Della Starza¹, S. Raponi¹, M. Nanni¹, P. Fazi⁴, E. Crea⁴, A. Neri⁴, G. Specchia³⁰, A. Guarini¹, A. Cuneo³¹, R. Foà¹

¹Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome; ²Department of Hematology, Pugliese Ciaccio Hospital, Catanzar; ³Italian Group for Adult Hematologic Diseases (GIMEMA), Rome, ⁴Hematology Department, Foundation IRCCS Ca’ Granda Ospedale Maggiore

Allogeneic and Autologous Transplantation

C026

HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STROMAL CELLS TO TREAT STEROID-REFRACTORY ACUTE GVHD III/IV OR OVERLAP SYNDROME: INTERIM ANALYSIS OF A MULTICENTER PHASE I/II STUDY

D. Taurino¹, M. Micò¹, A. Algarotti¹, A. Grassi¹, C. Pavoni¹, F. Onida², N. Mordini³, M.L. Ferrari¹, F. Lussana¹, M.C. Finazzi¹, C. Capelli⁴, M. Intronà⁴, A. Rambaldi⁵

¹Hematology and Bone Marrow Transplantation Unit, ASST Papa Giovanni XXIII, Bergamo, Italy; ²BMT Center - Hematology Unit, IRCCS Ospedale Maggiore Policlinico Di Milano - University of Milan, Milan, Italy; ³Santa Croce e Carle, UO Ematologia, Cuneo, Italy; ⁴USS Centro di Terapia Cellulare G. Lanzani, UOC Ematologia ASST Papa Giovanni XXIII, Bergamo, Italy; ⁵University of Milan, Milan, Italy and ASST Papa Giovanni XXIII, Bergamo, Italy

Background: Treatment of patients developing steroid-refractory acute GvHD (SR-aGvHD) after allo-HSCT remains an unmet clinical need and a major therapeutic challenge. Mesenchymal stromal cells (MSC) induce immunosuppression and reduce the inflammatory status initiated by aGvHD. We performed an interim analysis of a multicenter, prospective, non-randomized, phase I/II study, planned to test the safety and activity of human umbilical cord, third-party MSC given sequentially after Pentastatin for the treatment of SR-aGvHD grade III-IV and the overlap syndrome.¹

Patients and Methods: UC MSC were produced under GMP conditions as previously described.² The patients underwent infusion of Pentostatin for 3 consecutive days, followed by 3 MSC infusions weekly. Between October 2013 and May 2018 we enrolled 27 allo-HSCT adult recipients with SR-aGvHD (n 25, 9 patients had grade III and 16 had grade IV) or overlap syndrome (n 2), transplanted from HLA-identical sibling donors, haploidentical donors, MUD or cord blood. Target organ involvement included skin (n 11), gastrointestinal tract (GI) (n 23) and liver (n 12); 64% of patients had multiple organ involvement.

Results: Response by day 30 after the final MSC dose, was evaluable in 59% of patients: 26% achieved CR, 22% PR and 11% NR; 10 patients died before reaching the evaluation time of response: 8 patients for aGvHD and 2 for haematological disease; 1 patient was lost to follow-up. The patients with single organ involvement showing 67% of ORR (67% RC, 33% PR). No response was observed in 2 cases of overlap syndrome. Patients achieving CR had improved OS vs patients in PR/NR (80% vs 50% at 1 year). For patients with grade III and IV SR-aGvHD, CR rates were 67% and 31% respectively. We observed relapse of underlying malignancy in 5 patients. At last follow-up, 30% of patients were alive with no GvHD in 71% of them; 52% of patients died from TRM (aGvHD, overlap syndrome, infections, cGvHD). Two patients with uncontrolled GvHD has developed Transplant Associated Microangiopathy; 85% patients developed infectious complications, in most cases virus and fungal related.

Conclusions: The UC MSC-infusion were well tolerated without any toxic event. As expected in patients with SR-aGvHD, a high rate of infectious complications were observed. The ORR and the OS are promising and this warrants planning a future pivotal trial.

References

1. EUDRACT n. 2012-000582-21, NCT02032446
2. Capelli C et al Cytotherapy, 2011, 13, 786-801

C027

ACUTE GRAFT VERSUS HOST DISEASE: REDUCED INCIDENCE AND PREDICTIVE FACTORS

A. Di Francesco¹, A. Raiola², A. Dominietto², C. Di Grazia², F. Gualandi², M. Van Lint², P. Chiusolo¹⁻³, L. Laurenti¹⁻³, F. Sorà¹⁻³, S. Giammarco¹, E. Metafuni¹, E. Angelucci², A. Bacigalupo¹⁻³

¹Istituto di Ematologia, Fondazione Policlinico Universitario A Gemelli IRCCS; ²IRCCS AOU San Martino IST; ³Università Cattolica del Sacro Cuore, Italy

Background: Acute Graft versus Host Disease (GvHD) is a major complication of allogeneic HSCT. Its prophylaxis has significantly changed

Policlinico di Milan; ⁵Hematology Division, Department of Medicine, University of Padua; ⁶DH Ematologico, Oncologia, Ospedale Cardinal Massaia, Asti; ⁷Department of Hematology, SS. Antonio e Biagio e Cesare Arrigo Hospital, Alessandria; ⁸Division of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino; ⁹Hematology Unit, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena; ¹⁰Institute of Hematology-Centro di Ricerca Emato-Oncologica (CREO), University of Perugia; ¹¹Division of Hematology, Dept. of Translational Medicine, Università del Piemonte Orientale, Novara; ¹²Department of Hematology, Azienda Ospedaliera Universitaria Città della Salute e della Scienza, Torino; ¹³Department of Haematology, Azienda Ospedaliera Bianchi Melacrinò Morelli, Reggio Calabria; ¹⁴Division of Hematology, P.O. V. Fazzi, Lecce; ¹⁵Università degli Studi di Perugia, A.O.S. S. Maria, Terni; ¹⁶A.O.U. S. Giovanni Battista A.O. Maurizio-Umberto I, Turin; ¹⁷Department of Hematology, CTMO University, Parma; ¹⁸AUSL - IRCCS S. Maria Nuova - Reggio Emilia; ¹⁹Institute of Hematology "Seragnoli" University of Bologna; ²⁰Division of Hematology, Azienda Ospedaliera Papardo, Messina; ²¹Hematology, Dep. of Medical Science, Surgery and Neuroscience, University of Siena; ²²Department of Oncology and Hematology, Infermi Hospital, Rimini; ²³Hematology Section, Cosenza Hospital, Cosenza; ²⁴Division of Hematology, Azienda Policlinico-OVE, Catania; ²⁵Fondazione Policlinico Universitario A. Gemelli, Roma; ²⁶Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia; ²⁷Hematology and Bone Marrow Transplantation Unit, IRCCS Ospedale Policlinico San Martino, Genova; ²⁸Division of Hematology and CTMO, Businco Hospital, Azienda Ospedaliera Brotzu, Cagliari; ²⁹Division of Hematology, Santa Maria delle Croci Hospital, Ravenna; ³⁰Department of Emergency and Transplantation, Hematology Section, University of Bari Medical School; ³¹Hematology Section, St. Anna University Hospital, Ferrara, Italy

The potential for improved efficacy with the addition of rituximab (R) to ibrutinib (IBR) was investigated in previously untreated and unfit patients with CLL in a prospective multicenter study of the GIMEMA group (LLC1114). The inclusion criteria were as follows: previously untreated CLL requiring treatment, Cumulative Illness Rating Scale (CIRS) score >6 and/or creatinine clearance <70 mL/min. IBR, 420 mg once daily continuously, and R, 375 mg/sqm, on day 1 of months 1-6 were given during the first 6 months of the study; thereafter, IBR was given as a single agent until progression or severe toxicity, or complete response with no evidence of minimal residual disease (MRD) or for a maximum duration of 6 years. Between March 2015 and April 2017 143 unfit patients with CLL were included in this study. The median follow-up of patients was 22.8 months, the median age 73 years, the median CIRS score, 6. Binet stage B/C was present in 81% of patients, increased beta-2 microglobulin in 71%. Del(17p) or del(11q) were recorded in 9% and 14% of cases, respectively, TP53 mutations in 15% and an IGHV unmutated status in 51%. Response to the IBR+R combination was achieved by 114/142 (81%) patients with a CR in 20%, a L-PR in 9% and a PR in 52%. Undetectable MRD by flow cytometry (<10⁻⁴) in the peripheral blood and bone marrow was found in 3 patients. Treatment failure was considered in 7 (5%) patients with no response and in 14% who discontinued treatment due to other reasons than non-response. The PFS and OS rates at 24 months were 91% (95% CI, 85.20-96.69) and 94% (95% CI, 88.93-98.55), respectively. PFS and OS were not influenced by the response to IBR+R (PFS and OS at 12 months, CR vs L-PR/PR: 100% vs 98.5%; p=0.44), nor by the IGHV mutational status (mutated vs unmutated IGHV, PFS at 24 months: 92% vs 88%; p=0.77; OS at 24 months: 94% vs 93%; p=0.71). Patients with del(17p) and/or TP53 mutations showed a shorter, though not significantly, survival (TP53 disruption, absent vs present, PFS at 24 months: 93% vs 77%; p=0.13; OS at 24 months: 95% vs 87%; p=0.22). Grade ≥ 3 adverse events of interest were: infections, in 19 (13%) patients, cardiac disorders, in 14 (9.7%; AF, 7%), and bleeding events in 6 (4%). The first analysis of the GIMEMA LLC1114 protocol indicates that in unfit CLL patients the IBR+R combination was relatively well tolerated and resulted in a higher rate of early CR than observed in patients treated with IBR given as a single agent.

C025

ABSTRACT WITHDRAWN

in time.

Aim of the study: Determine the cumulative incidence of GvHD and its outcome, in 3 periods: <2000; 2000-2010; >2010, together with predicting factors.

Methods: We analyzed 3126 patients allografted in 2 transplant Centers between 1976 and 2018. Median age increased from 32 (1-66) to 42 years (9-71) to 50 years (range 13-74) (<0.00001).

Results: GvHD timing: The median day of onset of GvHD before year 2000 was day +14 (2-90), between 2001 and 2010 it was day +19 (2-98) and beyond year 2010 it was day +33 (2-98) (p<0.0001). There were 237 patients developing GvHD before day 14 in year<2000, 71 patients between 2001 and 2010 and only 8 patients beyond 2010 (p<0.0001). GvHD cumulative incidence. The cumulative incidence (CI) of GvHD grade II-IV was 47% before 2000, 24% between 2000 and 2010, 16% after 2010 (p<0.00001). The CI of GvHD grade III-IV was 13%, 5%, 4% respectively (p<0.001). Survival at 5 years of patients with GvHD grade II has remained unchanged (52%, 44%, 55%, respectively, p=0.2); survival of patients with GvHD grade III-IV was 15%, 30% and 23% respectively, p=0.003. GvHD and donor type. GvHD grade II-IV was recorded in HLA identical siblings as 25%, 19% and 14% (p=0.0005), and in alternative donors as 43%, 34%, 21% (p<0.00001). Transplant related mortality (TRM). The CI of TRM for patients with grade II GvHD has not changed significantly (27%, 25%, 20%, p=0.1) whereas TRM for patients with GvHD grade III-IV has been reduced from 70% (<2000) to 49% (2001-2010) to 26% beyond year 2010 (p=0.002). Multivariate analysis on survival for patients with grade II GvHD. Significant predictors were the day of onset of aGvHD before day 14, advanced phase of the disease, transplant year before year 2000, patients age over 60 and a donor other than an HLA identical sibling. When adding together these negative predictors, the 5 year survival was 28% for patients with 3-5 negative predictors, as compared to 56% for patients with 0-2 negative predictors.

Conclusions: We show a very significant reduction of the CI of GvHD with time, together with a very significant delay of onset of the disease. TRM has been reduced in patients with grade III-IV GvHD, less so in patients with GvHD grade II. In the latter significant clinical predictors may allow the identification of patients at high risk, eligible for treatment protocols.

C028

THE ROLE OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH NPM1 ACUTE MYELOID LEUKEMIA

F. Lussana, C. Caprioli, P. Stefanoni, C. Pavoni, O. Spinelli, A. Michelato, K. Buklijas, A. Algarotti, C. Micò, A. Grassi, T. Intermesoli, A. Rambaldi

Unità di Ematologia e Trapianto di Midollo Osseo, Azienda Socio Sanitaria territoriale Papa Giovanni XXIII, Bergamo, Italy; Dipartimento di Oncologia ed Emato-Oncologia Università degli Studi di Milano, Italy

Background: Recent studies have shown a correlation between the persistence of NPM1 minimal residual disease (MRD) level and patient adverse outcome. Since the outcome of NPM1 mutated (NPM1mut) MRD positive patients is usually poor the choice of an allogeneic transplant (alloHSCT) is usually considered for these patients, although the exact benefit remains to be elucidated.

Methods: We analyzed the impact of alloHSCT in a cohort of 89 newly diagnosed NPM1mut AML patients consecutively treated according to the Northern Italy Leukaemia Group (NILG) protocol 02/06 [ClinicalTrials.gov Identifier: NCT00495287].

Results: As expected, the 3-years overall survival (OS) and disease free survival (DFS) were significantly inferior for patients with persistent MRD positivity after the second consolidation cycle compared to MRD negative patients (OS 45% vs 84%, p=0.001; DFS 42% vs 76%, p=0.006). Among MRD negative patients a benefit was not observed in patients receiving alloHSCT compared to patients not undergoing alloHSCT in CR1 (OS 89% vs 81%, p=0.59 and DFS 80% vs 75%, p=0.87). In contrast, for patients with persistent MRD positivity after consolidation, the 3-years OS and DFS were superior, although not statistically significant, for patients undergoing alloHSCT compared to those who did not (52% vs 31%, p=0.45 and 54% vs 17%, p=0.24, respectively). The therapeutic benefit of alloHSCT was mainly limited by a significant incidence of relapse during the first year after alloHSCT

40% (95%CI 24%-56%). Finally, post-transplant outcomes seem to be superior among patients undergoing alloHSCT with low MRD positivity before transplant (OS 83% vs 33%, p=0.08; DFS 83% vs 38%, p=0.02) in comparison to those undergoing transplantation with high levels of MRD positivity (OS 43% vs 30%, p=0.65; DFS 46% vs 30%, p=0.70).

Conclusions: Consolidative alloHSCT improves outcomes compared to standard chemotherapy in patients at higher risk for leukemia relapse due to the persistence of NPM1mut MRD positivity. However, since the therapeutic benefit of alloHSCT is only partial due to the significant incidence of relapse after alloHSCT, clinical studies evaluating experimental pre-transplant or preemptive treatments after transplantation are warranted.

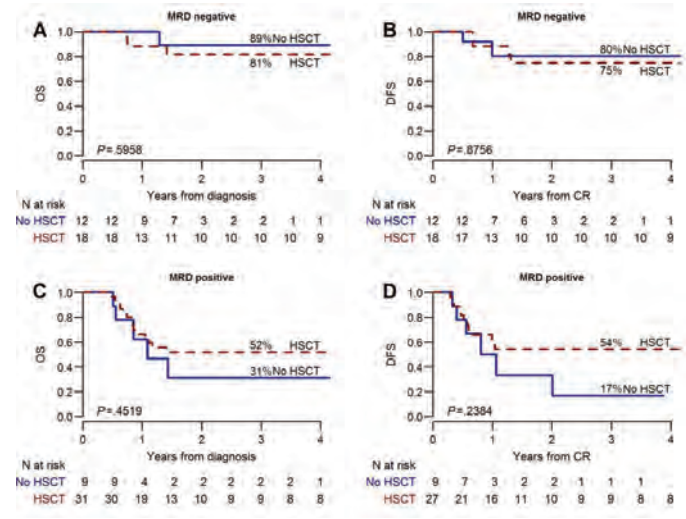


Figure 1.

C029

IMPACT OF THE DONOR/RECIPIENT ALLELIC HLA MATCHING LEVEL IN THE ITALIAN UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE OBSERVATIONAL TRIAL ON BEHALF OF GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO - CELLULE STAMINALI EMOPOIETICHE E TERAPIE CELLULARI (GITMO), ITALIAN BONE MARROW DONOR REGISTRY (IBMDR) AND ASSOCIAZIONE ITALIANA DI IMMUNOGENETICA E BIOLOGIA DEI TRAPIANTI (AIBT)

A. Picardi¹, N. Sacchi², V. Miotti³, F. Lorentino⁴, E. Oldani⁵, A. Rambaldi⁵, M. Sessa⁶, B. Bruno⁷, M. Cerno⁸, P. Bernasconi⁹, L. Vago⁴, W. Arcese¹, F. Benedetti¹⁰, P. Pioltelli¹¹, L. Farina¹², G. Saporiti¹³, P. Chiusolo¹⁴, F. Zallio¹⁵, F. Fagioli¹⁶, G. Papalinetti¹⁷, C. Borghero¹⁸, A.M. Carella¹⁹, F. Narni²⁰, D. Russo²¹, A. Olivieri²², T. Lamparelli²³, G. Specchia²⁴, S. Guidi²⁵, A.P. Iori²⁶, G. Milone²⁷, M. Andreani²⁸, A.M. Gallina², F. Patriarca³, S. Mammoliti²⁹, F. Ciceri⁴, F. Bonifazi⁶

¹Università di Roma Tor Vergata - Dipartimento di Biotecnologia e Prevenzione; ²IBMDR Ospedale Galliera; ³Azienda Universitaria Ospedaliera di Udine; ⁴Haematology and BMT Unit IRCCS San Raffaele; ⁵ASST Papa Giovanni XXIII; ⁶S. Orsola Malpighi; ⁷AOU Città della Salute e della Scienza; ⁸Ospedale Santa Maria della Misericordia; ⁹IRCCS Policlinico San Matteo; ¹⁰University of Verona; ¹¹Ospedale San Gerardo; ¹²IRCCS Istituto Nazionale dei Tumori; ¹³Fondazione Ospedale Maggiore Policlinico; ¹⁴Fondazione Policlinico Universitario Agostino Gemelli; ¹⁵Azienda Ospedaliera SS Antonio e Biagio e C Arrigo; ¹⁶Ospedale Regina Margherita - Università di Torino; ¹⁷Ospedale Civile C Borghero; ¹⁸USSL 6; ¹⁹CSS Hospital IRCCS; ²⁰Azienda Ospedaliera Universitaria; ²¹Università di Brescia - Dipartimento di Scienze Cliniche e Sperimentali; ²²Università Politecnica delle Marche; ²³Ospedale Policlinico San Martino; ²⁴Dipartimento di Trapianto di Organi e Emergenza dell'Università di Bari; ²⁵Università degli Studi di Firenze; ²⁶Policlinico Umberto I -Università La Sapienza; ²⁷Ospedale Ferrarotto; ²⁸Ospedale Pediatrico Bambino Gesù; ²⁹Studio Trials Clinici GITMO, Italy

Introduction: The effect of allelic incompatibility and the identification of "permissive" and "non permissive" HLA mismatching (mm) in the unrelated allogeneic hematopoietic stem cell transplant (HSCT) from a

volunteer donor are still controversial. Aims of this prospective observational Italian analysis was to elucidate the impact of allelic HLA mm on unrelated HSCT and to identify “permissive” and “non permissive” I and II class HLA mm loci.

Methods: From January 2012 to December 2015, 1789 pts with haematological malignancies received unrelated HSCT in most cases for AML (56%) in early phase (47%). Donor/Recipient (D/R) age was 29 (18-57) and 49 (18-70) years. As conditioning regimen, GVHD prophylaxis and stem cell source 71% of pts received myeloablative conditioning, 76% anti-Thymo, CSA and MTX and 80% PBSC. Median follow up for survivors was 38 months (1-76). Regarding to the matching, 50% of pairs were 10/10, 38% showed 1 mm for A, B, C, DRB1 or DQB1 in 249, 141, 173, 2 and 112 cases, respectively and 12% was 8/10 HLA matched or less.

Results: Overall 90% and 79% of pts achieved PMN and PLTS engraftment within 30 and 90 days, respectively. The probabilities of 3yrOS, 3yrPFS and 3yrGRFS were 52%, 42%, and 30%. The 3yrTRM was 26%, the CI of aGVHD >2 was 26% and the 3-yr CI of extensive cGVHD was 10%. Multivariate analysis showed that, compared to 10/10, 9/10 and ≤8/10 HLA-matched were associated with worse outcomes in terms of OS (p=0.04/p=0.007), GRFS (p=0.005/p=0.07), TRM (p=0.007/p<0.0001), grade 3-4aGVHD (p=0.0001/p=0.01) and cGVHD (p=0.005/p=0.35). In the pairs with a single HLA mm with a frequency > 5%, the presence of A02:01 in the pts showed a significant higher risk of TRM (p= 0.03) and worst OS (p=0.04). Age of pts > 49 years (p<0.0001), advanced disease (p<0.0001), HCT-CI >1 (p=0.01) were related to a hazard risk of 1.4, 2, 1.2 for OS and 1.6, 1.75, 1.4 for TRM. The Italian D/R origin resulted in minor grade 2-4 aGVHD (p=0.001) and cGVHD (p=0.002).

Conclusions: 10/10 HLA matching remains a significantly favourable prognostic factor for OS, TRM, GRFS and acute/chronic GVHD but there are no significant differences between 8/10 and 9/10 matching unrelated transplants. HLA A02:01 as single mm seems to play a “non permissive” role. The Italian D/R origin is related to minor development of GVHD probably due to the matching of the extended MHC haplotypes in individuals of the same origin.

C030

OUTCOME OF ALLOGENEIC-HSCT IN 441 ADULT PATIENTS WITH PH-POSITIVE-ALL IN THE ERA OF TKI: A RETROSPECTIVE ANALYSIS OF THE ITALIAN BLOOD AND MARROW TRANSPLANTATION SOCIETY (GITMO)

A. Candoni¹, A. Rambaldi², R. Fanin¹, A. Velardi³, W. Arcese⁴, F. Ciceri⁵, D. Lazzarotto¹, F. Lussana⁶, J. Olivieri¹, G. Grillo⁷, M. Parma⁸, B. Bruno^{9,10}, F. Sora¹¹, P. Bernasconi¹², R. Saccardi¹³, R. Foà¹⁴, M. Sessa¹⁵, P. Bresciani¹⁶, F. Giglio⁵, A. Picardi⁴, A. Busca¹⁰, S. Sica¹¹, E. Diral⁸, A.A. Colombo¹², S. Tringali¹⁷, S. Santarone¹⁸, G. Irrera¹⁹, S. Mancini²⁰, F. Zallio²¹, M. Malagola²², F. Albano²³, A.M. Carella²⁴, A. Olivieri²⁵, C. Tecchio²⁶, A. Dominiotto²⁷, A. Vacca²⁸, R. Sorasio²⁹, E. Orciuolo³⁰, A.M. Risitano³¹, A. Cortelezzi³², S. Mammoliti³³, E. Oldani³⁴, F. Bonifazi¹⁵, on behalf of GITMO

¹University of Udine, Santa Maria Misericordia Hospital, Hematology and SCT Unit, Udine, Italy; ²Università degli Studi di Milano and Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Oncology-Hematology, Bergamo, Italy; ³University of Perugia, Hematology and Clinical Immunology, Perugia, Italy; ⁴University 'Tor Vergata', Rome Transplant Network, Rome, Italy; ⁵University Vita-Salute San Raffaele, Hematology and BMT Unit San Raffaele Scientific Institute, Milano, Italy; ⁶Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Oncology-Hematology, Bergamo, Italy; ⁷Ospedale Niguarda Ca' Granda, Hematology Oncology, Milano, Italy; ⁸Ospedale San Gerardo, Clinica Ematologica e Centro Trapianto di Midollo, Monza, Italy; ⁹Università di Torino, Dip. di Biotecnologie Molecolari e Scienze per la Salute, Torino, Italy; ¹⁰Presidio Molinette - AOU Città della Salute e della Scienza, Trapianto Allogeneico di Cellule Staminali, Dip. Oncologia, Torino, Italy; ¹¹Fondazione Policlinico Universitario 'A. Gemelli', Istituto di Ematologia, Roma, Italy; ¹²Fondazione IRCCS Policlinico San Matteo, Centro Trapianti SC Ematologia, Pavia, Italy; ¹³Careggi University Hospital, Centro Trapianti di Midollo Osseo, Firenze, Italy; ¹⁴Sapienza University, Policlinico 'Umberto I', Cellular Biotechnologies and Hematology, Roma, Italy; ¹⁵University Hospital S. Orsola-Malpighi, Institute of Hematology 'L. and A. Seragnoli', Bologna, Italy; ¹⁶University of Modena and Reggio Emilia, Section of Hematology, Modena, Italy; ¹⁷AOR Villa Sofia-Cervello, UOS Trapianto di Midollo, Palermo, Italy;

¹⁸Ospedale Civile, Dip. di Ematologia, Medicina Trasfusionale e Biotecnologie, Pescara, Italy; ¹⁹Grande Ospedale Metropolitano 'Bianchi-Melacrino-Morelli', Centro Unico Regionale Trapianto Cellule Staminali e Terapie Cellulari 'A. Neri', Reggio Calabria, Italy; ²⁰Azienda Ospedaliera San Camillo Forlanini, Centro Trapianti di Cellule Staminali, Roma, Italy; ²¹Azienda Ospedaliera S. Antonio e Biagio, Unità Trapianti Midollo, Alessandria, Italy; ²²University of Brescia, ASST-Spedali Civili, University of Brescia, Unit of Blood Diseases and Stem Cell Transplantation, Brescia, Italy; ²³University of Bari, Hematology and Bone Marrow Transplantation, Bari, Italy; ²⁴Fondazione IRCCS Casa Sollievo della Sofferenza, UO Ematologia e Centro Trapianti TMO, San Giovanni Rotondo, Italy; ²⁵University of Marche-Ospedali Riuniti, Hematology and Bone Marrow Transplantation, Ancona, Italy; ²⁶University of Verona, Haematology - Bone Marrow Transplant Unit, Verona, Italy; ²⁷IRCCS Ospedale Policlinico San Martino, U.O. Ematologia e Centro Trapianti, Genova, Italy; ²⁸P.O. 'Roberto Binaghi', U.O. Ematologia-CTMO, Cagliari, Italy; ²⁹Azienda Ospedaliera S. Croce e Carle, SC Ematologia, Cuneo, Italy; ³⁰Azienda Ospedaliero Universitaria Pisana, UO Ematologia, Pisa, Italy; ³¹Università di Napoli, Clinical Medicine and Surgery-BMT Unit, Napoli, Italy; ³²Università degli Studi e Ospedale Maggiore Policlinico di Milano, Milano, Italy; ³³GITMO Clinical Trial Co-Ordinator, Genova, Italy; ³⁴Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Oncology-Hematology, Bergamo, Italy

Background: We conducted a retrospective, nationwide analysis to describe the clinical outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) undergoing allogeneic hematopoietic stem cell transplantation (HSCT) after being treated with a TKI based therapy.

Methods: A total of 441 patients were included. The median age at HSCT was 44 (range: 18-70). All 441 patients (100%) received TKI before HSCT (performed between 2005-2016); 404 (92%) were in cytologic complete remission (CR) while 37(8%) had an active disease at the time of HSCT. Molecularly Measurable Residual Disease (MRD) was negative in 147 patients (36%) at the time of HSCT. The donor was unrelated in 46% of cases. The prevalent source of SC was peripheral blood(70%). The conditioning regimen was myeloablative in 82% of cases (TBI-based in 50%) and included ATG in 51% of cases.

Results: With a median follow-up after HSCT of 39.4 months the probability of Overall Survival (OS) at 1, 2 and 5 years was 69.6%, 61.1%, and 50.3%, respectively, with a median OS of 62 months. Progression Free Survival (PFS) at 1, 2 and 5 years was 60.2%, 52.1% and 43.7%, respectively. OS and PFS were significantly better in patients with CR and MRD-negativity at the time of transplant compared to patients with CR but MRD-positive (50% OS not reached vs 36 months, p=0,015; 50% PFS not reached vs 26 months, p=0.003). The cumulative incidence of relapse (CIR) at 5 years was significantly lower in patients with CR and MRD-negativity (19.5% vs 35.4%, p=0.001). The non relapse mortality (NRM) after 2, and 5 years was 20.7% (95%CI: 17-24.7) and 24.1% (95%CI: 20-28.5), respectively. The subgroup of patients with MRD-negative both at HSCT and at 3rd month after HSCT had a better outcome (5 year OS 70%). Conversely, the 37 patients who underwent HSCT with active Ph+ALL had a median OS and PFS of 7 and 5 months.

Conclusions: The median OS of all patients with Ph+ALL, treated with TKI based therapy and allografted in recent years at the GITMO Centers, is 62 months. The outcome of Ph+ALL patients undergoing HSCT after TKI therapy has improved (with a 2 yrs NRM of 20,7%), particularly for those achieving a molecular remission before transplant (50% OS and PFS not reached). HSCT still remains a standard of care consolidation treatment for Ph+ALL and only prospective randomized trials can suggest a survival benefit of non transplant based treatment strategies. [ClinicalTrials. NCT03821727].

Chronic Myeloid Leukemia

C031

LONG-TERM MORTALITY RATE FOR CARDIOVASCULAR DISEASE IN 656 CHRONIC MYELOID LEUKAEMIA PATIENTS TREATED WITH SECOND- AND THIRD-GENERATION TYROSINE KINASE INHIBITORS

G. Caocci, O. Mulas, M. Annunziata, L. Luciano, E. Abruzzese, M. Bonifacio, E.M. Orlandi, F. Albano, S. Galimberti, A. Iurlo, P. Pregno, N. Sgherza, B. Martino, G. Binotto, F. Castagnetti, A. Gozzini, M. Bocchia, C. Fozza, F. Stagno, M.P. Simula, F. De Gregorio, M. Trawinska, L. Scaffidi, C. Elena, I. Attolico, C. Barate, D. Cattaneo, F. Pirillo, G. Gugliotta, A. Sicuranza, M. Molica, G. La Nasa, R. Foà, M. Breccia

Department of Medical Sciences and Public Health University of Cagliari Businco Hospital, Department of Medical Sciences and Public Health University of Cagliari Businco Hospital, Haematology Unit Cardarelli Hospital Haematology Unit "Federico II" University of Naples, Haematology Unit Sant'Eugenio Hospital Tor Vergata University; Department of Medicine Section of Hematology University of Verona; Haematology Unit Fondazione IRCCS Policlinico S. Matteo University Hospital; Haematology and Transplants Unit University of Bari; Department of Clinical and Experimental Medicine Section of Hematology University of Pisa; Haematology Unit Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico; Haematology Unit Azienda Ospedaliera Universitaria Città della Salute e della Scienza, Haematology and Transplant Center Casa Sollievo della Sofferenza Hospital San Giovanni Rotondo, Haematology Unit Azienda Ospedaliera Bianchi-Melacrino-Morelli; Haematology Unit University of Padova; Department of Experimental Diagnostic and Specialty Medicine S. Orsola-Malpighi Hospital University of Bologna; Haematology Unit AOU Careggi University of Florence; Haematology Unit Azienda Ospedaliera Universitaria Senese University of Siena; Department of Clinical and Experimental Medicine University of Sassari; Haematology Unit Ferrarotto Hospital; Department of Medical Sciences and Public Health University of Cagliari Businco Hospital, Haematology Unit "Federico II" University of Naples; Haematology Unit Sant'Eugenio Hospital Tor Vergata University; Department of Medicine Section of Hematology University of Verona; Haematology Unit Fondazione IRCCS Policlinico S. Matteo University Hospital; Haematology and Transplants Unit University of Bari; Department of Clinical and Experimental Medicine Section of Hematology University of Pisa, Haematology Unit Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico; Haematology Unit Azienda Ospedaliera Universitaria Città della Salute e della Scienza, Department of Experimental Diagnostic and Specialty Medicine S. Orsola-Malpighi Hospital University of Bologna; Haematology Unit Azienda Ospedaliera Universitaria Senese University of Siena; Haematology Unit Ferrarotto Hospital, Division of Haematology Department of Cellular Biotechnologies and Haematology Policlinico Umberto I Sapienza University; Department of Medical Sciences and Public Health University of Cagliari Businco Hospital; Division of Haematology Department of Cellular Biotechnologies and Haematology Policlinico Umberto I Sapienza University; Division of Haematology Department of Cellular Biotechnologies and Haematology Policlinico Umberto I Sapienza University, Italy

Introduction: Cardiovascular (CV) diseases represent the leading cause of premature death, responsible for 35% of deaths under 75 years and 29% of deaths under 65 years, being ischemic heart disease (IHD) the leading single cause. Limited information is available regarding the rate of long-term CV mortality in chronic myeloid leukemia (CML) patients treated in the real-life practice with second- and third-generation tyrosine kinase inhibitors (2ndG/3rdG TKIs) nilotinib, dasatinib, bosutinib and ponatinib. The primary endpoint of this study was to establish the incidence of mortality related to CV adverse events and the PYLL parameter in a Italian cohort of CML patients. The secondary endpoint was to evaluate the standardized mortality ratio (SMR) following IHD.

Methods: We considered 656 adult CP-CML patients diagnosed and treated consecutively with 2ndG/3rdG TKI, frontline or with subsequent lines of treatment in 19 Italian centres, between 2012 and 2017. The CV-free survival was estimated from diagnosis to the date of death occurred for CV complications. PYLL to CV disease provided a measure of premature mortality and was calculated by summing-up deaths occurring at each age and multiplying that result by the number of the remaining years up to the selected age limit of 75 years. The SMR was used to compare the mortality risk following IHD of the cohort of CML

patients to that of the Italian population. An SMR greater than 1.0 indicates that there were "excess deaths" compared to what was expected

Results: Overall 37 deaths were recorded. The 15-year OS was $83.3 \pm 3.6\%$. No significant difference in OS was observed according to the TKI administered. The 15-year CV-mortality free survival was $93 \pm 2.8\%$ (Figure 1). Age ≥ 65 years ($p=0.005$) and a positive history of CV disease ($p=0.04$) were significantly associated to a lower CV-mortality free survival. CV disease accounted 16.5% and 5% of potential years of life lost (PYLL) in male and female patients, in comparison with 18% and 12% of Italian population, respectively. The standard mortality ratio (SMR) following ischemic heart disease (IHD) was 3.9 in males and 3.8 in female patients with excess deaths observed in comparison with the population of control.

Conclusions: Although life expectancy in CML is close to that observed in the general population, our data are a reminder that IHD remains an important cause of death in CML patients treated with 2ndG/3rdG TKIs. These findings emphasize the need to personalize prevention strategies based on CV risk.

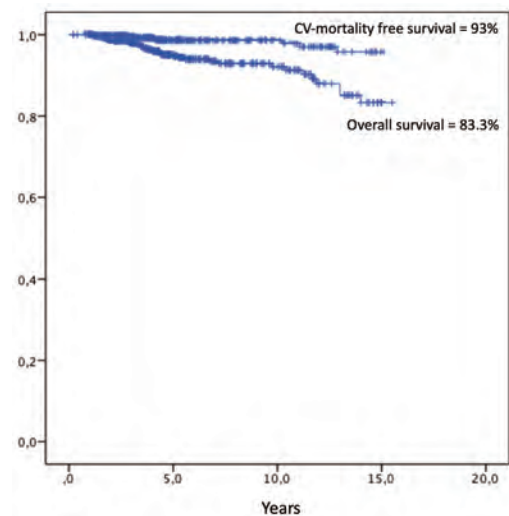


Figure 1.

C032

SWITCH TO GENERIC IMATINIB: IMPACT ON MOLECULAR RESPONSES AND SAFETY IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

E. Scalzulli, M. Porrazzo, F. Stocchi, G. Colafigli, M. Molica, D. Diverio, R. Latagliata, R. Foà, M. Breccia

Hematology, Department of Translational and Precision Medicine, Policlinico Umberto 1, Sapienza University, Rome, Italy

Background: Since July 2017, generic imatinib has been introduced in Italy for the treatment of patients affected by chronic myeloid leukemia (CML). The switch from branded to generic imatinib has been investigated in terms of efficacy and safety but with contrasting results.

Aim: We analyzed 168 chronic phase CML patients treated with branded imatinib for a median time of 12 years (range: 1-16) at a single institution who switched to one generic formulation (imatinib Accord) in order to assess the safety and impact on molecular responses.

Results: Male and female patients were respectively 58% and 42%, with a median age of 52 years (range 18-82). Sokal risk was low/intermediate/high in 63%, 33% and 4% of cases, respectively, and 25% of patients had received a previous therapy (7% only hydroxyurea, 66% interferon, 27% hydroxyurea and interferon). Median duration of generic imatinib treatment was 19 months (range 4-22). Twenty-seven % of patients were in MR3 and 73% in deep molecular responses (MR4-4.5) at the time of switch. After 12 months of treatment with generic imatinib, 140 patients were evaluable for response: 33/140 (23.6%) and 107/140 (76.4%) were respectively in MR3 and deep molecular response. When the degree of response was compared to the best molecular response observed with branded imatinib, it was found that 85% of patients maintained stability of response, 6.4% improved and 10% of patients had a molecular fluctuation from deep molecular

response to MR3. Only 1 patient lost the MR3 and no patient switched to another TKI for inefficacy. In terms of safety, 34/168 (20%) of patients reported new or worsening side effects, (15% grade 3, 74% grade 2, 11% grade 1 according to CTCAE). The most frequent were dyspepsia in 32.3% of patients (11/34), muscle cramps in 25% (8/34 patients), conjunctival hyperemia in 18% (6/34), diarrhea in 15% (5/34), skin rash in 9% (3/34), arthralgia in 9% (3/34) and elevated creatinine levels in 3% (1/34). Only 2 patients reversed to branded imatinib due to toxicity (1 developed gynecomastia and salivary glands hypertrophy and the other due to persistent gastrointestinal symptoms). Overall, 18 patients (11%) discontinued generic imatinib treatment for intolerance (n=4) or treatment-free remission attempt (n=14).

Conclusions: Our data showed that the switch to generic imatinib in patients who have been previously treated with branded imatinib appears to maintain efficacy, although a proportion of patients experienced new or worsened side effects.

C033

PREDICTIVITY OF EARLY MOLECULAR RESPONSE LEVELS AND BCR-ABL TRANSCRIPT TYPE ON STABLE DMR ACHIEVEMENT IN CML PATIENTS TREATED WITH IMATINIB

M. Tiribelli¹, M. Bonifacio², G. Binotto³, D. Griguolo⁴, L. Scaffidi², L. Frison³, M.C. Miggiano⁴, E. Calistri⁵, M. Stulle⁶, E. De Biasi⁷, R. Sartori⁸, R. Stella¹, I. Tanasi², M. Ruggeri⁴, D. Damiani¹, G. Semenzato³, M. Krampera², R. Fanin¹

¹Division of Hematology and Bone Marrow Transplantation, Department of Medical Area, University of Udine; ²Department of Medicine, Section of Hematology, University of Verona; ³Department of Medicine, Hematology Section, Padua University School of Medicine; ⁴Hematology Department, San Bortolo Hospital; ⁵Hematology Unit, Ca' Foncello Hospital; ⁶Division of Hematology, Azienda Sanitaria Universitaria Integrata di Trieste; ⁷Hematology Unit, Camporampiero Hospital; ⁸Hematology Department, Castelfranco Veneto Regional Hospital, Italy

Stable deep molecular response (sDMR), is considered a prerequisite for treatment discontinuation in chronic myeloid leukemia (CML) patients (pts) treated with tyrosine kinase inhibitors (TKIs). Depth and kinetics of MR during the first year of therapy correlate with the probability of sDMR, but the impact of BCR-ABL transcript type across different levels of early MR on subsequent sDMR have not yet been reported. We retrospectively analysed 185 chronic phase CML pts (male=109; median age at diagnosis: 60 yrs, range 20-85; Sokal score: low=74, intermediate=85, high=25, unknown=1) treated frontline with imatinib 400 mg daily. MR was defined as BCR-ABL^{IS} ratio >1%-≤10% (MR1), >0.1%-≤1% (MR2), >0.01%-≤0.1% (MR3) and ≤0.01% (MR4 or better). DMR was defined as MR4 or better, sDMR as DMR lasting ≥2 years and at least a Q-PCR every 6 months. Frequencies were compared by Fisher's exact test. Univariate and multivariate regression analysis were performed using the competing risk model of Fine and Gray. Death and switch to other TKIs for resistance to imatinib were considered competing events. BCR-ABL transcripts were b2a2 (n=74, 40%), b3a2 (n=89, 48%), or b2a2/b3a2 (n=22, 12%). Depth of MR at 3 months was: no EMR=51 (28%), MR1=67 (36%), MR2=56 (30%), MR3=11 (6%). At 6 months pts with no MR, MR1, MR2, MR3, and MR4 were 36 (19%), 44 (24%), 53 (29%), 39 (21%) and 13 (7%), respectively. After a median imatinib duration of 50 months (range 3-165), 86 (46%) pts never achieved DMR, 33 (18%) had an unstable DMR and 66 (36%) reached a sDMR. Pts with MR2 or MR3 at 3 months had a 5-yrs cumulative incidence of sDMR of 70.0% (95%CI: 54-80.5%), superior to those with MR1-no MR (p=0.006) Similarly, 5-yrs cumulative incidence of sDMR was significantly different across level of MR at 6 months, ranging from 13.4% (95%CI 0-29.4%) for MR1 to 78.6% (95%CI 56.1-89.6%) for MR3 or better (p<0.001). Pts with b3a2 or b3a2/b2a2 had a higher probability of sDMR compared to those with b2a2 (60.3% vs 44.6%, p=0.018). Considering transcript type across different depth levels of MR, we found that among pts with MR1 at 3 months, those with b3a2 had a significantly higher probability of sDMR (39.5% vs 16.7%, p=0.029).

In conclusion, pts with very good MR after 3 or 6 months of imatinib, defined as BCR-ABL ≤1% and ≤0.1% respectively, have a high probability of achieving a sDMR and that, among pts with BCR-ABL ≤10% but >1% at 3 months, b3a2 transcript identifies pts with a higher possibility of sDMR.

C034

AURORA KINASE A/MDM2-MEDIATED SETD2 LOSS OF FUNCTION IN CHRONIC MYELOID LEUKEMIA PATIENTS IN BLAST CRISIS INDUCES GENETIC INSTABILITY AND CAN BE THERAPEUTICALLY TARGETED

M. Mancini, S. De Santis, C. Monaldi, L. Bavaro, M. Martelli, F. Castagnetti, G. Gugliotta, G. Rosti, A. Iurlo, E. Abruzzese, M. Salvucci, P. Pregnò, A. Gozzini, M. Crugnola, F. Albano, M. Bonifacio, E. Calistri, M. Tiribelli, G. Binotto, A. Imovilli, E. Trabacchi, S. Galimberti, C. Barate, E. Tenti, M. Baccarani, G. Martinelli, M. Cavo, S. Soverini

Department of Experimental, Diagnostic and Specialty Medicine - DIMES, Institute of Hematology "L. and A. Seràgnoli", Bologna, Italy; UO Onco-ematologia, Fondazione IRCCS Ca' Granda - Ospedale Policlinico, Milano, Italy; S. Eugenio Hospital, Tor Vergata University, Rome, Italy; Azienda Azienda USL di Romagna, Ravenna, Italy; Ospedale Universitario Molinette San Giovanni Battista, Torino, Italy; Hematology, Firenze, Italy; Azienda Ospedaliero-Universitaria di Parma, Italy; Dipartimento dell'Emergenza e dei Trapianti di organi (DETO), Università di Bari, Italy; Department of Medicine, Section of Hematology, University of Verona, Italy; Hematology Unit, Ca' Foncello Hospital, Treviso, Italy; Division of Hematology and BMT, Department of Experimental and Clinical Medical Sciences, Azienda Ospedaliero-Universitaria di Udine, Udine, Italy; Padua School of Medicine, Department of Medicine, Hematology and Clinical Immunology, Padova, Italy; Arcispedale S. Maria Nuova, Azienda Ospedaliera di Reggio Emilia, Reggio Emilia, Italy; Azienda Unità Sanitaria Locale, Piacenza, Italy; Clinical and Experimental Medicine, Hematology, University of Pisa, Italy; UO Ematologia - AOU Pisana, Pisa, Italy; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola, Italy

Inactivating mutations in the SETD2 tumor suppressor occur in solid tumors and acute leukemias. SETD2 trimethylates histone H3 Lysine 36 (H3K36Me3) playing a key role in transcription, splicing, homologous recombination (HR), mismatch repair. In Systemic Mastocytosis, we have demonstrated that SETD2 loss of function occurs at the post-translational level. Reduced or null SETD2 and H3K36Me3 was detected in 83/96 (86%) pts with blast crisis (BC) CML as compared to a pool of healthy donors and to chronic phase (CP) pts at diagnosis. Proteasome inhibition in primary cells from pts with undetectable SETD2 restored H3K36Me3 and led to accumulation of hyper-ubiquitinated SETD2. Moreover, it induced apoptosis and reduced clonogenic growth. In K562 cells (SETD2/H3K36Me3^{low}), co-immunoprecipitation (co-IP) performed before and after proteasome inhibition showed that SETD2 interacts with MDM2 and, as a result, it is hyper-ubiquitinated. MDM2 inhibition by SP-141 resulted in cytostatic effects and rescued SETD2 expression and activity. The latter was also achieved by siRNA-mediated silencing of MDM2, suggesting that MDM2 is implicated in SETD2 reduced stability. Co-IP also showed that SETD2 interacts with Aurora Kinase A (AKA) a S/T kinase frequently overexpressed in CML. We found that AKA phosphorylates SETD2, and both pharmacological inhibition by Danusertib and siRNA-mediated knock-down rescued SETD2 expression and activity. To investigate whether SETD2/H3K36Me3 loss contribute to genetic instability, K562 and LAMA 84 (SETD2/H3K36Me3^{high}) cells were studied by Western blotting and immunofluorescence to assess H2AX and Rad51 in steady state and after sub-lethal DNA damage by UV exposure. The same studies were performed after siRNA silencing of SETD2 for 3 months. Cells with low or silenced SETD2 had significantly higher levels of H2AX and were unable to induce HR repair. Clonogenic assays in LAMA 84 before and after SETD2 silencing showed that reduction of clonogenic growth after proteasomal or MDM2 inhibition is indeed SETD2-dependent. Our results were confirmed in cells from both CP (n=2) and BC (n=4) CML pts showing different levels of SETD2 expression and activity. Phosphorylation by AKA and ubiquitination by MDM2 contribute to SETD2 non-genomic loss of function in BC CML. Loss of SETD2/H3K36Me3 results in increased DNA damage and impaired HR repair. Restoring physiological H3K36Me3 may help improve the outcome of this critical subset of pts.

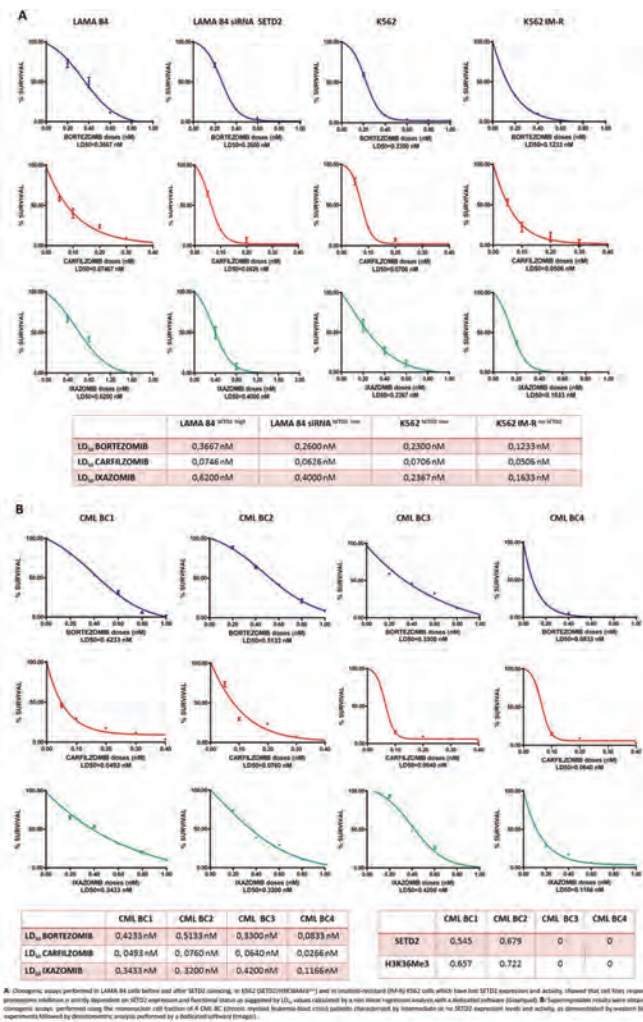


Figure 1.

C035
PRO-ATHEROTHROMBOTIC EFFECT INDUCED BY TYROSINE-KINASE INHIBITORS FOR FIRST-LINE TREATMENT OF CML PATIENTS: RESULTS OF A PROSPECTIVE MULTICENTER STUDY (KIARO TRIAL)

A. Sicuranza, C. Zuanelli Brambilla, I. Ferrigno, E. Abruzzese, A. Iurlo, D. Cattaneo, S. Galimberti, A. Gozzini, F. Stagno, A. Russo Rossi, N. Sgherza, L. Luciano, C. Bucelli, F. Di Martino, D. Tocci, S. Ciofini, M. Bocchia

Department of Medicine Surgery and Neuroscience, Hematology Unit; Department of Medical Biotechnologies, University of Siena, Siena; S.Eugenio University Hospital, Rome; Hematology Division, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; Department of Hematology, Clinical and Experimental Medicine, University of Pisa, Pisa; Hematology Unit, Careggi University Hospital, Florence; Department of Clinical and Molecular Biomedicine (MEDBIO), Ferrarotto Hospital, AOU Policlinico - V. Emanuele, University of Catania, Catania; Hematology and Bone Marrow Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari; Division of Hematology, IRCCS Ospedale Casa Sollievo Sofferenza, San Giovanni Rotondo; Hematology, Federico II University, Naples, Italy

Tyrosine kinase inhibitors (TKI) revolutionized treatment of chronic myeloid leukemia (CML), but some patients (pts) still suffer from potentially severe TKI-related toxicity. Of relevance is cardiovascular (CV) toxicity, mostly associated to Nilotinib (NILO). Previously, we retrospectively confirmed a higher CV risk for pts NILO-treated, particularly if harboring the unfavorable OLR1 polymorphism and we found a NILO-associated pro-inflammatory effect. We now report clinical, ge-

netic and serologic results of a prospective multicenter Italian trial including CML pts treated with first-line TKIs (IMA, NILO, DASA). Aims of the study are: to assess CV toxicity in CML pts in which first-line TKI choice was made according to CV risk guidelines; to assess pts' genetic predisposition to CV events; to confirm a pro-inflammatory effect NILO-induced. Clinical CV risk Score (S) and Integrated Score (IS), obtained combining clinical and genetic data from Single Nucleotide Polymorphisms (SNPs) associated to CV risk, were calculated. Clinical and biological parameters and plasma samples for IL6, IL10, TNF and oxLDL assessments, were collected at diagnosis and at 1, 3, 6, 12 months (mos) of treatment. 177 naive CML pts starting IMA, NILO or DASA treatment, were enrolled. After a median follow-up of 22.4 mos (range 35 days-5.4 years), a CV event occurred in 5 pts, with a median time to event of 15 mos (range 44 days-31.4 months). NILO treated pts had lower CV risk at diagnosis (S=6.9±2.8) compared to DASA (S=8.1±3.2; p=0.042) or IMA-treated (S=9.9±4.9; p=0.019). 3/57 (5.2%) NILO pts developed CV toxicity, as opposed to 1/83 (1.2%) IMA-treated (HR 2.9, 95% CI 1.8-4.4; p=0.021) and 0 out of 37 DASA-treated pts. A CV event occurred in a pt during IMA 23 mos after being switched to Bosutinib, thus was not attributed to IMA. IL10 levels were higher after 6 and 12 mos of IMA or DASA compared to NILO. Also IL6/IL10 and TNF /IL10 ratios in IMA and DASA treated pts were lower, compared to NILO. OxLDL levels increased after 12 mos of NILO, not during IMA or DASA. This study confirms a higher CV risk for pts treated with NILO, even in a contemporary setting where pts were carefully selected for having a low pre-treatment CV risk. This may be a consequence of the NILO-induced pro-inflammatory status confirmed in this study. The few CV events registered did not allow any correlation between SNPs and increased CV risk; a longer follow-up is necessary to explore a possible genetic role.

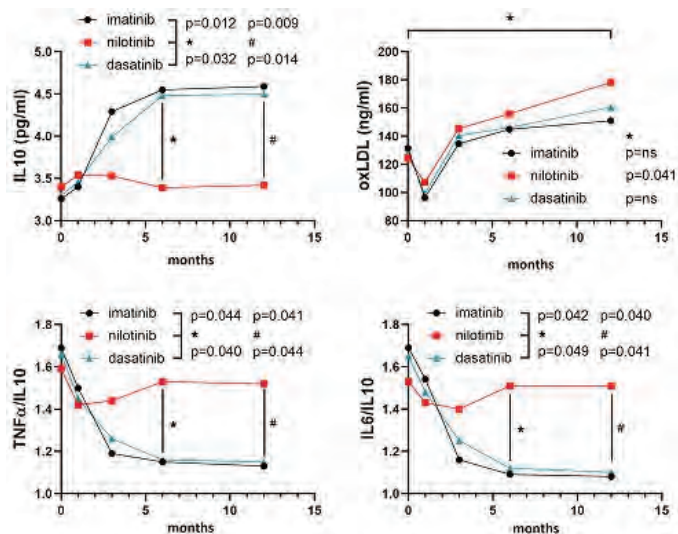


Figure 1.

Anemia and Thalassemia

C036

RAVLIZUMAB VERSUS ECULIZUMAB IN ADULTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: A PROSPECTIVE ANALYSIS ON THE DEEPNESS OF C5 INHIBITION BASED ON PHARMACOKINETICS/PHARMACODYNAMICS AND BREAKTHROUGH HEMOLYSIS OBSERVED IN TWO PHASE 3 RANDOMIZED, MULTICENTER STUDIES

A.M. Risitano, R. Peffault de Latour, S.T. Rottinghaus, P. Hillmen, S. Ortiz, J. Szer, H. Schrezenmeier, R. Aguzzi, A. Hill, J.W. Lee, A.G. Kulasekararaj, R.A. Brodsky

Department of Clinical Medicine and Surgery, Bone Marrow Transplantation Program, Federico II University, Naples, Italy; Hôpital Saint-Louis, Paris, France; Alexion Pharmaceuticals, Inc, Boston, MA; St James's University Hospital, Leeds, United Kingdom; Clinical Haematology, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, VIC, Australia; Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg-Hessen and University Hospital Ulm, Ulm, Germany; Department of Hematology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea; Department of Haematological Medicine, King's College Hospital, National Institute for Health Research and Wellcome King's Clinical Research Facility, London, United Kingdom; Division of Hematology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Background: In two large, phase 3 clinical studies, ravulizumab (RAV), a new C5 inhibitor administered i.v. every 8 weeks, achieved non-inferiority in all primary and key secondary endpoints to eculizumab (ECU) in complement inhibitor-naïve and -experienced patients (pts) with PNH. The objective of this analysis was to characterize RAV PK and PD, and the return of hemolytic disease activity during anti-C5 treatment (breakthrough hemolysis, BTH).

Methods: Pts in study 301 (NCT02946463; Blood 2019;133:530) were naïve to C5 inhibitor therapy; pts in study 302 (NCT03056040; Blood 2019;133:540) were stable on ECU treatment (900mg q2w) for ≥ 6 months. Pts received weight-based dosing of RAV q8w or the approved ECU dose (900 mg q2w) for 183 days. PK/PD outcomes included serum drug concentrations, total and free C5 levels. BTH was defined as ≥ 1 new or worsening symptom/sign of intravascular hemolysis in the presence of LDH ≥ 2 times the ULN.

Results: In study 301, 246 pts received RAV (n=125) or ECU (n=121); in study 302, 195 pts received RAV (n=97) or ECU (n=98). Complete suppression of free C5 (serum concentrations < 0.5 g/mL) was attained by the end of first RAV infusion and was sustained throughout the entire 183-day treatment period for all pts at all time points in both studies. In contrast, mean free C5 concentrations did not consistently remain < 0.5 g/mL with ECU in either study. In studies 301 and 302, 15 (12.4%) and 7 (7.1%) ECU-treated pts experienced ≥ 1 individual post-baseline serum free C5 level ≥ 0.5 g/mL over the 183-day treatment period. RAV was associated with fewer episodes of BTH than ECU in both studies. In study 301, 5 (4.0%) vs 13 pts (10.7%) in RAV and ECU groups experienced BTH, respectively. BTH events temporally associated with elevations in free C5 (C5 ≥ 0.5 g/mL) were 0/5 vs 7/15, whereas those temporally associated with infections were 4/5 and 6/15 (including 2 with high free C5) in RAV and ECU groups, respectively. In study 302, BTH events were observed in 5 ECU pts; four of 7 BTH events were temporally associated with high free C5 and 3/7 with infection.

Conclusions: RAV, while shown to be non-inferior to ECU, was associated with consistent free C5 suppression below the free C5 threshold in all pts whereas ECU was less consistent. These results support the concept that RAV treatment resulted in more effective C5 inhibition than ECU, eventually leading to a decreased rate of BTH as compared to ECU by eliminating free C5-associated BTH.

C037

ABSTRACT WITHDRAWN

C038

PROGNOSTIC AND PREDICTIVE IMPACT OF SMALL PNH CLONES IN A LARGE COHORT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA: A SINGLE-CENTER EXPERIENCE

B. Fattizzo, A. Dunlop, G. Levati, D. Consonni, G. Mufti, J. Marsh, W. Barcellini, A. Kulasekararaj

Hematology, King's College Hospital; Hematology, Fondazione IRCCS Ospedale Maggiore Policlinico di Milano; Epidemiology, Fondazione IRCCS Ospedale Maggiore Policlinico di Milano, Italy

The use of high sensitive FLAER improved the detection of very small PNH clones ($< 1\%$) in aplastic anemia (AA)/myelodysplastic syndromes (MDS). Here we aimed to evaluate the prevalence of PNH clones $> 0.01\%$ in AA/MDS patients tested at a single tertiary center, and to assess their impact on disease prognosis, occurrence of thrombosis, and response to current therapies. We retrospectively collected clinical and laboratory features of 869 MDS and 531 AA patients tested from March 1998 till October 2017.

Table 1.

	MDS		p	AA		p
	PNH- N=693	PNH+ N=176		PNH- N=204	PNH+ N=327	
Males	424 (61.2)	91 (51.7)	0.02	107 (52.5)	157 (51.1)	NS
Females	269 (38.7)	85 (48.3)	0.02	97 (47.5)	160 (48.9)	NS
Median age years	63 (11-92)	59 (19-89)	< 0.0004	46 (6-91)	43 (9-7)	NS
Hematologic values	N=693	N=176		N=204	N=327	
Anemia (Hb < 10) N(%)	258 (37.2)	81 (46)	0.04	107 (52.5)	158 (48.3)	NS
Thrombocytopenia (PLT < 100) N(%)	272 (39.2)	106 (60.2)	< 0.0001	6 (2.9)	241 (73.7)	< 0.0001
Neutropenia (ANC < 1500) N(%)	320 (46.17)	94 (53.4)	NS	121 (59.3)	198 (60.5)	NS
Median LDH U/L	193 (79-1864)	233 (133-1520)	< 0.00001	187 (78-748)	223 (70-3102)	< 0.00001
monosomy 7 N(%)	28 (4)	14 (7.5)	0.04	5 (2.5)	8 (2.4)	NS
complex karyotype N(%)	44 (6.3)	6 (3.2)	NS	-	-	-
Treated patients N(%)	365 (52.6%)	108 (61.3%)		161 (78.9)	323 (98.8)	
Chemotherapy N(%)	191 (27)	29 (16)	< 0.0001	-	-	-
CSA N(%)	40 (11)	37 (34)	0.0001	97 (60)	273 (84)	< 0.0001
ATG N(%)	10 (3)	13 (12)	0.0002	72 (45)	245 (76)	< 0.0001
Androgen N(%)	-	-	-	10 (6)	3 (0.9)	0.002
Azacytidine N(%)	123 (34)	19 (18)	0.002	-	-	-
Eltrombopag N(%)	8 (2)	5 (5)	NS	11 (6.8)	23 (7.1)	NS
HSCT N(%)	141 (39)	40 (37)	NS	55 (34)	82 (25)	0.05
Eculizumab N(%)	0 (0)	7 (6)	< 0.0001	0 (0)	48 (14.8)	< 0.0001
Outcome	N=693	N=176		N=204	N=327	
MDS Progression N(%)	34 (4.9)	1 (0.6)	0.003	14 (6.9)	7 (2.1)	0.01
AML evolution N(%)	88 (12.7)	12 (6.8)	0.01	6 (2.9)	6 (1.8)	NS
Thrombosis N(%)	37 (5.3)	16 (9)	0.05	11 (5.4)	21 (6.4)	NS
Death N(%)	308 (44.4)	48 (27.3)	< 0.0001	70 (34.3)	38 (11.6)	< 0.0001
MDS type and IPSS risk	N=693	N=176				
RCMD/RCUD N(%)	271 (39)	55 (31.3)	NS			
RA N(%)	28 (4)	4 (2.3)	NS			
RA del5q N(%)	23 (3.3)	3 (1.7)	NS			
HypoMDS N(%)	90 (13)	67 (38.1)	< 0.0001			
RAEB1/2 N(%)	199 (28.7)	36 (20.5)	0.03			
RARS/RARS-T	34 (4.9)	3 (1.7)	NS			
Other MDS-F N(%)	41 (5.9)	8 (4.60)	NS			
IPSS low N(%)	281 (40.6)	59 (33.5)	NS			
IPSS int1 N(%)	237 (34.2)	85 (48.3)	< 0.0001			
IPSS int2 N(%)	147 (21.2)	22 (12.5)	0.01			
IPSS high N(%)	28 (4)	10 (5.7)	NS			

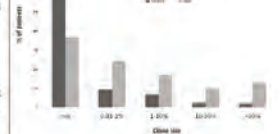


Table 1 shows characteristics of MDS and AA patients, divided according to PNH positivity, which was less frequent in MDS versus AA (20.3% vs 61%). Focusing on MDS, PNH+ cases were significantly more hypoplastic, and showed deeper cytopenias and higher LDH levels. Considering clone size (negative, 0.01-1%, 1.01-10%, 10.01-50%, and $> 50\%$ on granulocytes), we observed a significant worsening of cytopenia and raise of LDH along with clone size increase. As regards therapy, PNH+MDS showed significantly higher response rates to immunosuppressive therapies (ATG and CyA, 84% vs 44.7%, $p=0.01$) and to HSCT (71% vs 56.6%, $p=0.09$) compared to PNH-, and the cumulative probability of response to any treatment significantly improved along with clone size increase (from 52 to 100%, $p=0.03$). PNH+MDS showed lower rate of evolution, and longer OS [mean 11.9+0.7 years (10.5-13.3) vs 7.3+0.3 (6.6-7.9), $p<0.0001$], confirmed in multivariable analysis. However, PNH+MDS had a higher incidence of thrombotic events (from 5% in PNH- to 50% in PNH+ with clone size $> 50\%$, $p<0.0001$). Similarly to MDS, PNH+AA showed deeper thrombocytopenia and higher LDH, and showed higher response rates to any therapy (97 vs 77% for HSCT, $p=0.01$; 78 vs 50% for IST, $p<0.0001$; and

88% vs 65% considering any treatment, $p < 0.0001$). They also showed lower rate of MDS progression and death ($p = 0.01$ and $p < 0.0001$), and longer OS [mean 15.8 ± 0.43 years (14.9-16.7) vs 6.5 ± 0.35 (5.8-7.21), $p < 0.0001$], confirmed in multivariable analysis. Prevalence of PNH clones of any size is high in patients with MDS and AA. We firstly show a positive impact of PNH clone positivity on response to IST and HSCT in a large MDS series and confirmed this data in AA. The presence of a PNH clone also correlated with lower disease progression and better OS. Clone size analysis suggests that even small clones (0.01-1%) have a clinical and prognostic significance.

C039

ANEMIA IN EXTREMELY LOW GESTATIONAL AGE NEONATES: TRANSFUSING ALLOGENEIC UMBILICAL CORD BLOOD RED CELL CONCENTRATES SAVE PHYSIOLOGICAL LEVELS OF FETAL HEMOGLOBIN

L. Teofili, M. Bianchi, C. Pellegrino, P. Pinci, P. Papacci, C. Giannantonio, C.G. Valentini, B.M. Ricerca, A. Molisso, B. Carducci, G. Vento, V. De Stefano

Fondazione Policlinico Universitario A.Gemelli IRCCS, Roma, Italy

Repeated blood transfusions in extremely low gestational age (ELGA) neonates have alleged for an increased risk of prematurity-related diseases (PRD), resulting in lifelong invalidating disabilities. Adult and fetal hemoglobin (HbA and HbF, respectively) deeply differ regarding their oxidative properties, nitric oxide generation, and DNA cleavage susceptibility. It is therefore conceivable that HbA delivered through transfusions might worsen the oxidative damage underlying PRD. Accordingly, Stuchfield et al. observed a link between %HbF levels and the incidence of retinopathy of prematurity, the most frequent PRD (Eye, 2017). We developed a preterm-customized transfusion approach, based on allogeneic umbilical cord blood (UCB). This inexpensive strategy exploits UCB units collected as solidary donations from full-term healthy babies at our UCB Bank. The units with low TNC content are converted to HbF-enriched RBC concentrates (UCB-RBC) for premature babies. We have previously run a pilot study to assess feasibility and safety of this approach (Bianchi et al. 2015; Teofili et al. 2018). We are currently running a clinical prospective study aimed at demonstrating that UCB-RBC may restrain the HbF decrease caused by adult blood transfusions (A-RBC) (NCT03764813). The study has a duration of 12 months, with an anticipated population of 25 neonates. Here, we report the preliminary data (Figure 1, red circles for A-RBC, green circles for UCB-RBC).

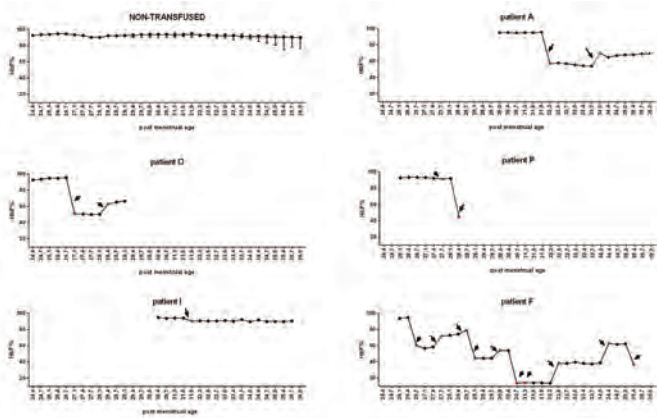


Figure 1.

Overall, 17 ELGA neonates have been enrolled (median postmenstrual age 30.0 weeks, range 24.4 to 30.6; median birth weigh 1110 g, range 550-1545). In total, 17 transfusions have been delivered to 5 patients (9 UCB-RBC and 8 A-RBC). UCB-RBC and A-RBC transfusions elicit in recipients similar hematocrit increase. Moreover, the intervals between transfusions are similar in both products. As expected, each A-RBC transfusion caused a progressive decrease of HbF, which varied according to the burden of the previous transfusions: on average, after

a single transfusion, the HbF valued reduced of 39% (IQR 45-34). Conversely, similar levels of HbF were detected following UCB-RBCs transfusions, as compared to age-matched non-transfused neonates, with median HbF values of 91%, IQR 90-92 and 92%, IQR 91-93, respectively. These data show that UCB-RBCs can be used to prevent the HbF loss in anemic preterm neonates, and prompt future randomized trials evaluating the clinical impact of this transfusion strategy on PRD.

The authors thank Genitin and Olgiate Onlus.

C040

EVIDENCE BASED USE OF ERYTHROPOIETIN IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: A MULTICENTER INTERNATIONAL STUDY

B. Fattizzo, G. Levati, L. Languille, F.R. Mauro, B. Jilma, A. Patriarca, A. Hill, S. Berentsen, M. Michel, W. Barcellin

Ematologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; Department of Internal Medicine, national referral center for adult immune cytopenias, Henri Mondor University Hospital, Assistance Publique Hôpitaux de Paris, Créteil, France; Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy; Medical University of Vienna, Department of Clinical Pharmacology, Vienna, Austria; Haematology Unit, AOU Maggiore della Carità, Novara, Italy; Hematology, Leeds Teaching Hospital, Leeds, UK; Department of Research and Innovation, Haugesund Hospital, Norway

Bone marrow reticulocyte compensation in autoimmune hemolytic anemia (AIHA) is a recently recognized determinant of outcome. Erythropoietin (EPO) has been anecdotally used to ameliorate marrow response, but predictors of outcome are not known. In this study we aimed to evaluate EPO efficacy and its predictors in a cohort of AIHA patients. Data on AIHA cases who had received EPO were retrospectively collected using a preformed survey. Efficacy was evaluated at 15 and 30 days, and then at 3, 6 and 12 months; Hb response was considered partial (PR, > 2 g/dL Hb increase or > 10 g/dL) or complete (CR, > 12 g/dL). 29 cases followed from Jun 2007 to Feb 2019 at 7 centers in Italy, France, Norway, Austria, and UK were included. Main AIHA types (warm, cold, mixed, and DAT negative) were present, and 3 cases were secondary to a lymphoproliferative disorder (1 non-Hodgkin lymphoma, 1 Waldenstrom macroglobulinemia, and 1 chronic lymphocytic leukemia; not active and without specific treatment at the moment of the study).

Table 1.

Age years, median(range)	68 (35-92)
M/F	16/13
CAD, N(%)	12 (41.4)
WAIHA IgG, N(%)	5 (17.2)
WAIHA IgG+C, N(%)	9 (31)
MIXED, N(%)	2 (7)
DAT neg, N(%)	1 (3.4)
Haematologic parameters at diagnosis	
Hb g/L, median(range)	73 (41-118)
LDH U/L, median (range)	468 (193-6000)
Ret $\times 10^9/L$, median(range)	122 (57-310)
BMRI, median(range)	69 (35-193)
EPO, median(range) N=15	35 (8-670)
Previous therapy lines	
steroids, N(%)	22 (76)
rituximab, N(%)	19 (66)
splenectomy, N(%)	2 (7)
immunosuppressor, N(%)	13 (45)
time from diagnosis to EPO days, median (range)	1740 (209-1760)
time on EPO days, median (range)	209 (21-2464)
Haematologic parameters at EPO initiation	
Hb g/L, median(range)	87 (62-109)
LDH U/L, median (range)	338 (193-1030)
Ret $\times 10^9/L$, median(range)	117 (34-310)
BMRI, median(range)	85 (30-222)
EPO, median (range) N=17	27 (9.3-620)
Concomitant therapy, N(%)	20 (69)
Response rates, ORR (%), CR/PR	
day+15, N=29	18(64), 3/15
day+30, N=23	16(69), 4/12
month+3, N=24	17(71), 9/8
month+6, N=15	9(60), 9/0

Patients' characteristics are shown in Table 1: all patients had received at least one previous therapy, and the majority (69%) started EPO because of non-response to ongoing treatment (steroids 15, immunosuppressor 4, sutimlimab 1). 6 patients had received rituximab during the 3 months before EPO start (median 1 month, range 0-5). At EPO initiation, 21% of cases displayed severe anemia, 73% had inadequate reticulocytosis (i.e. BMR_I<121) and 89% (of 18 tested) showed inappropriately low endogenous EPO. Patients were treated for a median of 7 months and responses were observed in about 70% of cases at month+1 and +3, with a median Hb and reticulocyte increase of 21.5 (2-48) g/L (p<0.001) and 25(0-220)x10⁹/L at month+1; and 29 (0-66) g/L (p<0.001) and 49 (0-195)x10⁹/L at month+3, respectively. Notably, 64% of patients responded as soon as at day+15; this finding supports a direct activity of EPO although recent/concomitant treatments may have contributed. At last follow up, 13 cases had discontinued EPO: 6 for long standing CR and 7 because of NR(3 with hemolytic flares). Response to EPO was associated to primary AIHA (73 vs 33% in secondary), inadequate reticulocytosis (76 vs 50% with adequate reticulocytosis), and not-warm (85 vs 50% in warm cases) not transfusion dependent cases (76 vs 50% transfusion dependant). In conclusion, EPO is effective in about 70% of AIHA patients unresponsive to ongoing treatments, particularly in cases with inadequate reticulocytosis.

Hodgkin Lymphoma

C041

BRENTUXIMAB VEDOTIN IN THE TREATMENT OF ELDERLY HODGKIN LYMPHOMA PATIENTS AT FIRST RELAPSE OR WITH PRIMARY REFRACTORY DISEASE: A PHASE 2 STUDY OF FIL ONLUS

M. Marangon¹, L. Argnani¹, A. Re², A. Lleshi³, M. Bonfichi⁴, A. Pinto⁵, N. Bianchetti², C. Pellegrini¹, V. Stefoni¹, P.L. Zinzani¹

¹Institute of Hematology "L. e A. Seràgnoli" University of Bologna; ²Department of Hematology ASST Spedali Civili di Brescia; ³National Cancer Institute; ⁴Division of Hematology Fondazione IRCCS Policlinico San Matteo; ⁵Hematology-Oncology and Stem Cell Transplantation Unit National Cancer Institute Fondazione Pascale IRCCS, Italy

Older age (≥60 years) has consistently been identified as an independent adverse prognostic factor for Hodgkin lymphoma (HL) survival in population-based studies and clinical trials in the last several decades. Elderly HL patients are significantly underrepresented in clinical trials and have a markedly inferior prognosis compared with younger patients. Brentuximab vedotin (BV) is an antibody–drug conjugate linking the microtubule-disrupting agent monomethylauristatin E to an anti-CD30 antibody. BV monotherapy yields an objective response rate (ORR) of 75% in relapsed HL, with a subset of patients having durable remissions at 5 years. In a retrospective analysis of BV activity in patients aged ≥60 years with relapsed HL, ORR was 56%. Although higher rates of adverse events such as anemia, fatigue, and neuropathy were seen in older compared with younger patients, BV was tolerable overall, and a significant proportion of older patients had clinical benefit. Based upon this favorable experience, our phase II study evaluated the efficacy and safety of BV as a single agent in elderly patients at first relapse or with primary refractory HL. This was a single-arm, open-label, multicenter, clinical trial. The primary endpoint of this study was the ORR. Main secondary endpoints were: duration of response, complete remission rate, progression free and overall survival at 1 year and type, incidence, severity, seriousness, and relatedness of any adverse events occurring during the study period. ClinicalTrials.gov identifier NCT02227433. Twenty patients were enrolled and 18 were evaluable for analyses. ORR was 52.9% (4 complete response and 5 partial response). With a median follow-up of 24.9 months, median PFS was 8.8 months and median OS was 21.7 months. 1-year PFS and OS were 40% and 68.8%, respectively. Prolonged disease control (more than 12 months) was registered in two patients. Seven patients had early treatment discontinuation due to toxicity - mainly due to neuropathy grade II-III - suggesting that this subset of frail patients should be carefully monitored during treatment with BV. To our knowledge, our study is one of the first trials evaluating efficacy and safety of BV in elderly patients affected by HL in first relapse or refractory to first line treatment. BV can represent an effective and quite tolerable chemo-free therapeutic regimen for elderly patients with relapsed or refractory HL.

C042

RECURRENCE OF HODGKIN LYMPHOMA AFTER 5 YEARS FROM FRONT-LINE TREATMENT: A NOT SO FAVORABLE RELAPSE

S. Viviani¹, A. Liaskas², G. Rizzuto¹, M.K. Angelopoulou², C. Giatra³, F. Kontopidou⁴, M.N. Dimopoulou², A. Mussetti¹, A. Guidetti¹, M.P. Siakantaris², J. Asimakopoulos², M. Arapaki², P. Triantafyllou², C. Chatzidimitriou², T. Yannikos², P. Korkolopoulou⁵, G. Rassidakis⁶, M. Dimou⁷, E. Variami⁸, P. Panayiotidis⁷, M. Bakiri⁸, T. Karmiris⁸, K. Konstantopoulos², G.A. Pangalis⁹, A. Cabras¹⁰, P. Corradini¹¹, T.P. Vassilakopoulos²

¹Department of Medical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano; ²Department of Haematology and Bone Marrow Transplantation, National and Kapodistrian University of Athens; ³Department of Haematology, Evangelismos General Hospital, Athens; ⁴Second Department of Internal Medicine, National and Kapodistrian University of Athens; ⁵First Department of Pathology, National and Kapodistrian University of Athens, Laikon General Hospital; ⁶Department of Oncology-Pathology, Karolinska Institutet, Stockholm; ⁷First Propaedeutic Department of Internal Medicine, National and Kapodistrian University of Athens, Laikon General

Hospital; ⁸First Department of Internal Medicine, National and Kapodistrian University of Athens, Laikon General Hospital; ⁹Department of Haematology, Athens Medical Center, Psychikon Branch; ¹⁰Department of Pathology Fondazione IRCCS Istituto Nazionale dei Tumori di Milano; ¹¹School of Medicine, University of Milan

Background: HL is considered cured if a complete remission (CR1) lasting ≥ 5 years is achieved after front-line therapy (1T). Few data on prognostic factors and long-term outcome of very late relapses (VLRs), occurring after ≥ 5 years from CR1, are available. **Aims:** To evaluate long-term outcome and prognostic factors for Freedom From Second Progression (FF2P) and Overall Survival after Relapse (O2S) of VLRs in an international, multicenter, retrospective study. **Methods:** Pts with biopsy-proven VLRs were retrospectively identified from the databases of 5 centers. FF2P and O2S were estimated by Kaplan-Meier method. Univariate logistic regression analysis and Cox model were used to identify variables predictive of survival.

Results: A total of 103 pts were identified; males 69%; median age: 49 years (range, 19-81), age ≥ 65 years 18%; B symptoms 38%; extranodal disease 25%; anemia 30%. Relapse occurred after a median of 118 months (range, 60-420) from 1T start and 24% relapsed after >15 years. The initial histologic subtype was: NS 42%, MC 41%, LR 3% and NLP 13%. Retreatment with the same chemotherapy (CT) regimen was given in 24% of cases, non-cross resistant regimens in 45%, CT followed by ASCT in 26%, and radiotherapy (RT) alone in 6%. After a median follow-up of 72 months, the 5- and 10-year FF2P and O2S were 55%, 50%, and 78%, 55%, respectively. Among 37 deaths, 21 were due to HL, 11 to second tumors, 4 to unrelated causes and 1 to toxicity of salvage CT. Long-term CR was achieved in 5/6 pts treated with RT alone, while retreatment did not significantly affect FF2P and O2S. In pts <65 years intention-to-treat with ASCT was associated with higher -although not statistically different- 5-year FF2P compared to conventional salvage CT alone (82% vs 56%, $p=0.57$). At 10-years FF2P was 59% and 56%, and O2S 84% and 59%, respectively ($p=0.19$). In multivariate analysis, anemia at VLRs was independent predictor of FF2P ($p=0.025$), while anemia and age ≥ 65 years were independent prognostic factors for O2S ($p=0.042$ and $p<0.001$, respectively). Long-term outcome of pts with anemia and/or age ≥ 65 years at VLR (41% of the whole population) was dismal: 10-year FF2P and O2S 28% and 35%. **Conclusions:** Long-term survival of HL VLRs is limited, because a non-negligible number of pts succumb to second tumors. Prognosis is particularly unfavorable in pts with advanced age and/or anemia at VLRs. ASCT should be considered despite the long duration of CR1 in pts <65 years old.

C043

A NOVEL CAR.CD30 T CELL THERAPY ASSOCIATED WITH LONG PERSISTENCE AND HIGH ACTIVITY AGAINST CD30 POSITIVE LYMPHOMAS

M. Guercio¹, C. Quintarelli^{1,2}, D. Orlando¹, I. Boffa¹, S. Caruso¹, Z. Abbaszadeh¹, S. Di Cecca¹, K. Bovetti¹, A. Camera¹, M. Sinibaldi¹, R. De Vito¹, L. Vinti¹, B. De Angelis¹, F. Locatelli^{1,3}

¹Ospedale Pediatrico Bambino Gesù, Roma; ²Università degli Studi di Napoli Federico II, Napoli; ³Università La Sapienza, Roma, Italy

The prognosis of many patients with chemotherapy-refractory or multiply-relapsed CD30+ Non-Hodgkin's Lymphoma (NHL) or Hodgkin lymphoma (HL) still remain poor, and novel therapeutic approaches are desirable to address this unmet clinical need. We designed and pre-clinically validated a Chimeric Antigen Receptor (CAR) construct characterized by a novel anti-CD30 single-chain variable fragment cassette linked to CD3 ζ by signaling domains of two costimulatory molecules, namely either CD28.4-1BB or CD28.OX40. We also added in the construct a Δ CD34 antigen, as a selectable marker and the inducible Caspase-9 suicide gene as a safety switch. The anti-tumor efficacy and persistence of both CAR.CD30 modified T cells were evaluated by *in vitro* experiments, as well as in vivo NSG mouse models. Independently from the costimulatory domains CD28/OX40 or CD28/4-1BB, both retroviral vectors showed similar transduction efficiency of T cells (86.50 \pm 5.08% and 79.30 \pm 5.33%, respectively). Nevertheless, CAR.CD30 incorporating CD28.OX40 co-stimulation was associated with more stable expression of the CAR over time, during extensive *in vitro* culture (84.72 \pm 5.30% vs 63.98 \pm 11.51% CAR+ T cells

at 30 days after transduction; $p=0.002$). This finding was also associated with the evidence that CD28.OX40 T cells showed a superior anti-lymphoma *in vitro* activity as compared to CD28.41BB T cells, when challenged at very high tumor/effector ratio (8:1) (37.45 \pm 27.65% vs 62.85 \pm 10.90% of residual tumor respectively; $p=0.03$) and after multiple stimulations by tumor. Moreover, antigen-specific stimulation was associated with higher levels of IFN γ (8306.03 \pm 3745.85pg/ml), IL2 (13492.68 \pm 5837.77 pg/ml) and TNF α (17661.00 \pm 11113.27 pg/ml) production by CD28.OX40 compared to CD28.41BB T cells (6617.81 \pm 3025.67 pg/ml, $p=0.040$; 7616.67 \pm 4464.06 pg/ml, $p=0.008$; 5824.63 \pm 1823.73 pg/ml, $p=0.02$; respectively). In NSG mouse models, we proved that CD28.OX40 T cells had superior anti-tumor control and persistence than 28.41BB T cells, leading to a significant reduction of bioluminescence at day 45 (3.32 \times 10 vs 2.29 \times 10, $p=0.04$) and an increased overall survival of treated mice (60% vs 10%, at 180 days, $p=0.0014$). Overall, these data indicate that, in the context of CAR.CD30 T cells, the costimulatory combination of CD28.OX40 is crucial for improving both persistence and antitumor efficacy of the approach.

C044

GLOBAL LONGITUDINAL STRAIN (2D-GLS) IN LYMPHOMA PATIENTS TREATED WITH CHEMOTHERAPY (CT)+/- MEDIASTINAL RADIOTHERAPY (RT): EARLY SUBCLINICAL CARDIOTOXICITY IN THE CARDIOCARE PROSPECTIVE OBSERVATIONAL STUDY

B. Botto¹, M. Levis², L. Orsucci¹, V. De Luca², S. Bartoncini², M. Giorgi³, A. Chiappella¹, S. Ferrero⁴, A. Fava³, F. Cavallo⁴, D. Caracciolo⁴, S. Vicentini³, A. Gastino², G. Furfaro², M. Nicolosi¹, G. Priolo¹, P. Pregno¹, A.R. Filippi², U. Ricardi², U. Vitolo¹

¹SC Ematologia A.O.U. Città della Salute e della Scienza; ²Dipartimento di Oncologia, Radioterapia Oncologica, Università di Torino; ³Dipartimento di Cardiologia, A.O.U. Città della Salute e della Scienza; ⁴SCDU Ematologia, Università di Torino, Italy

Treatments-related cardiotoxicity is a critical issue in long term lymphoma survivors, and its early identification is important to prevent clinically relevant cardiac events. Echocardiography including 2D-GLS, seems to be an effective tool in detecting preclinical systolic changes in the cardiac function even when the ejection fraction is preserved. Cardiacare study is an ongoing monocentric prospective observational study aimed at investigating early subclinical chemo and radiation-induced changes in left ventricular function (LV) using 2D-GLS. The planned accrual is 100 patients, 50 treated with chemotherapy alone (CT-alone) and 50 with combined modality treatment (CMT) including CT + mediastinal RT. Patients aged 18-70, affected with either HD or DLBCL or primary mediastinal lymphomas (PML) were eligible. Patients received an echocardiographic assessment including 2D-GLS at baseline, after chemo, after radiotherapy (if planned), and 3 months after the end of treatment. Paired samples T test correlations were applied to evaluate GLS changes at each time-point. The cumulative dose of anthracycline and the adsorbed radiation dose of whole heart and cardiac substructures were assessed for each patient. So far, fifty-two patients (24 in CT-alone group and 28 in CMT group) have been enrolled and included into this preliminary analysis. No patients experienced a significant reduction of LV ejection fraction during the entire treatment period. Median dose of anthracycline was 500 mg in CT arm vs 400 mg in CMT arm. A marginal reduction of 2D-GLS was seen after chemo for patients in CT-alone arm ($p=0.06$), but not for patients in CMT arm ($p=0.94$). A marginal reduction of GLS after CT was observed also for patients with Age >40 ($p=0.056$) and receiving >4 cycles ($p=0.055$). After RT, a significant reduction of 2D-GLS was found in those receiving: A) maximum dose to interventricular septum >10 Gy ($p=0.006$), to lateral wall of the LV >8 Gy ($p=0.002$) and to whole LV >11 Gy ($p=0.002$); B) mean dose to whole heart >4.5 Gy ($p=0.007$), ≥ 2 Gy to interventricular septum ($p=0.01$), to lateral wall of the LV ($p=0.02$) and to whole LV ($p=0.006$). 2D-GLS is a promising tool to detect early cardiotoxicity in lymphoma patients. Preliminary results suggest a correlation of both anthracyclines and radiation dose with preclinical heart damage. The completion of Cardiocare study, and a future correlation with clinical events are needed to support and strengthen these preliminary assumptions.

C045

A GENE EXPRESSION-BASED SCORE TO PREDICT INTERIM PET POSITIVITY IN HODGKIN LYMPHOMA PATIENTS TREATED WITH ABVD

S. Luminari¹, B. Donati², M. Casali³, A. Fama¹, B. Puccini⁴, M. Zanelli⁵, R. Valli⁵, R. Santi⁶, F. Vergoni⁶, A. Ruffini⁷, V. Berti⁸, L. Rigacci⁹, A. Ciarrocchi², F. Merli¹

¹Hematology AUSL IRCCS; ²Laboratory of Translational Research AUSL IRCCS; ³Nuclear Medicine AUSL IRCCS; ⁴Hematology Azienda Ospedaliero Universitaria Careggi; ⁵Pathology AUSL IRCCS; ⁶Pathological Histology and Molecular Diagnostics Azienda Ospedaliero Universitaria Careggi; ⁷Gruppo Amici Dell'Ematologia Foundation; ⁸Nuclear Medicine University of Firenze; ⁹Hematology and Stem Cell Transplant AO San Camillo Forlanini, Italy

Introduction: Response-adapted therapy through the use of FDG-PET after 2 cycles of ABVD (interim PET-iPET) is an effective strategy in Hodgkin Lymphoma (HL). However, this approach may cause 2 months of suboptimal treatment. The objective of this study was to use gene-expression profiling to build and validate a model to predict iPET positivity (iPET+).

Methods: Consecutive untreated HL patients (pts) with available diagnostic tumor sample who underwent iPET after 2 ABVD were identified and used to define the training and the validation set. Expression of 770 immune related genes was analyzed by digital expression profiling (Nanostring Technology). iPET was centrally reviewed according to the five-point Deauville scale (5PS), and was used as primary study endpoint. Clinical events were used as secondary endpoint. An iPET+ predictive model was derived by multivariate regression analysis and assessed in a validation set.

Results: A training set of 121 and a validation set of 113 pts were identified, with 23 iPET+ cases in each group. iPET+ HLs have a distinctive transcriptional profile at diagnosis that discriminate these lesions from iPET-. A list of 13 genes was found significantly associated with iPET+. This signature comprises two functionally distinct, stromal-related nodes. Lymph mono-ratio (LMR) was also associated to iPET+. In the training cohort a 5-gene and LMR integrated score predicted interim iPET+ (AUC 0.88 95%CI, 0.80-0.96). Model score was used to divide the training cohort into quartiles showing that the vast majority of iPET+ patients segregated within Q4th (Q4th -76.2%, Q3th -9.5%, Q2th -14.2% and Q1th -0%). Model performance was confirmed in the validation set (AUC 0.67 95%, 0.47-0.84). Furthermore, score-based quartile separation in this cohort, confirmed the preferential distribution of iPET+ pts within Q4th. Merging the training and validation set, 34 events were identified out of 172 pts (16 pts who underwent therapy escalation after iPET+ and 18 with relapse). iPET score was higher in pts with event vs those without event. Correlation of iPET score with pts survival was not informative because the adoption of intensified therapies in iPET+ cases (46% of iPET+) likely corrected the higher risk of progression in these pts.

Conclusions: In HL interim metabolic response measured with PET after 2 ABVD cycles is associated with a genetic signature and can be predicted applying an integrated gene-based model on the diagnostic biopsy.

Infections

C046

ANTI-BACTERIAL PROPHYLAXIS CAN BE SAFELY OMITTED IN ACUTE MYELOID LEUKEMIA DURING POST INDUCTION APLASIA: A RETROSPECTIVE SINGLE CENTER STUDY

I. Urbino¹, C. Frairia¹, E. Audisio¹, S. Aydin¹, S. D'Ardia¹, E. Messa², G. Iovino¹, B. Nicolino², U. Vitolo¹

¹Department of Oncology and Hematology Città della Salute e della Scienza di Torino; ²Hematology Unit ASL-TO 4, Italy

Aim: Acute myeloid leukemia (AML) patients (pts) are at high risk of infections during post induction neutropenia. Anti-bacterial prophylactic approach is the standard of care in many institutions. Recently many concerns raised about the emergence of multiresistant pathogens. Aim of the present study was to evaluate the safety of omitting antibacterial prophylaxis in AML induction aplasia.

Methods: The present retrospective single center study enrolled consecutive diagnosed AML (not APL) patients from June 2001 until March 2019 treated with intensive induction chemotherapy. Patients were divided in two groups according to anti-bacterial prophylaxis (levofloxacin 500 mg OD) given (Group A (GA), 343 pts from June 2001 to December 2016) or not (Group B (GB), 80 pts from January 2017 to March 2019). Primary endpoint was the incidence of early induction death; secondary endpoints the number of fevers, bacteremias and septic shocks. Due to the difference in the group size and the different periods of observation, a case control analysis comparing 80 sex and age matched patients from each group was performed. Statistical analysis was done using Fisher's exact test.

Results: A total of 423 AML patients with a median age of 54 (18-76) years were enrolled. Complete remission was achieved in 298 pts (70%), 97 were resistant (23%) and 27 died (6%) during induction. Baseline characteristics were equally distributed in both groups with the exception of age (median 53 vs 60 years in GA and GB, respectively p<0.0001). Early induction deaths were 22 in GA (6%) and 5 in GB (6%) (p>0.99). A total of 313 pts developed fever (91%) in GA and 77 (96%) in GB (p=0.17). The incidence of bacteremias was 26% (n=89) in GA compared to 32.5% (n=26) in GB (p=0.26); among them gram-negative infections were 32% (n=30) vs 36% (n=11), whereas gram-positive infections were 68% (n=63) vs 64% (n=20) in GA and GB, respectively (p=0.83). A septic shock was recorded in 5% (n=17) vs 10% (n=8) in GA and GB, respectively (p=0.11). The case control analysis confirmed these results, showing no significant difference for all endpoints.

Conclusions: In the present study the omission of levofloxacin prophylaxis was safe and did not increase the incidence of early induction deaths. Neither the incidence of fever, bacteremias, septic shocks nor the rate of gram-negative or gram-positive cultures were statistically significant different in the two groups. Prospective studies are needed to confirm these data.

C047

RESPIRATORY VIRUSES INFECTIONS ARE A SIGNIFICANT CLINICAL PROBLEM IN HAEMATOLOGICAL PATIENTS WITH UNDERESTIMATED ADVERSE OUTCOME: A SINGLE INSTITUTION 9-YEARS EXPERIENCE

A. Passi¹, C. Pagani¹, D. Gramegna¹, R. Daffini¹, C.P. Pollara², L. Signorini³, D. Tuzzo⁴, E. Accorsi¹, C. Cattaneo¹, F. Castelli³, G. Rossi¹

¹Ematologia ASST Spedali Civili di Brescia; ²Laboratorio di Microbiologia e Virologia ASST Spedali Civili di Brescia; ³Malattie Infettive ASST Spedali Civili di Brescia; ⁴Anestesia e Rianimazione 1 ASST Spedali Civili di Brescia, Italy

Respiratory viruses infections (RVI) are an underestimated problem among haematological (haem) patients (pts) with upper respiratory tract infections (URTI). Frequent pulmonary complications and scarcity of effective treatments cause severe clinical presentation and unfavorable outcome. We have analyzed frequency and outcome of RVI diagnosed from Jan 2011 to Mar 2019 at our Institution. Pts with URTI symptoms were tested for Influenza (Flu), Parainfluenza (PIV), Respiratory Syncytial Virus (RSV), Coronavirus (CoV), Adenovirus (ADV), Rhinovirus (RhV) and Metapneumovirus (MPV) by virus DNA detection on nasal

swab. Variables considered were: age, gender, underlying haem disease, type of treatment and of RVI, neutropenia, lymphopenia, pulmonary infiltrates (PI), respiratory failure (RF), concomitant bacterial or fungal infection (CI), intensive care unit (ICU) admission. We detected 154 RVI in 151 pts (M/F: 91/59; median age 63) with acute leukemia (64), lymphoma (48), myeloma (35), other haem diseases (8), mainly during winter. Only PIV occurred throughout the year with a peak in autumn. Flu was isolated in 74 pts, PIV in 23, RSV in 42, CoV in 15, ADV in 6, RhV in 2 and MPV in 5. PI were present in 86 (55%) cases, particularly in MPV (100% p 0.042) and PIV (82% p 0.005) infections. CI was diagnosed in 64 cases and was associated with RSV infection, RF and ASCT at multivariate analysis (p=0.038, p=0.017, p=0.01 respectively). RF (need for oxygen supplementation or non-invasive ventilation) occurred in 45 pts, associated with PI and fungal CI at multivariate analysis (p<0.001 and p=0.01 respectively). ICU admission was needed in 13 pts. Mortality at 30 days was 11% (16/151); mortality attributable to RVI was 7% (10/151) with no differences among different RVI. At univariate analysis, advanced haem disease (p0.002), PI (p 0.018), CI (p 0.004), RF and ICU admission (p<0.0001) were associated to adverse outcome and CI, advanced haem disease and ICU admission confirmed independent prognostic value at multivariate analysis (p=0.04, p=0.017 and p<0.0001 respectively). RVI severely impact on haem pts regardless of type of disease and virus, with PI in more than 50% of cases and frequent RF. Overall mortality is at least comparable with that of bacterial infections and only slightly lower than in pulmonary aspergillosis. Careful monitoring of RVI, particularly during winter, early diagnosis and prevention strategies should be urgently implemented in haem pts.

C048

IMPACT OF INVASIVE ASPERGILLOSIS OCCURRING DURING REMISSION-INDUCTION THERAPY ON OUTCOME OF ACUTE MYELOID LEUKEMIA (SEIFEM 2012B STUDY).

A. Candoni¹, F. Farina², K. Perruccio³, R. Di Blasi⁴, M. Criscuolo⁴, C. Cattaneo⁵, M. Delia⁶, D. Lazzarotto¹, G. Dragonetti⁴, R. Fanci⁷, B. Martino⁸, M.I. Del Principe⁹, L. Potenza¹⁰, N. Vianelli¹¹, A. Chierichini¹², M. Garzia¹³, G. Nadali¹⁴, L. Verga¹⁵, M.V. Dubbini¹, A. Busca¹⁶, L. Pagano⁴ on behalf of SEIFEM Group

¹Cattedra di Ematologia, Azienda Sanitaria-Universitaria Integrata di Udine; ²IRCCS Ospedale San Raffaele, Milano; ³Oncoematologia Pediatrica, Ospedale SM Misericordia, Perugia; ⁴Istituto di Ematologia, Fondazione Policlinico Universitario A. Gemelli-IRCCS-Università Cattolica del Sacro Cuore, Roma; ⁵Divisione di Ematologia, ASST-Spedali Civili di Brescia, Brescia; ⁶Sezione di Ematologia, Dipartimento dell’Emergenza e dei Trapianti d’Organo-Università di Bari; ⁷Unità Funzionale di Ematologia, Azienda Ospedaliero-Universitaria Careggi e Università di Firenze; ⁸UOC Ematologia, Ospedale Bianchi Melacrinò Morelli, Reggio Calabria; ⁹Cattedra di Ematologia, Dipartimento di Biomedicina e Prevenzione, Università degli Studi di Roma ‘Tor Vergata’, Roma; ¹⁰UOC Ematologia, Dipartimento di Scienze Mediche e Chirurgiche Materno infantili e dell’Adulto, Università di Modena e Reggio Emilia, Modena; ¹¹Istituto di Ematologia, Dipartimento di Onco-Ematologia, Policlinico S. Orsola-Malpighi Università di Bologna; ¹²UOC Ematologia Azienda Ospedaliera S. Giovanni Addolorata, Roma; ¹³UOC Ematologia, Ospedale San Camillo, Roma; ¹⁴UOC di Ematologia, Azienda Ospedaliera Universitaria Integrata di Verona; ¹⁵Clinica Ematologica, Ospedale San Gerardo, ASST Monza, Università Milano Bicocca, Milano; ¹⁶Ematologia, A.O.U. Città della Salute e della Scienza di Torino, Dipartimento di Oncologia, SSD Trapianto allogenico di cellule staminali, Torino

Background: Acute myeloid leukemia (AML) patients are at high risk of invasive aspergillosis (IA) and required a mould active antifungal prophylaxis during induction chemotherapy (CHT). Although IA risk factors have been identified, few data are available on impact of IA, occurring during first remission induction phase, on overall AML outcome.

Patients and Methods: The primary endpoint of this prospective, multicentric, case-control study, was to evaluate if IA, occurring during first remission-induction CHT, can affect treatment schedule and, consequently, patient overall survival (OS). We identified 40 AML patients (cases) who developed IA during induction phase, 31 probable (67%) and 9 proven (33%) IA. These cases were matched with a control group (80 AML) without IA, balanced according to age, type of induction CHT, AML characteristics and cytogenetic-molecular risk factors. The overall response rate to induction CHT was the same in the 2 groups.

Results: In the 40 cases with IA, the overall response rate (ORR) to antifungal treatment was favorable (ORR 80%) but it was significantly affected by the achievement of leukemia complete remission (CR) with induction CHT. In fact, in cases with AML responsive to induction CHT, complete responses of IA to antifungal therapy were 96% compared to 21% in cases of AML not responsive to induction CHT (p<0.0001). The adherence to schedule and full doses of CHT was reported in 35% of cases (14/40) and in 76% of controls (61/80) (p=0.0001). After induction CHT a significant higher number of cases (15/40; 37.5%) compared to controls (21/80; 26%) could not receive additional cycles of CHT (p=0.01). The IFI related mortality was 22.5%. Comparing OS of 40 cases with the OS of the 80 controls, the median OS of cases was significantly worse with a difference of 12.3 months (12.1 vs 24.4 months, p=0.04-Figure 1A). However, the occurrence of IA during induction phase did not have a significant impact on the OS of cases who achieved a CR with induction CHT which are able to proceed, despite the IA, with their intensive therapeutic program, achieving the same OS as the control group with an AML in CR (p=ns-Figure 1 B).

Conclusions: These data showed that IAs during induction CHT can delay the subsequent therapeutic program and has a significant impact on OS of AML, specifically in those patients with IA occurring during induction phase who did not achieved a CR of AML with the first course of CHT (Figure 1 C).

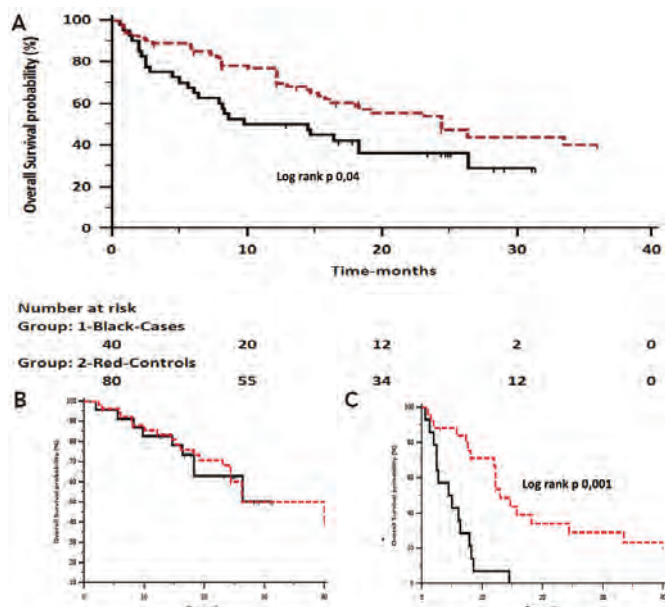


Figure 1. Os of 40 cases compared to 80 controls.

C049

FUNGAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM IN ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENTS. EPIDEMIOLOGY AND CLINICAL OUTCOME

G. Facchin¹, A. Candoni¹, A. Busca², D. Lazzarotto¹, C. Cattaneo³, G. Nadali⁴, N. Klimko⁵, M.I. Del Principe⁶, C. Castagnola⁷, L. Verga⁸, E. Calore⁹, M.E. Zannier¹, D. Capelli¹⁰, K. Perruccio¹¹, L. Melillo¹², R. Fanin¹, L. Pagano¹³

¹Hematology and Stem Cell Transplantation Azienda Sanitaria Universitaria Integrata di Udine; ²Hematology AO Città della Salute e della Scienza; ³Hematology Spedali Civili; ⁴Hematology AOUI Policlinico GB Rossi; ⁵Metchnikov North-Western State Medical University; ⁶Hematology Dpt of Biomedicine and Prevention University Tor Vergata; ⁷Onco-hematology Fondazione ICRRS Policlinico San Matteo; ⁸Hematology Ospedale S. Gerardo; ⁹Hematology pediatric section Dpt di Salute della Donna e del Bambino Azienda Ospedaliero-Universitaria; ¹⁰Hematology Azienda Ospedaliero-Universitaria Ospedali Riuniti; ¹¹Pediatric onco-hematology section Ospedale SM Misericordia; ¹²Hematology, IRCCS Casa Sollievo della Sofferenza; ¹³Istituto di Ematologia Polo Onco-Ematologico Fondazione Policlinico A. Gemelli-IRCCS Università Cattolica del Sacro Cuore

Background: Invasive Fungal Infections of the Central Nervous System (IFI-CNS) are rare and life-threatening complications and no specific epidemiologic data are available in Allo-SCT recipients.

Patients and Results: We describe the incidence, clinical characteristics and outcome of IFI-CNS detected, over a 7 years period (2010-2016), in 13 Allo-SCT Centers. There were 24 consecutive IFI-CNS in 3866 Allo-SCT with an incidence of 0,62%. All cases were proven(58%) or probable(42%) according to the EORTC criteria. Median age of patients (pts) was 39 yrs and acute leukemia was the most common underlying disease(71%); 54% of pts had severe neutropenia at the onset of IFI and there was a concomitant extra CNS involvement in 75% of cases; 16 pts had received an Allo-SCT from unrelated donors, 4 from a HLA-id family donor and 4 from a haplo donor. Of 14 proven cases 12 were caused by moulds (7 aspergillosis, 3 mucormycosis, 1 fusariosis, 1 scedosporidiosis) and 2 by yeasts (1 cryptococcosis and 1 trichosporonosis). All the 10 probable infections were caused by *Aspergillus* spp. The fungal biomarkers on cerebrospinal fluid (CSF) were performed in 11/24(46%) of pts and were positive in 73%. The brain biopsy was performed in 8% of cases and a biopsy from other involved sites was performed in 21% of pts. All pts received antifungal therapy (AT) mainly with amphotericin(58%) or voriconazole(46%). A combination AT was administered in 33% of cases. The AT was coupled with neurosurgery only in 17% of pts. The overall response rate (ORR) to AT was 38%. Median follow-up was 7 months with a 12 mths overall survival (OS) probability of 23% (Figure 1).

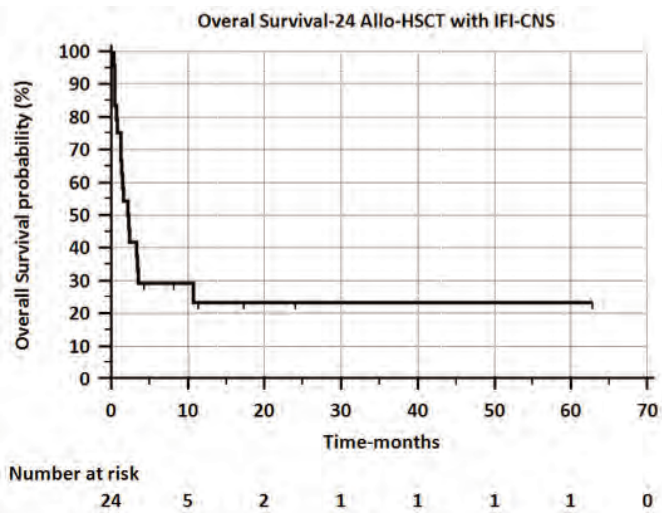


Figure 1.

The overall mortality was 75%(18/24) and the IFI-CNS attributable mortality was 44%(8/18). **Conclusions:** a)IFI-CNS remains a rare infectious complication after Allo-SCT with an incidence of 0,62% in this

recent survey. The most important causative agent remains *Aspergillus* sp and a concomitant extra CNS involvement (mainly lung) is common. b)The CSF fungal biomarkers were highly informative (positive in 73% of our case series). This underlines that diagnostic lumbar puncture should be encouraged when we suspect an IFI-CNS to confirm the diagnosis. c)The ORR to the AT remains poor (38% in the present analysis) and the OS at 12 months was only 23%. New drugs with better permeability in the CNS and less interactions with immunosuppressive agents (such as isavuconazole) could improve the outcome of IFI-CNS in Allo-SCT recipients.

C050

LATENT TUBERCULOSIS INFECTION IN ADULTS WITH ACUTE LEUKEMIA AND APLASTIC ANEMIA: A RETROSPECTIVE SINGLE CENTER EXPERIENCE

F. Bettelli¹, D. Giusti¹, M. Morselli¹, A. Paolini¹, E. Colaci¹, V. Nasillo¹, A. Messerotti¹, V. Pioli¹, L. Arletti¹, A. Gilioli¹, M. Celli¹, F. Donatelli¹, S. Iotti¹, H. Catellani¹, L. Galassi¹, C. Colasante¹, D. Vallerini¹, P. Barozzi¹, I. Lagreca¹, R. Maffei¹, G. Riva¹, E. Franceschini², C. Mussini², R. Marasca¹, F. Narni¹, L. Potenza¹, M. Luppi¹, F. Forghieri¹

¹Section of Hematology, Department of Medical and Surgical Sciences; ²Section of Infectious Diseases, University of Modena and Reggio Emilia, Azienda Ospedaliero-Universitaria di Modena, Italy

Patients (pts) with hematologic malignancies are at risk to develop active tuberculosis (TB). However, scanty information is currently available on prevalence and management of latent TB infection (LTBI) in pts with newly diagnosed acute leukemia or aplastic anemia (AA), especially from Western Countries. We retrospectively analyzed the prevalence of LTBI, by the interferon- release assay (IGRA) Quantiferon®-TB (QFT) test, and the incidence of TB reactivation in 269 HIV-negative consecutive pts (median age 58 years, range 15-83), with either intensively treated acute leukemia (AML 196, ALL 62), or severe AA (11) receiving immunosuppression. Most pts (91.4%) were Caucasian, while 8.6% of subjects was foreign born. Tuberculin skin test was not performed. Over a 9-year period (2010-2018), QFT results at diagnosis were available for 261 pts. Overall, QFT test was positive in 20 subjects (7.7%), namely 17 with AML and 3 with ALL, only 2 of them foreign born. The overall rate of indeterminate QFT results due to low positive control values was 9.9%. QFT test yielded positive results in 4 of 10 cases with previous TB contact. Among the 20 positive pts, LTBI was documented in 19 cases, whereas a 35-year old Italian woman presented with active pulmonary TB at AML diagnosis. Radiological findings consistent with LTBI, such as lung nodules or calcifications, were observed in 7 of 19 (36.8%) positive cases. Pts with LTBI were significantly older than those with negative QFT test ($p=0.027$). Oral isoniazid (INH) 300 mg/day, with concurrent pyridoxine, was administered for a median of 6 months (range 0.2-18) to 18 LTBI pts, while receiving intensive chemotherapy, including HSCT in 6 cases. Subsequent lung infections were observed in 10 pts with LTBI, without evidence of active TB. INH toxicity occurred in 6/18 cases (33.3%), namely hepatotoxicity (3), psychosis (1), skin toxicity (1) and peripheral neuropathy (in 2 pts with ALL concurrently receiving vincristine), leading to either temporary or definitive drug withdrawal in 3 and 2 pts, respectively. Our observational monocenter study showed, using an IGRA as screening tool, a low prevalence of LTBI (7.3%) in newly diagnosed acute leukemia pts from Italy. None of these cases developed TB reactivation despite severe immunodeficiency. INH prophylaxis was feasible and active. However, prospective controlled studies are warranted to further investigate efficacy and ideal duration of INH therapy for LTBI in hematologic pts.

Hemostasis, thrombosis, thrombocytopenia and platelet diseases

C051

ELTROMBOPAG AS SECOND LINE THERAPY IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ATTEMPT TO ACHIEVE LONG-TERM REMISSION. UPDATED RESULTS OF A PHASE II, MULTICENTER, PROSPECTIVE STUDY BY GIMEMA GROUP (THE ESTIT STUDY)

E. Lucchini¹, F. Palandri², S. Volpetti³, N. Vianelli², G. Auteri², E. Rossi⁴, A. Patriarca⁵, G. Carli⁶, W. Barcellini⁷, F. Rotondo⁸, U. Consoli⁹, F. Valeri¹⁰, C. Santoro¹¹, E. Crea¹², M. Vignetti¹², E. Boggio¹³, U. Dianzani¹³, I. Giardini¹⁴, M. Carpenedo¹⁵, F. Rodeghiero¹⁶, R. Fanin³, F. Zaja¹

¹S.C. Ematologia, Azienda Sanitaria Universitaria Integrata; ²Institute of Hematology "L. and A. Seràgnoli" S. Orsola-Malpighi; ³Clinica Ematologica, Centro Trapianti e Terapie Cellulari "C. Melzi", DAME, Università degli Studi; ⁴Istituto di Ematologia, Fondazione Policlinico A. Gemelli IRCCS, Università Cattolica; ⁵U.O. di Ematologia, AOU Maggiore della Carità; ⁶Hematology Department, Ospedale San Bortolo; ⁷UOC Ematologia, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico; ⁸Hematology Unit, Infermi Hospital; ⁹UOC Ematologia ARNAS Garibaldi; ¹⁰Regional Center for Hemorrhagic and Thrombotic Diseases, Haematology Unit, City of Health and Science University Hospital of Molinette; ¹¹Department of Cellular Biotechnology and Hematology, Sapienza University; ¹²GIMEMA Foundation; ¹³Laboratory of Immunology, Department of Health Sciences, Università del Piemonte Orientale; ¹⁴Clinical and Experimental Pharmacokinetics Lab, Diagnostic Medicine Dep, IRCCS Policlinico San Matteo; ¹⁵Hematology and Transplant Dept, ASST Ospedale San Gerardo; ¹⁶Hematology Project Foundation, affiliated to the Dept. of Hematology of the San Bortolo Hospital, Italy

Durable remissions after discontinuation of thrombopoietin (TPO) receptor-agonists have been described in 10-30% of ITP patients (pts), suggesting their immunomodulatory activity. The aim of this prospective multicenter study is to evaluate the effect of eltrombopag (ELT) as an anticipated second-line short-term treatment in pts with newly diagnosed (ND) or persistent (P) ITP. Pts aged ≥18 years with ND or P ITP relapsed or refractory to standard first-line therapy with active disease were included. The study consisted of period of treatment (PT, 24 weeks (wk)), period of tapering and discontinuation (PTD, wk 25-32), period of observation (PO, wk 33-52). Complete response (CR), response (R) and no response were defined according to IWG standard criteria.

Table 1. Treatment free remission.

	Evaluable patients	ORR (R + CR) (%)	CR (%)	NR (%)	Too early*
Week 24	51	34 (67)	17 (33)	17 (33)	/
Tapering and discontinuation in patients with response					
Week 28	34	23 (68)	8 (23)	11 (32)	/
Week 32	34	17 (50)	7 (21)	17 (50)	/
Week 36	32	15 (47)	7 (22)	17 (53)	2
Week 40	30	10 (33)	8 (26)	20 (66)	4
Week 44	29	9 (31)	7 (24)	20 (69)	5
Week 48	28	8 (29)	6 (21)	20 (71)	6
Week 52	28	8 (29)	5 (18)	20 (70)	6

*: patients who have not yet achieved the timepoint for evaluation

The primary end-point was treatment-free remission (TFR): the proportion of pts who, in R or CR at the end of PT, was able to taper down and discontinue ELT, maintaining the remission during the PO. Secondary objectives included the relationship between baseline TPO serum level and response, and modifications of immunological parameters. Between 24/02/16 and 05/05/18, 55 pts were enrolled by 10 GIMEMA Italian Centers. 3 pts didn't meet the inclusion criteria; 1 was excluded for protocol violation. Of the 51 evaluable pts, 61% were females. Median age was 65 years (range 21-90). 43% had ND ITP. Median baseline platelet count was 19x10⁹/L. Median baseline TPO level, evaluable in 44/51 pts, was 47.6 pg/mL (range 31.3-607.7). The overall response rate (ORR) at wk 24 was 67% (33% CR). All the 34 responders completed

the PTD: 17 (50%) maintained the response (21% CR), while 50% relapsed. At the time of data cut-off, 6/34 pts have not completed yet the PO. 28/34 pts were evaluable at the end of PO: TFR was observed in 8/28 responders (29%) - 8/45 evaluable pts (18%). 20 pts relapsed: 17 during the PTD and 3 during the PO. No relationship between baseline TPO level, ORR, CR and TFR was found; 23 pts (45%) reported a total of 65 adverse events. In conclusion, after 6 months of therapy the ORR to ELT in pts with ND or P ITP is 67% (33% CR). Among pts who start the tapering, the ones that don't relapse within the PTD are more likely to achieve longer TFR. For pts who don't maintain the response, ELT can be a steroid-sparing agent used as a bridge to the chronic phase. TPO serum levels are not adequately increased in ITP pts; baseline TPO level doesn't predict TFR.

C052

EFFICACY AND IMPROVEMENT OF SURVIVAL WITH ANTICOAGULANT THERAPY IN 149 CASES OF SPLANCHNIC VENOUS THROMBOSIS

G. Micucci¹, S. Rupoli¹, G. Tarantino², A. Fiorentini¹, I. Federici¹, G. Svegliati Baroni², E. Morsia¹, K.B. Garvey¹, A.R. Scortechini¹, A. Olivieri¹

¹Clinic of Hematology, Polytechnic University of Marche Region, Ancona; ²Clinic of Gastroenterology and Hepatology, Polytechnic University of Marche Region, Ancona, Italy

Splanchnic vein thrombosis (SVT) is an unusual-site thrombosis commonly observed in patients with malignancies, cirrhosis, and acute abdominal inflammatory conditions. Although being crucial for SVT, the benefit/risk ratio of anticoagulant treatment (AT) in these settings remains uncertain. A cohort of 149 SVT patients (cirrhotic and non-cirrhotic) were prospectively followed from 1998 to 2018. After an adequate variceal prophylaxis, we started as soon as possible an AT with LMWH or AVK; the dosage was tailored on the patient-specific risk of bleeding. Main outcomes evaluated were major bleeding (MB), recurrent thrombosis and recanalization. Cirrhosis, solid cancer and myeloproliferative neoplasms were the most common underlying disease (79%, 36% and 9%). Despite the fact we found F2-F3 esophageal varices in 39% and thrombocytopenia in 62% of patients, 82% were anticoagulated: 55% with LMWH (prophylactic or therapeutic doses) and 32% with AVK (TTR 64%). The median follow-up was 14 months (1-232) and the median duration of therapy was 11 months (1-240). During AT, the incidence rate was 3.2/100 pt-y for MB events and 2.5/100 pt-y for thrombotic events. In the first 19 months of observation, a low rate of MB occurred (20%; median not reached). Similar cumulative risk of bleedings was observed in patients with or without anticoagulant therapy. Thirty-five patients died for causes not related to AT: one patient for fatal cerebral bleeding. Follow up imaging, showed complete recanalization in 54 patients (45%), partial in 19 patients (16%), stable thrombosis in 35 (29%), progression or recurrent thrombosis in 11 patients (9%). The probability of recanalization was 50% at 18 months (95% CI: 12.9-23), without significant differences between cirrhotic and non-cirrhotic group and between the type of AT (AVK or LMWH). Recanalization was lower in patients who were not treated (p=0.063). At multivariate analysis, long-term AT resulted independently associated with a higher overall survival according to Cox-Regression analysis, adjusted for albumin levels, Child-Pugh score, HCC and recanalization (Odds ratio 1.88; 1.02-3.45, 95% CI; p=0,04). A patient-tailored management together with an interdisciplinary approach improves the clinical outcome of SVT patients, achieving both high rate of early recanalization and low rates of MB with AT; moreover long-term AT is correlated with higher overall survival.

C053**DEFIBROTIDE EXPOSURE MODIFIES GENE PROFILE EXPRESSION OF ENDOTHELIAL CELLS IN RESPONSE TO LIPOPOLYSACCHARIDE**

L. Teofili, N. Orlando, P. Chiusolo, C.G. Valentini, M. Bianchi, S. Sica, V. De Stefano

Fondazione Policlinico Universitario A. Gemelli IRCSS, Roma, Italy

Endothelial cell (EC) damage plays a pathogenetic role in several life threatening complications of hematopoietic stem cell transplantation (HSCT), such as sinusoidal obstruction syndrome/venoocclusive disease (SOS/VOD), thrombotic microangiopathy, or graft versus host disease. Chemo- and radio-therapy toxicity, increased circulation of microbial products, allogeneic lymphocyte reaction or exposure to immunosuppressive drugs can activate several EC pathways, including inflammation, adhesion, coagulation and apoptosis. Defibrotide (DF) is used to treat SOS/VOD in HSCT patients and early administration is associated with a better outcome. We explored the gene profile of EC in response to lipopolysaccharide (LPS) and its modulation by DF exposure. Endothelial colony forming cells (ECFCs) were obtained from umbilical cord blood (Ingram *et al.* Blood 2004;104:2752). Confluent ECFCs at passages II to III were exposed for 6 h to LPS (100 ng/ml, 4 experiments), with or without DF (100 ng/ml). In additional 5 experiments cells were pre-treated for 2 h with DF before adding LPS. Total RNA was extracted and reverse transcribed and genotype profile was evaluated using Human Endothelial Cell Biology array (PAHS-015Z, SABiosciences, Qiagen). Data were analyzed through RT2 Profiler PCR array data analysis template v3.5 (SABiosciences, Qiagen). Relative fold regulation (FC) in gene expression were calculated using the Ct (cycle threshold) method applying a cut-off of 2. DF induced in resting cells an up-regulation of KIT and VEGFA (FR 2.70 and 2.2 respectively). LPS produced a substantial up-regulation of several genes involved in cell adhesion, inflammatory response, coagulation and platelet activation, apoptosis, vasoconstriction and vasodilatation (CCL2, CX3CL1, ICAM, IL6, IL7, Tissue factor, KIT, PGFRA, SELE, VCAM1, with a FR range between 2.5 and 57.4). Nevertheless, the contemporary exposure of cells to DF and LPS failed to significantly modify this gene profile response to LPS. Conversely, DF pre-incubation significantly softened the cell response to LPS, with a reduced expression of the majority of genes usually up-regulated by LPS. In some cases (tissue factor, CX3CL1) the DF pre-incubation maintained the expression of these genes at levels comparable to resting cells.

On the whole, these observations contribute to explain the more favorable outcome of SOS/VOD when DF therapy is early started.

The study was supported by Jazz Pharmaceutical.

C054**INCIDENCE OF THROMBOSIS IN A COHORT OF PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA: EFFECT OF TREATMENTS**

E. Rossi, A. Ciminello, A. Di Giovanni, T. Za, S. Betti, F. Bartolomei, F. Di Landro, V. De Stefano

Istituto di Ematologia, Università Cattolica, Fondazione Policlinico A. Gemelli IRCCS, Roma, Italy

A higher risk of thrombosis has been reported in patients with primary immune thrombocytopenia (ITP). There is concern that the use of thrombopoietin receptor agonists (TPO-RA) further increase this risk. This study is aimed to gain further insights into the risk of thrombosis in ITP patients and the effects of treatments. We analysed the medical records of 172 ITP patients (M/F 61/111, median age 59 yrs, range 4-94) referred to our Center from 1990 to 2018 and with a follow-up > 3 months. Platelet increasing treatments were categorized as steroids (prednisone, high dose-dexametasone), TPO-RA (romiplostim, eltrombopag), other treatments (rituximab, immunosuppressive drugs). The total duration of each treatment was estimated by computing the time in which individual patients received the drugs; the time off-treatment was computed too. The incidence rate (IR) of thrombosis was estimated for each period; the time when patients received combined treatments (e.g. steroids and TPO-RA) was not considered for the analysis. Twenty-seven patients of 172 had a first thrombosis (15.6%) over a total observation time of 880 pt-yrs, with an IR of thrombosis of 3.0 per 100 pt-yrs. Thrombosis involved venous vessels in 22 cases and ar-

teries in 5 cases; recurrent thrombosis occurred in 10 patients. Nine first events occurred out of therapy, 9 on steroids, 5 on TPO-RA, and 2 after rituximab administration. Two additional venous thromboses occurred after splenectomy. The IR of thrombosis per 100 pt-yrs in the absence of treatment was 1.5; the IR per 100 pt-yrs during steroid treatment, TPO-RA treatment, and other treatments were 5.8 (p=0.003 vs no treatment), 3.9 (p=0.09), and 14.2 (p=0.0007), respectively. No difference was found between the IRs of thrombosis on steroids and TPO-RA (p=0.48). After exclusion of the pt-yrs on oral anticoagulants or LMWH because of previous VTE or atrial fibrillation the IR of thrombosis on TPO-RA was 4.6 per 100 pt-yrs and remained substantially unchanged in the other groups (p=0.73 vs IR on steroids and p=0.03 vs IR without treatment). In conclusion in our ITP monocenter cohort the IR of thrombosis was 3 per 100 pt-yrs, confirming previous observations; a significant further increase of thrombosis was found during the periods on treatment, whatever drug employed. Therefore, the thrombotic tendency seems associated with the activity of disease and need of treatment rather than a specific drug treatment.

C055**THE TRANSITION FROM CHILDHOOD TO ADULTHOOD IN CHRONIC IMMUNE THROMBOCYTOPENIA PATIENTS: THE ROLE OF SPLENECTOMY AND THROMBOPOIETIN RECEPTOR AGONISTS IN A SINGLE CENTER EXPERIENCE**M. Carpenedo¹, D.P. Bernasconi², M. Spinelli³, S. Pezzatti¹, M. Jankovic⁴, R. Renso¹

¹U.O Ematologia e Trapianto ASST Ospedale San Gerardo di Monza; ²Bio-statistica ed Epidemiologia Clinica Università di Milano Bicocca; ³U.O Pediatria Fondazione MBBM Ospedale San Gerardo Monza; ⁴Università di Milano Bicocca, Italy

Introduction: Children with ITP will develop a chronic disease (CD) (plt < 100x10⁹/L >12 mos) in 20-30% of cases. No specific studies have been published about transition from ITP started in childhood (CH) to adulthood (AH).

Aims and Methods: To present a single center retrospective survey on ITP pts diagnosed in CH (< 18 yrs) who developed a CD, need at least one line of therapy and were sent to adult ITP office from Jan 2013 to Feb 2019.

Results: Our Pediatric Dept accounts for a mean of 37 newly ITP diagnosis/year, with 32.8% of pts developing a CD. During the observation time, 60 pts was expected to enter AH with a CD. Overall 28 pts (14 F) were sent to Adult ITP office at pediatrician's discretion, 46.6% of expected (Table 1) at a median age of 21 yrs (median IQR 19-24). Median age at ITP diagnosis was 8.5 yrs (median IQR 3.75-14). The median n of lines of treatment received in CH was 1 (median IQR 1-2.25). The median follow up from diagnosis after the transition to AH is 18 yrs (4-39 yrs), the median n of lines at data cut-off was 2.5 (1-5). 11 patients were treated with TPO-RA (8 pts with Elt since CH, 2 pts with Elt and 1 Rom in AH). Splenectomy (Spln) was offered to all pts treated with TPO-RA to avoid chronic treatment, and to other selected pts with low plt count and history of bleeding. 4 pregnant pts were treated with steroids and IVIG, had natural labour, no adverse events and their babies had all normal plt count but one who recovered 4 weeks later. Overall a CR, PR and NR was achieved in 14, 9 and 5 pts respectively (Figure 1). During TPO-RA treatment a CR was obtained in 9/11 pts and 10/10 after Spln. Median time since diagnosis to Spln was 8 yrs (1 to 18 yrs), with a median follow up of 41 months (IQR 16-47.50). By univariate analysis Spln was the only factor significantly related to CR or PR (Figure 2), even after stratification by age group at ITP diagnosis (>8.5 yrs: RR 3.5- CI 1.085-11.2 p 0.021; < 8.5 yrs: RR 10- CI 1.55- 64.1, p=0.005).

Conclusions: The transition from CH to AH in chronic ITP pts leads clinicians to challenges related to growing age. However only a minority of children with ITP developed a CD which required a prolonged treatment. TPO-RA seems to be effective and well tolerated but chronic administration has limited compliance. Spln, even if performed in AH after many yrs since diagnosis allows to achieve a stable CR sparing young adults from chronic treatment.

Table 1.

PI ID	Sex	Reason for being sent to adult office	Age at 1st diagnosis (yrs)	Age at arrival in adult office	Tx received in childhood	Survival to date cut-off
1	F	TPO-RA ongoing	12	21	P, IVIG, Rom	P, IVIG, Rom, spl
2	F	TPO-RA ongoing	17	21	P, IVIG, Elt	P, IVIG, Elt, Rom, Spl
3	F	TPO-RA ongoing	13	19	P, Elt	P, Rom, spl, Splen
4	M	TPO-RA ongoing	9	22	P, IVIG, Elt	P, IVIG, Elt, Rom, Splen
5	M	bleeding	17	18	Deza	Deza, Rom, Elt, Splen
6	M	bleeding	9	22	P, IVIG	P, IVIG, Splen
7	M	TPO-RA ongoing	2	19	P, IVIG, Elt	P, IVIG, Elt, splen
8	M	low plt count	4	20	P, IVIG	P, IVIG
9	F	pregnancy	12	24	P	Deza
10	M	bleeding	2	37	P, IVIG, splen	P, IVIG, splen, accessory splen removal
11	F	TPO-RA ongoing	13	20	P, Elt	P, Elt, Splen
12	M	low plt count	16	19	P	P
13	F	low plt count	14	24	P	P
14	F	pregnancy	12	31	P	P
15	M	low plt count	3	19	P	P
16	F	low plt count	1	21	P	P
17	F	pregnancy	3	24	P	P
18	F	low plt count	14	20	P	Deza, Elt Splen
19	M	TPO-RA ongoing	3	20	P, IVIG, RTK, Elt	P, IVIG, RTK, Elt
20	M	TPO-RA ongoing	4	18	P, IVIG, Elt	P, IVIG, Elt
21	M	low plt count	19	22	P	P
22	M	low plt count	5	21	P	P
23	F	low plt count	12	21	P	P
24	M	low plt count	14	19	P	P
25	F	low plt count	17	22	P	P
26	M	low plt count	8	23	P	P
27	F	low plt count	4	20	P	P
28	F	pregnancy	4	27	P	P + IVIG

P= prednisone
RTK= rituximab
IVIG= intravenous immunoglobulin
Splen= splenectomy
Spl= splenectomy
Elt= eltrombopag
Rom= romiplostim
Spln= splenectomy
Deza= dasatinib

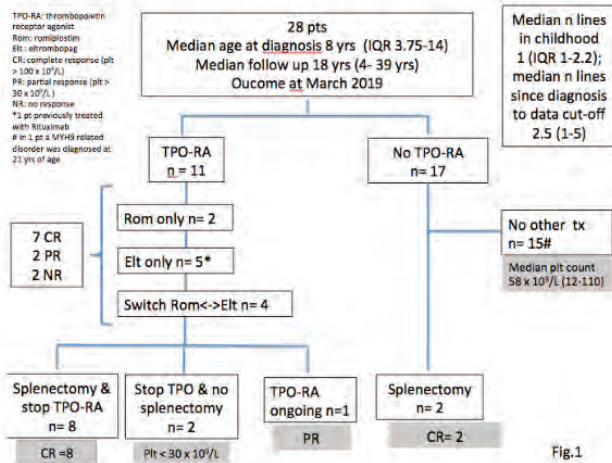
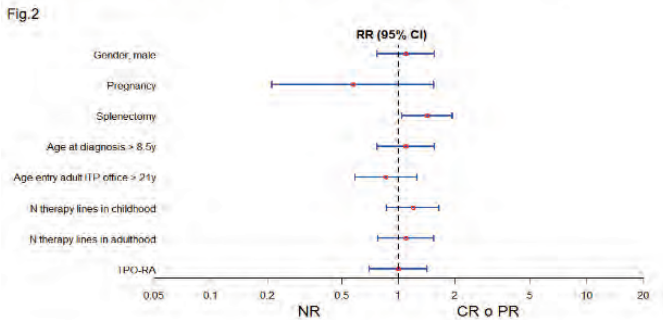


Fig.1



Figures 1 and 2.

Acute Lymphatic Leukemia

C056

A DASATINIB-BLINATUMOMAB COMBINATION FOR THE FRONT-LINE TREATMENT OF ADULT PH+ ALL PATIENTS. PRELIMINARY RESULTS OF THE GIMEMA LAL2116 D-ALBA TRIAL; ON BEHALF OF GIMEMA AL WORKING PARTY

S. Chiaretti, R. Bassan, A. Vitale, L. Elia, A. Piciocchi, F. Ferrara, M. Lunghi, F. Fabbiano, M. Bonifacio, N. Fracchiolla, P. Salutari, A. Mancino, M. Vignetti, A. Rambaldi, R. Foà

Department of Translational and Precision Medicine, Sapienza University of Rome, Hematology Unit, Ospedale dell'Angelo and Ospedale Ss Giovanni e Paolo, Fondazione GIMEMA – Franco Mandelli Onlus; Division of Hematology, Cardarelli Hospital, Division of Hematology, Amedeo Avogadro University of Eastern Piedmont and Ospedale Maggiore della Carità, Division of Hematology and Bone Marrow Transplantation; Ospedali Riuniti Villa Sofia-Cervello, Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, UO Onco-Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore; Policlinico, Clinical Haematology, Department of Haematology, Transfusion Medicine and Biotechnology, "Spirito Santo"; City Hospital, Hematology and Bone Marrow Transplant Unit, ASST-Papa Giovanni XXIII

The management of adult patients of all ages with Ph+ acute lymphoblastic leukemia (Ph+ ALL) has changed following the introduction of tyrosine kinase inhibitors (TKI). Patients who become minimal residual disease (MRD)-negative have a significantly better survival. To increase the rate of MRD-negative patients, the multicenter phase 2 front-line chemo-free induction/consolidation trial D-ALBA (GIMEMA LAL2116) based on the combination of dasatinib and blinatumomab was designed for patients aged 18 years or older (with no upper age limit). Prior to dasatinib, patients received a steroid pre-phase; dasatinib was administered as induction for 85 days. Patients then received a consolidation treatment with blinatumomab, continuing dasatinib. A minimum of 2 cycles was mandatory, while the administration of up to 3 additional cycles relied on response to treatment and medical decision. CNS prophylaxis was carried out during the entire treatment. Post-consolidation treatment was open.

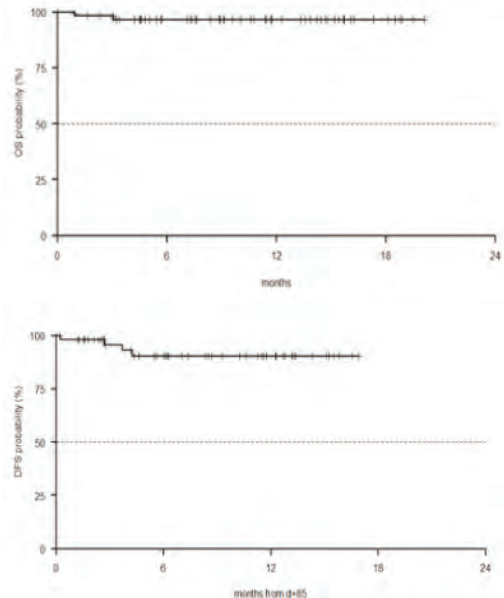


Figure 1. 12-months OS and DFS.

The primary endpoint was the rate of patients who achieved a complete molecular remission (CMR) or a positive non-quantifiable (PNQ) response after 2 cycles of blinatumomab; secondary endpoints included disease-free survival (DFS) and overall survival (OS). Between May 2017 and January 2019, 63 patients were enrolled. Median age was 54.5 years (24.1-81.7), the median white blood cell count (WBC) was 46.2x10⁹/l (8.61-355.2) and 66.1% of cases carried the p190 fusion. Median fol-

low-up is 9.14 months (0-20.1). 61 patients completed induction, 51 the 1st cycle of blinatumomab, 44 the 2nd, 30 the 3rd, 24 the 4th and 16 the 5th cycle. Two patients went off protocol and 1 died during induction. At the end of induction, 16/56 patients (28.6%) had a molecular response (6 CMR and 10 PNQ). At the primary endpoint, 23/42 patients (54.8%) had a molecular response (13 CMR and 10 PNQ); the rate of molecular responses increased after subsequent cycles: 73% and 77% after the 3rd and 4th cycle, respectively. ABL1 mutations were screened in 11 cases with a MRD increase: 6 were WT, while 5 harbored ABL1 mutations: 4 T315I and 1 E255K. All mutations but 1 occurred prior to blinatumomab and were abolished by its administration, confirming its efficacy also in ABL1 mutated cases. Three patients experienced a relapse (1 hematologic, 2 extra-hematologic). Eight patients were allografted: no transplant-related mortality has been recorded. The 12-months OS and DFS are 96.7% and 90.5% (Figure 1).

C057

PROSPECTIVE COMPARISON OF SANGER SEQUENCING VS NEXT GENERATION SEQUENCING FOR ROUTINE BCR-ABL1 KINASE DOMAIN MUTATION SCREENING IN PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

S. Soverini¹, M. Martelli¹, L. Bavaro¹, C. Papayannidis¹, S. Sica², F. Sorà², F. Albano³, S. Galimberti⁴, C. Baratè⁴, M. Rondoni⁵, E. Abruzzese⁶, M. Annunziata⁷, S. Russo⁸, D. Mannina⁹, M. Stulle¹⁰, A. Imovilli¹¹, A. Curti¹, M. Bonifacio¹², L. Scaffidi¹², D. Ferrero¹³, C. Basilico¹⁴, G. Reddiconto¹⁵, G. Mineo¹⁶, M.A. Laginestra¹⁷, S.A. Pileri¹⁸, F. Mignone¹⁹, A. Percesepe²⁰, G. Martinelli²¹, M. Cavo¹

¹Hematology "L.e A. Seràgnoli", University of Bologna; ²Hematology Department, Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore; ³Hematology, Dept. of Emergency and Organ Transplantation - University of Bari; ⁴Hematology, Department of Clinical and Experimental Medicine, University of Pisa; ⁵Hematology, Santa Maria delle Croci Hospital; ⁶Hematology, S. Eugenio Hospital; ⁷Hematology, Cardarelli Hospital; ⁸Internal Medicine, AOU Policlinico di Messina; ⁹Hematology and BMT Unit, San Raffaele Scientific Institute IRCCS; ¹⁰Hematology Unit; ¹¹Hematology, Arcispedale Santa Maria Nuova IRCCS; ¹²Hematology, University of Verona; ¹³A.O.U. Città della Salute e della Scienza, University of Turin; ¹⁴ASST dei Sette Laghi, Presidio di Varese Osp Circolo Fondazione Macchi; ¹⁵Hematology Unit, Vito Fazzi Hospital; ¹⁶Hematology Unit; ¹⁷Unit of Anatomic Pathology, University of Bologna; ¹⁸Hematopathology Unit, European Institute of Oncology; ¹⁹Department of Science and Innovation Technology (DISIT), University of Piemonte Orientale; ²⁰Department of Medicine and Surgery, University of Parma, Medical Genetics Unit; ²¹Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS

Background: BCR-ABL1 mutations are frequent in Philadelphia-positive (Ph+) Acute Lymphoblastic Leukemia (ALL) patients (pts) who are refractory or resistant to tyrosine kinase inhibitor (TKI) therapy. Routinely, Sanger sequencing (SS) is used for mutation screening but Next Generation Sequencing (NGS) may have considerable advantages.

Aims: To assess the feasibility and informativity of NGS as compared to SS for routine BCR-ABL1 mutation screening of a prospective series of de novo and TKI-resistant Ph+ ALL patients (MutationALL study).

Methods: Between May 2015 and February 2019, we used NGS (lower detection limit, 3%) in parallel with SS to analyze a consecutive series of 160 Ph+ ALL pts who were either newly diagnosed (n=44) or had relapsed/refractory disease on TKI therapy (n=116).

Results: De novo pts positive for mutations were 0/44 by SS and 3/44 (7%) by NGS. All the 3 pts received TKIs effective against the low level mutations they had and achieved remission. Relapsed/refractory pts positive for mutations were 71/117 (61%) by SS and 89/117 (76%) by NGS. NGS identified low level mutations in 18 pts who were negative for mutations by SS. All of them had minimal residual disease (MRD)-positivity to 1st- (n=10) or 2nd-line (n=2) therapy or after transplant (n=6). Most importantly, 37/71 (52%) pts showed one or more low burden mutations in addition to the dominant ones detectable by SS. Each low burden mutation detected by NGS could be recognized as poorly sensitive either to the TKI the pt was receiving at the time of testing, or to the previous TKI(s). Out of 37 pts, 28 had hematological relapse and 9 had MRD-positivity. Overall, pts with multiple mutations were 25/71 (35%; up to 4 mutations) by SS and 56/89 (63%; up to 13 mutations) by NGS. Mutation complexity correlated with the number of

lines of therapy received. T315I was the most frequent mutation; it was detected in 35 (50%) of the 71 mutated pts by SS and in 24 additional pts by NGS (66% of mutated pts). NGS could resolve the clonal complexity of 44/51 pts who had multiple mutations at different codons. Among these, 36 pts had one or more (up to 4) compound mutations (CMs). CMs included most frequently T315I or F317L.

Conclusions: In Ph+ ALL, i) approximately half of the pts positive for mutations by SS harbor additional TKI-resistant low level mutations detectable by NGS; ii) CMs are frequent. NGS may thus provide a more accurate picture of mutations complexity, and should replace SS.

C058

DIGITAL-DROPLET PCR AND NEXT-GENERATION-SEQUENCING AS NEW TOOLS FOR MINIMAL RESIDUAL DISEASE ASSESSMENT IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

I. Della Starza, L.A. De Novi, A. Santoro, D. Salemi, O. Spinelli, M. Tosi, M. Cavalli, R. Soccia, V. Apicella, L. Cafforio, A. Vitale, M. Vignetti, F. Fabbiano, A. Rambaldi, R. Bassan, S. Chiaretti, A. Guarini, R. Foà

Hematology, Department of Translational and Precision Medicine, "Sapienza" University, Division of Hematology and Bone Marrow Transplantation, Ospedali Riuniti Villa Sofia-Cervello, Hematology and Bone Marrow Transplant Unit, ASST-Papa Giovanni XXIII, GIMEMA Foundation, Hematology Unit, Ospedale dell'Angelo and Ospedale SS Giovanni e Paolo, Department of Molecular Medicine, "Sapienza" University, Italy

Real-time quantitative PCR (RQ-PCR) is widely used for the molecular monitoring of minimal residual disease (MRD) in both childhood and adult acute lymphoblastic leukemia (ALL). Digital-droplet-PCR (ddPCR) and next-generation-sequencing (NGS) represent advanced molecular tools with the potential to overcome some limitations of conventional approaches and provide additional information. We analyzed by RQ-PCR, ddPCR and NGS 117 ALL follow-up (FU) samples at four time-points, from 44 patients enrolled in the GIMEMA LAL 1913 protocol in order to define the discriminating power of the new methods: RQ-PCR analyses followed the EuroMRD Consortium guidelines, ddPCR and NGS were performed as described (Della Starza, 2016; Cavalli, 2017; Faham, 2012; Kotrova M, 2017). Overall, 94/117 samples (80.4%) resulted concordant by both RQ-PCR and ddPCR, while 23/117 samples (19.6%) were discordant. Most of the discordances occurred positive-not-quantifiable (PNQ) or negative (NEG) samples: 15/117 were RQ-PCR-PNQ, 5 of which were ddPCR-positive-quantifiable (Q) and 10 were ddPCR-NEG. In the remaining 8 discordant FU samples, 6 were RQ-PCR-NEG/ddPCR-Q and a relapse occurred, 1 patient was RQ-PCR-Q/ddPCR-NEG and 1 sample was RQ-PCR-NEG/ddPCR-PNQ and no recurrence was documented. ddPCR significantly reduced the proportion of PNQ samples if compared to RQ-PCR - 1/117 (0.9%) vs 15/117 (13%), p=0.0002 - and also quantified the disease in 8% of samples (6/76) that were RQ-PCR-NEG (Table 1).

Table 1. MRD stratification by RQ-PCR and ddPCR: concordant samples, corresponding to 80, 4%, are highlighted in bold. Among the discordant samples between RQ-PCR and ddPCR, 9 were also analysed by NGS: ^1 sample RQ-PCR-NEG/ddPCR-NEG resulted NGS Q with a MRD level of 10⁻⁵; § 1 sample RQ-PCR-PNQ/ddPCR-NEG was also NGS-NEG; # 3 ddPCR-Q samples have been confirmed Q by NGS; *4 ddPCR-Q samples resulted Q also by NGS.

		RQ-PCR			Total
		Neg	PNQ	Q	
ddPCR	Neg	69[^]	10 [§]	1	80
	PNQ	1	0	0	1
	Q	6 [#]	5 [*]	25	36
Total		76	15	26	117

MRD NGS analyses were performed in 41 samples from 15 patients: 18/41 samples proved Q and 23/41 were NEG. The concordance rate of ddPCR/NGS and RQ-PCR/NGS was 98% (40/41 samples) and 78%

(32/41 samples), respectively. Moreover, 9/41 samples were RQ-PCR/ddPCR discordant: in 4 RQ-PCR-NEG, 3 were ddPCR/NGS-Q, 1 was ddPCR-NEG and NGS-Q and experienced a relapse; 4 RQ-PCR-PNQ/ddPCR-Q resulted NGS-Q and a relapse occurred; 1 RQ-PCR-PNQ sample was ddPCR/NGS-NEG and no recurrence was documented. ddPCR and NGS may be pivotal to univocally define low MRD-positive samples that are a primary unmet need in the FU of ALL. We observed 3 relapses in FU samples classified as PNQ or NEG by RQ-PCR and that proved ddPCR-Q and/or NGS-Q. At variance, no relapses have so far been recorded in patients whose FU samples were RQ-PCR-PNQ and proved ddPCR/NGS-NEG. An advanced MRD assessment (PNQ or NEG vs Q) will help to refine the risk classification of ALL patients and to further optimize treatment algorithms.

C059

PROSPECTIVE DIAGNOSIS OF PH-LIKE ALL ENABLES EFFICIENT TKI THERAPY IN PATIENTS WITH ABL-CLASS ALTERATIONS

I. Tanasi¹, I. Ba³, M. Bonifacio¹, M. Krampera¹, H. Dombret², J. Soulier³, N. Boissel², E. Clappier³

¹Department of Medicine, Section of Hematology, University of Verona; ²Hematology Department, Saint-Louis Hospital, AP-HP; ³Hematology Laboratory, Saint-Louis Hospital, AP-HP

Introduction: Ph-like acute lymphoblastic leukemia (ALL) is a recently identified high-risk subtype of B-precursor ALL lacking known recurrent chromosomal rearrangements and characterized by several genetic abnormalities and poor outcome.

Aims and Methods: In this retrospective, observational, multicenter study, we analyzed a series of 24 Ph-like ALL patients harboring ABL-class rearrangements with absence of complete hematologic response (CHR, n=7) or absence of complete molecular response (CMR, n=12) after induction (frontline cohort), or relapsed disease (n=5). Patients were treated with ABL inhibitors, imatinib, dasatinib or ponatinib, in association to conventional chemotherapy. Ph-like patients were identified according to the multistep strategy implemented at the Hematology Lab of Hopital Saint-Louis (Paris, France).

Results: 23 out of 24 patients (95%) achieved CHR and 17 patients (70%) achieved a MRD level below 10⁻⁴. In the frontline cohort 7 out of 8 primary refractory patients subsequently achieved CR. The 3-years OS for patients in the frontline cohort (n=19) was 77% (95%CI: 50-91). All patients treated at relapse (n=5) achieved CHR, including two patients who were refractory to several lines of therapy. A MRD level below 10⁻⁴ was achieved in 3 patients of whom two could proceed to HSCT and remained alive in remission.

Conclusions: We report the largest cohort of patients with Abl-family kinase rearrangement exposed to diverse TKI frontline or at relapse. The prospective diagnosis allows the addition of TKI to conventional chemotherapy in Ph-like ALL patients which seems to be of particular benefit for patients treated frontline.

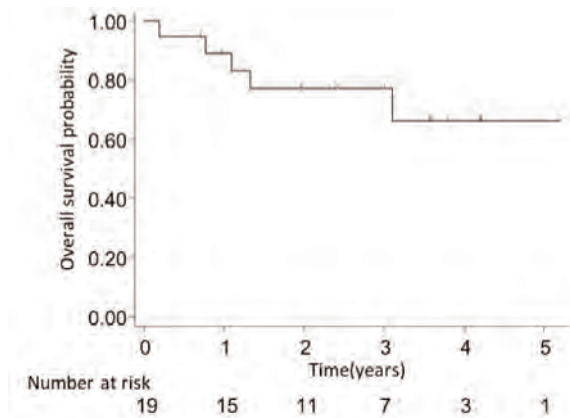


Figure 1.

C060

MOLECULAR CHARACTERIZATION OF THE MECHANISMS OF UNRESPONSIVENESS TO BLINATUMOMAB IMMUNOTHERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

V. Robustelli¹, C. Papayannidis¹, S. Vitali³, D. Remondini³, C. Sartor¹, G. Martinelli⁴, M.C. Abbenante⁵, G. Marconi¹, A. Curti², S. Paolini¹, A. Ghelli Luserna Di Rorà⁴, A. Ferrari⁴, M. Cavo¹, C. Terragna²

¹Università degli studi di Bologna - DIMES; ²Istituto di Ematologia L. e A. Seragnoli - Policlinico Sant'Orsola-Malpighi; ³Università degli studi di Bologna - DIFA; ⁴IRST - Istituto scientifico romagnolo per lo studio e la cura dei tumori; ⁵Ospedale Casa Sollievo della sofferenza, Italy

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease characterized by the sequential acquisition of genetic aberrations driving the leukemic clone's onset and maintenance. Although standard treatments have improved disease prognosis, most adult patients (pts) with B-cell ALL have worse outcome and finally relapse. The introduction of monoclonal antibodies (Ab) has both increased OS rates and reduced the need of intensive and prolonged chemotherapy in relapsed/refractory (R/R) ALL. Blinatumomab (BLIN) is a BiTE Ab construct that redirects CD3-expressing T-cells to CD19-expressing leukemic cells. To describe both the immunologic and the cellular mechanisms potentially involved in BLIN resistance we molecularly characterized BLIN-responder vs non responder B-ALL pts. The study included 18 B-ALL pts (median age 40), previously enrolled in Alcantara, Tower and compassionate protocols (as approved by Sant'Orsola-Malpighi EC). Molecular response was assessed by qPCR. GEP (HTA 2.0, Affymetrix) was performed on mononuclear cells isolated from 50 BM samples collected at baseline and after treatment. An original bio-informatic analysis pipeline was designed, which employs the linear mixed model (LMM) in order to compare responder vs non-responder expression profile, by taking into account all the possible bias interfering with the results. Eight pts responded to BLIN treatment, whereas 10 did not. By employing the LMM, the expression level (logratio >0/logratio <0), the FDR (cut-off 0.05) and the p-value (cut-off 0.05) of each gene, differentially expressed between responder and non-responder, was evaluated. A Principal Component Analysis (PCA) based on the 196 top ranked genes by LMM significantly labeled responder as compared to non-responder pts (Figure 1). The 150 genes with FDR <0.05 and p-value <0.05 potentially involved in resistance mechanisms were included in immune system regulation (CD200, CD86, CD4), cell-cycle checkpoint regulation (CDK6, CDK9), apoptosis modulation (BCL2) and B-cell development (CD34, CD22, CRLF2) pathways. The expression profile of pre-treated ALL pts highlighted both leukemic cell-related and immune system-related molecular features associated to BLIN resistance, which might suggest novel targets for combination therapy. A validation of results on larger cohorts of pts will be performed.

Acknowledgements: ELN, AIL, AIRC, Fond. del Monte BO-RA, RFO2018, Harmony EU Project, SYNtherapy EU Project

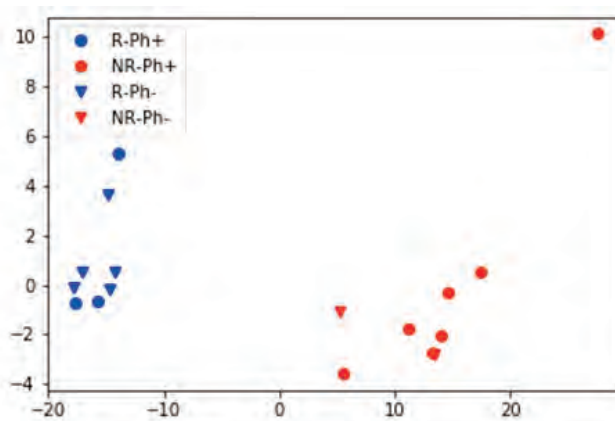


Figure 1.

Chronic Lymphatic Leukemia 2

C061

THE TREATMENT OF HAIRY CELL LEUKEMIA WITH A FOCUS ON LONG LASTING RESPONDERS TO CLADRIBINE: A THIRTY-YEAR EXPERIENCE FROM THE INSTITUTE OF HEMATOLOGY OF BOLOGNA

A. Broccoli, G. Gabrielli, C. Terragna, V. Stefoni, C. Pellegrini, B. Casadei, M. Marangon, A. Morigi, L. Nanni, G. Lolli, M. Carella, N. Fabbri, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

The treatment of hairy cell leukemia (HCL) has deeply changed over years. Purine analogs, namely cladribine (2CdA) now represent the treatment of choice. The BRAF V600E mutation is now regarded as the pathogenic event. One hundred and eighty-four patients were followed between 1986 and 2018 and treated according to era-specific guidelines. This is the largest monocentric series reported. Responses were classified by combining Consensus Resolution criteria and marrow immunohistochemistry. Patients were grouped according to the number of treatment lines they received (Table 1). Ten patients treated with front-line 2CdA and in complete response (CR) for at least 5 years were tested for the presence of the BRAF V600E mutation in peripheral blood by droplet digital PCR as a molecular marker for active disease.

Patients treated first line responded in 86% of cases, with 44% CR. Response rates remained high throughout the first 4 lines (84%, 81%, 79% for the second line onward, with CR in 38%, 37%, 15% of cases respectively), although decreasing progressively with the number of treatments received. One hundred and twenty-two patients received 2CdA as first line treatment, with a response rate of 86% and a CR rate of 54%. Among the 66 CR patients, 45 (68%) have never received further therapy: 11 patients are in continuous CR between 5 and 10 years after treatment, 14 between 10 and 20 years and 3 patients at more than 20 years. Median time-to-next treatment (TTNT) for patients receiving 2CdA was 8.2 years: partial responders had a significantly shorter median TTNT than CR patients (5.3 years versus median not reached at 25.8 years, $p=0.0001$). Seven patients in CR for more than 5 years after front line 2CdA were BRAF V6500E negative in peripheral blood. One of these displayed disease recurrence and required further treatment roughly 2 years later. Three patients were positive for the BRAF V600E mutation at 6.5, 8.4 and 13.7 years after treatment and developed an overt disease relapse between 4 months and 2 years.

In conclusion, patients with HCL require subsequent lines of therapy in more than 50% of cases. Purine analogs allow significant response rates when applied first line and upon retreatment. Some patients may enjoy long lasting responses after one course of 2CdA and display no evidence of BRAF V600E mutation in peripheral blood. A PCR-based evaluation of the allelic burden in peripheral blood may provide information regarding disease activity over time.

Table 1.

	I line 184 pts	II line 100 pts	III line 48 pts	IV line 33 pts	V line 18 pts	VI line 12 pts	VII line 8 pts	VIII line 5 pts
2CdA	122	67	28	18	3	2	1	0
Pentostatin	12	7	4	3	3	0	0	1
Interferon	40	11	4	3	2	1	2	0
Rituximab	0	12	9	7	5	4	0	1
Splenectomy	9	3	1	0	1	1	0	0
Vemurafenib	0	0	2	0	1	4	3	0
Moxetumomab	0	0	0	1	2	0	1	1
Chlorambucil	1	0	0	0	1	0	0	0
Cobimetinib + vemurafenib	0	0	0	0	0	0	0	1
Steroid	0	0	0	0	0	0	0	1
Rituximab + vemurafenib	0	0	0	1	0	0	0	0
Allogeneic transplant	0	0	0	0	0	0	1	0

C062

JAK2/STAT3 PATHWAY INHIBITION REDUCES CHRONIC LYMPHOCYTIC LEUKEMIA CELL VIABILITY

F. Severin, F. Frezzato, S. Pizzo, V. Trimarco, A. Visentin, S. Imbergamo, G. Semenzato, M. Facco, L. Trentin

Department of Medicine University of Padua, Veneto Institute of Molecular Medicine (VIMM), Padova, Italy

In Chronic Lymphocytic Leukemia (CLL), tumor progression is regulated by intrinsic events as well as by factors coming from the surrounding microenvironment. Several molecules released by microenvironmental partners signal through JAK (Janus kinases)-STAT (signal transducers and activators of transcription) pathway. The deregulation of JAK2/STAT3 axis may lead to aberrant activation of STAT3 and, as a result, to tumor development. By using specific inhibitors and co-culture systems, we are aimed at characterizing how the inhibition of the JAK2/STAT3 axis influences CLL lymphocyte viability and whether co-treatment with the currently used drug Ibrutinib can be boosted by targeting this pathway. B cells were collected from peripheral blood of 23 controls and 53 CLL patients and Mesenchymal Stromal Cells (MSCs) were obtained from 17 CLL patient bone marrow. We demonstrated that STAT3 was highly expressed in malignant with respect to normal B cells (1.3 ± 0.9 vs 0.6 ± 0.4 ; $p < 0.001$ Student's t Test) independently from prognosis. We showed that JAK2/STAT3 inhibitors AG490, Fedratinib and Stattic were able to induce a dose-dependent apoptosis in CLL cells bypassing microenvironment protection as we did not observed any differences in cell viability in CLL B cell cultured with JAK/STAT inhibitors as compared to leukemic B cells cultured with inhibitors in the presence of MSCs. AG490 and Fedratinib, targeting JAK2, inhibited the phosphorylation of SHP-1 at Ser591, activating the phosphatase. In turn, SHP-1 activation leads to Lyn Tyr396 inactivation. Treatment with Stattic did not affect Lyn and SHP-1 phosphorylation since this inhibitor acts downstream with respect to AG490. In fact, simultaneous administration of Ibrutinib leads to an increase of apoptosis only in Stattic ($62 \pm 23\%$ of apoptotic cells with Ibrutinib + Stattic vs $18 \pm 17\%$ with Stattic; $p < 0.01$, paired Student's t Test), but not in AG490 treated cells in presence or absence of MSCs. This confirms a possible dual role of JAK2 inhibition. Moreover, these data suggest that JAK2/STAT3 inhibitors could potentiate Ibrutinib administration effects disrupting microenvironmental sustainment. JAK2/STAT3 expression and the apoptosis due to AG490, Fedratinib and Stattic administration let us to demonstrate the importance of JAK2/STAT3 axis in the survival of the neoplastic clone. Furthermore, the strengthening of Ibrutinib effect could represent a starting point for the development of new therapeutic strategies in CLL.

C063

IBRUTINIB TREATMENT IS ASSOCIATED WITH A SHIFT OF THE TH2/TH1 T-CELL BALANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

M.C. Puzzolo, P. Mariglia, I. Del Giudice, N. Peragine, M.S. De Propriis, A. Piciocchi, V. Arena, F.R. Mauro, A. Guarini, R. Foà

Hematology-Department of Translational and Precision Medicine- Sapienza University; Department of Molecular Medicine- Sapienza University; GIMEMA Data Center- GIMEMA Foundation, Italy

Due to a significant homology between Bruton tyrosine kinase (BTK) and IL-2-inducible kinase (ITK), ibrutinib, by targeting ITK, may contribute to an *in vivo* T-cell polarization by reverting the Th2-dominant response observed in chronic lymphocytic leukemia (CLL). In order to gain insight into the role of ibrutinib in the Th2/Th1 T-cell balance, we investigated the modulation of T-cell cytokine production in CLL patients before and during ibrutinib treatment. Peripheral blood samples from 71 CLL patients enrolled in the front-line GIMEMA LLC1114 trial (ibrutinib+rituximab for 6 months, followed by ibrutinib maintenance) were evaluated in a longitudinal analysis for the T-cell cytokine production. Cytokine production of activated T cells was analyzed by flow cytometry using the Human Th1/Th2/Th17 Phenotyping Kit (BD Bioscience, San Jose, CA). Differences were evaluated by non-parametric tests (Wilcoxon, Chi-Squared and Fisher Exact). A total of 208 samples were analyzed at baseline and at subsequent time points under ibrutinib treatment: 71 samples prior to treatment (T0) and thereafter at day +14 (T14D; n=50), at month +8 (T8M; 30), month +12 (T12M; 25), month +18 (T18M; 22) and month

+24 (T24M; 10). Overall, we documented a progressive increase in the median percentage of CD3+CD4+IFN + T cells (Th1), with a decrease of CD3+CD4+IL-4+ T cells (Th2). The Wilcoxon paired test showed a significant decrease of the Th2/Th1 ratio already after 14 days of ibrutinib treatment that was maintained up to month +18 (T0 vs T14D $p=0.037$, T0 vs T8M $p=0.001$, T0 vs T12M $p=0.005$, T0 vs T18M $p=0.002$). In particular, Th2 cells were significantly reduced from T8M and persisted at T12M and T18M (T0 vs T8M $p=0.007$, T0 vs T12M $p=0.012$, T0 vs T18M $p=0.039$), whilst Th1 cells increased ($p=0.03$) and Th17 cells decreased ($p=0.006$) at T18M of ibrutinib treatment (Figure 1). ROC curve analysis showed a correlation between the decrease of the Th2/Th1 ratio below a cut-off value of 0.088 and the achievement of a complete response (CR) ($p=0.016$). No further significant correlations between the Th2/Th1 ratio and the baseline characteristics of CLL patients were found. Ibrutinib may shape the T-cell profile of CLL, limiting Th2 activation and polarizing T cells towards a Th1 phenotype after 18 months of treatment. The association between the decrease of the Th2/Th1 ratio and progressive CR achievement suggests the generation of a potential host anti-tumor immune activation.

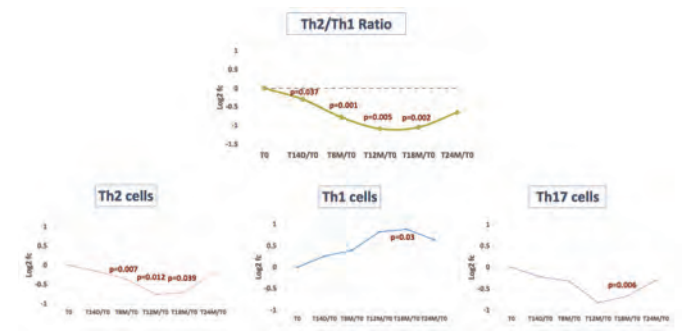


Figure 1.

C064

THE AGE, COMORBIDITY, ALBUMIN (ACA) SCORE IN ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IS AN EFFECTIVE TOOL FOR COMPREHENSIVE GERIATRIC ASSESSMENT

S. Molica¹, D. Giannarelli², L. Levato¹, M. Lentini¹, D. Levato¹, D. Zappalà¹

¹Dipartimento Onco-ematologico, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro; ²Unità di Biostatistica, IRCCS Regina Elena, Roma, Italy

The age, comorbidity, albumin (ACA) score, was originally developed in pts with DLBCL treated with R-CHOP. We attempted its validation in 108 elderly (65 years) CLL pts followed up at our institution. Median age of pts was 71 years (range, 65–90). According to Rai staging system 48 (44.4%) pts were in stage 0, 42 (38.8%) in stage I–II, and 18 (16.6%) in stage III–IV. Eighteen pts (16%) had a Cumulative Illness Rating Score (CIRS) score > 6, 14 (13%) Activity of Daily Living (ADL) score < 4, and 16 (15%) an Instrumental Activity of Daily Living (iADL) score < 5. Based on ACA score, which comprises 3 risk factors such as age >75 yrs, albuminemia <3.7g/dL, and Charlson Comorbidity Index [CCI] score > 3, pts were categorized into “excellent” (0 points), “good” (1 point), “moderate to poor” (2–3 points) groups. Median OS was not reached in pts of “excellent” group, while it was 113 and 87 mo. for pts of “good” and “moderate to poor” group, respectively ($p=0.0004$). An analysis of characteristics of pts stratified according to ACA score suggests that cases with “moderate to poor” score were more frequently older ($p<0.0001$), females ($p<0.01$), had higher B2-M levels ($p=0.02$), lower creatinine clearance ($p<0.0001$), ECOG PS > 1 ($p<0.0001$), higher CIRS score ($p<0.0001$), ADL < 4 ($p<0.002$) and iADL < 5 ($p<0.0001$). The same did not apply for high risk genetic features (i.e., U-IGHV and/or 11q del or 17 del) ($p=0.59$) equally found in pts with “excellent”, “good” and “moderate to poor” group ACA score ($p=0.59$). Limitations of ACA score are the followings: a) it does not include evaluation for functional status (i.e., ADL); b) it uses for the assessment of comorbidities CCI instead of CIRS score. We compared performance of ACA score with a frailty score which includes CIRS score and ADL. This previously developed model based on age >75 yrs, CIRS > 4 and ADL < 5 predicts for OS (median OS of fit, intermediate fit and

frail group was 146, 82 and 52 mo. $p<0.0001$). A comparison of OS performance between ACA score and frailty score gave similar Results: c-statistic (frailty score, 0.66; 95% CI, 0.50–0.82; ACA score, 0.68; 95% CI, 0.51–0.83; Akaike information criterion (AIC), 374 for both scores). In conclusion, ACA score is a simple and effective tool of comprehensive geriatric assessment in CLL able to predict clinical outcome of CLL pts. Performance of ACA is similar to that a frailty score which includes more time consuming tests such as ADL and CIRS.

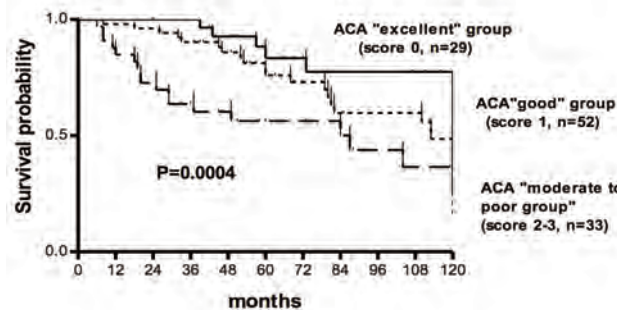


Figure 1.

C065

HYPOGAMMAGLOBULINEMIA AND INFECTIONS IN IBRUTINIB-TREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS: A PROSPECTIVE STUDY

R. Cassin¹, A. Noto¹, I. Silvestris¹, M. Barbieri¹, E. Cappello², S. Lonati¹, L. Baldini², A. Neri², G. Reda¹

¹Hematology Department, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan; ²Hematology Department, Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Italy

Background: Hypogammaglobulinemia (HypoG) affects up to 85% of patients with chronic lymphocytic leukemia (CLL) and contributes to susceptibility to infections. These have been reported in 56–82% of patients during ibrutinib therapy. IgG levels seem to remain stable during the first 6 months of therapy, but decrease thereafter. A transient increase in IgM and a sustained increase in IgA have been observed.

Aim: We evaluated changes in immunoglobulin levels and infection rate in a real life monocentric ibrutinib-treated CLL population.

Patients and methods: Between July 2014 and December 2018 we prospectively evaluated 58 CLL patients treated with ibrutinib at our Centre. At definite time points (before starting therapy, and after 6, 12, 18, 24, 36 months of therapy) we evaluated IgA, IgG and IgM levels. For suspected infection, we carried out analysis in order to highlight etiology. Infectious events were collected per CTCAE Version 4.03 grading. Primary endpoint was to describe overall infection rate and type, secondary endpoint to assess if HypoG could impact on infection occurrence during ibrutinib therapy.

Results: Fifty-eight patients were treated with ibrutinib in clinical practice. They were mainly males (69%), with a median age of 70 years and a number of previous therapies of 2.2. FISH was positive for 11q/17p deletion in 30% and IGHV unmutated in 53% of cases. Most patients (94%) received trimethoprim/sulfamethoxazole prophylaxis, while only 44% received acyclovir. At a median follow-up of 26.8 months, 31/58 (53%) of patients developed 44 infections: 90% were grade < 2, while 10% were grade > 3 events (one death in a patient with diagnosis of lung cancer). Respiratory tract was the most commonly involved site. All classes of immunoglobulin underwent progressive reduction: after 24 months of ibrutinib therapy, IgA were 63%, IgG 48% and IgM 56% of baseline. IgA < 70 mg/dl and IgM < 40 mg/dl were not associated with an increased risk of infection in our population. Unexpectedly, patients with IgG > 500 mg/dl had higher infection rate (61% versus 29%, $p=0.03$) than patients with IgG < 500 mg/dl. The former were less pretreated than the latter (0.97 versus 2.07 number of previous therapy) and time to infection was lower in the first group than in the second (13.3 vs 17.23 months).

Conclusions: HypoG doesn't impact on infection occurrence in ibrutinib treated patients. Prompt evaluation of suspected infection can reduce its rate and severity.

Myeloproliferative Neoplasms 2

C066

HOW RUXOLITINIB CAN CHANGE THE MIRNAS LANDSCAPE IN MYELOFIBROSIS

S. Galimberti, C. Baratè, F. Ricci, S. Balducci, F. Guerrini, S. Grassi, E. Ciabatti, S. Barsotti, M. Petrini

Dipartimento di Medicina Clinica e Sperimentale, Sezione di Ematologia, Università di Pisa, Italy

Background: MicroRNAs (miRNAs) are a family of small non coding RNAs that have been associated with many cell functions in several hematological diseases. In myelofibrosis (MF), it has been reported that 48 miRNAs were expressed in a different way in respect of healthy donors, and that hyper-expression of miR-494 might contribute to the megakaryocyte hyperplasia.

Aim: Because it is not yet clear what modulation of miRNAs can occur during treatment with ruxolitinib, we decided to identify eventual changes of miRNAs expression during ruxolitinib therapy.

Methods: miRNAs were extracted from bone marrow and peripheral blood samples from 4 patients with MF before starting ruxolitinib and after 3 months of therapy. As controls, 15 peripheral blood samples from healthy subjects and one sample from a bone marrow donor were employed. The expression of 675 miRNAs was assessed by the TaqMan@Low Density Arrays kit (ThermoFisher) and miRgator software was used to identify the respective target genes.

Results: No differences in terms of miRNAs expression levels were observed between bone marrow and peripheral blood. After 3 months of ruxolitinib, 9 miRNAs resulted up-regulated: miR-25, miR-145, miR-186, miR-142-3b, let-7b, miR-30d, miR-188, miR-942, and miR-378. Among targets genes of these miRNAs there are JAK2, MPL, TET2, ASXL1, CBL, IDH1, IDH2, IKZF1, EZH2. miRNA-145 has been reported to be up-regulated in PV, and let-7b is inversely correlated with JAK2V617F mutation. Moreover, miR-188 has been reported to suppress macrophage oxidation and plasma expression of pro-inflammatory factors. This anti-inflammatory effect could be also sustained by the miR-186, that has been reported to inhibit HIF-1alpha factor, involved in the inflammatory processes, and by the up-regulation of miR-145, that seems to inhibit TGFBR2 and reduce sepsis in a murine model. Moreover, the over-expression of miR-30d has been associated with a reduced proliferation in colon cancer, and miR-142 high levels play an anti-oncogenic activity in breast cancer. If it would be true also in MF, we could argue that ruxolitinib could also exert an anti-proliferative effect.

Conclusions: Even if only preliminary and on a very small number of cases, this study supports the possible role of miRNAs in the modulating the expression of genes involved in pathogenesis and progression of MF during treatment with ruxolitinib. This could help to better understand the action mechanisms of this important drug.

C067

IN SYSTEMIC MASTOCYTOSIS, MIDOSTAURIN TARGETS BOTH KIT AND AURORA KINASE A REVERTING H3K36ME3 DEFICIENCY AND SYNERGIZES WITH SECOND-GENERATION TYROSINE KINASE INHIBITORS

M. Mancini, C. Monaldi, S. De Santis, C. Papayannidis, M. Rondoni, L. Bavaro, M. Martelli, M.C. Abbenante, A. Curti, M.C. Fontana, R. Zanutti, M. Bonifacio, L. Scaffidi, L. Pagano, M. Criscuolo, F. Albano, F. Ciceri, C. Elena, P. Tosi, M. Delledonne, C. Avanzato, L. Xumerle, P. Valent, G. Martinelli, M. Cavo, S. Soverini

Department of Experimental Diagnostic and Specialty Medicine - DIMES, Institute of Hematology "L. e A. Seràgnoli", University of Bologna, Azienda USL della Romagna, Azienda Ospedaliera di Verona, Fondazione Policlinico Universitario A. Gemelli, Dipartimento dell'Emergenza e dei Trapianti di organi (DETO), Università di Bari, Servizio Immunoematologia Trasfusionale (SIMT), IRCCS Ospedale San Raffaele, Fondazione I.R.C.S.S. Policlinico San Matteo, U. O. di Oncologia ed Oncoematologia-Osp. Infermi Azienda Unità Sanitaria-Locale di Rimini; Dipartimento di Biotecnologie, Università degli Studi di Verona, Department of Hematology, Internal Medicine I, Medical University of Vienna, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola (FC)

We have previously reported that the HMC-1.1 and -1.2 mast cell leukemia (MCL) cell lines and many patients (pts) with advanced systemic mastocytosis (advSM) display H3K36Me3 deficiency as a result of non-genomic loss of function of SETD2. Proteasome inhibition restored SETD2 protein expression and H3K36me3, suggesting that a functional protein is produced but rapidly degraded. To understand the mechanisms underlying this phenomenon, we used an in silico approach to identify candidate SETD2-interacting proteins, followed by experimental confirmation by co-immunoprecipitation. We found that, after proteasomal inhibition, SETD2 co-immunoprecipitates with Aurora Kinase A (AKA). We also found that AKA was overexpressed and hyper-activated in 20 pts with advSM compared to 10 ISM pts and a pool of healthy donors, and that AKA can phosphorylate SETD2. Both pharmacological inhibition by Danusertib and siRNA-mediated knock-down of AKA rescued SETD2 expression and activity, raising the hypothesis that phosphorylation by AKA might be involved in proteasome-mediated degradation of SETD2. A forthcoming new standard of therapy in advSM is midostaurin, able to inhibit the activity of both wild type and D816V mutant KIT, as well as of various other kinases including AKA. Therefore we investigated if midostaurin effects may be addressed to AKA inhibition and consequent SETD2 restoration. To the purpose HMC-1 cells were treated with 5 μ M midostaurin for 24 h and AKA, SETD2 and H3K36me3 expression were evaluated by western blotting. Treatment with midostaurin was able to inhibit AKA activity by about 60%, partially restoring SETD2 expression and H3K36Me3. Moreover, midostaurin treatment of HMC-1 cells at μ molar doses induced cytostatic but not cytotoxic effects as shown by cell growth curves performed in liquid medium. Finally, we performed growth curves in liquid medium and clonogenic assays to evaluate the therapeutic potential of pharmacological combination of midostaurin with Nilotinib and Dasatinib in HMC-1 cells and in neoplastic mast cells from 3 patients with advSM and we observed in all cases synergistic effects at nM doses. Our results suggest that AKA-mediated post-translational modifications contribute to SETD2 non-genomic loss of function in advSM. Inhibiting AKA and c-Kit activity by midostaurin associated with a second generation TKI is a promising therapeutic strategy in patients with low SETD2 expression levels.

Supported by AIRC (project 16996) and AIL.

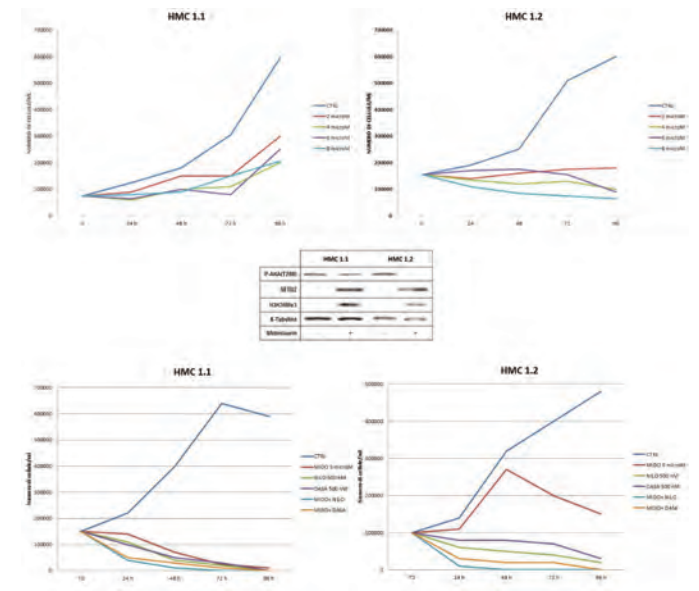


Figure 1.

C068

USE OF DIRECT ORAL ANTICOAGULANTS IN MYELOPROLIFERATIVE NEOPLASMS: A SINGLE CENTER EXPERIENCE

R. Renzo¹, V. Coccini², M. Carpenedo¹, A. Carrer¹, F. Rossi², C. Gambacorti-Passerini³, E.M. Elli¹

¹Hematology Division San Gerardo Hospital; ²Department of Transfusional Medicine San Gerardo Hospital; ³Hematology Division San Gerardo Hospital, Department of Medicine and Surgery University of Milano-Bicocca, Italy

Myeloproliferative neoplasms (MPN) are clonal disorders with high incidence of venous (VTE) and arterial thrombotic events (TE). Direct oral anticoagulants (DOAC) have been approved to treat and prevent TE and are potentially associated with a lower rate of bleeding with respect to vitamin K-antagonist (VKA). However, there are few evidences for use of DOAC in patients (pts) with active cancer, in particular in MPN setting. Here we analysed efficacy and safety of DOAC in MPN pts. We retrospectively identified from our MPN database of 792 pts, 38 (4.8%) treated with DOAC between 2013 and 2019. We collected biological and clinical data from diagnosis of MPN to last follow-up. The evaluation of TE and haemorrhagic events (HE) were performed according to current guidelines. Patient features are shown in Table 1. The majority of pts were JAK2V617F positive (84.2%). DOAC started after a median of 6 (1-27) years from MPN diagnosis, with a median age at start of DOAC (baseline) of 80 (41-98) years. Only 3 pts started DOAC before MPN diagnosis. DOAC were prescribed at standard dose and included: Dabigatran (9), Rivaroxaban (5), Apixaban (22), Edoxaban (2). Indications for DOAC use were non valvular atrial fibrillation (NVAf) in 28 and VTE in 10 pts, respectively. VTE included 5 pulmonary embolism, 4 deep and 1 recurrent superficial venous thrombosis. 29 (76.3%) pts received DOAC *de novo*, while 9 pts shifted therapy from VKA (all NVAf).

Table 1. Principal clinical and biological features in MPN patients treated with DOAC.

Sex, female/male, n (%)	25/13 (65.8%/34.2%)
Median age at diagnosis, years (range)	71 (40-92)
Myeloproliferative neoplasms (MPN), n (%)	
- Primary Myelofibrosis (PMF)	7 (18.4%)
- Polycythemia Vera (PV)	8 (21.0%)
- Essential Thrombocythemia (ET)	16 (42.2%)
- post-PV Myelofibrosis	3 (7.9%)
- post-ET Myelofibrosis	4 (10.5%)
Driver mutations, n (%)	
- JAK2 V617F	32 (84.2%)
- Calreticulin	4 (10.5%)
- MPL	1 (2.6%)
- no known mutations	1 (2.6%)
Median age at baseline, years (range)	80 (41-98)
Indications for DOAC treatment, n (%)	
- non valvular atrial fibrillation (NVAf)	28 (73.7%)
- thrombotic events: Pulmonary Embolism (PE)	5 (13.2%)
Deep venous thrombosis (DVT)	4 (10.5%)
Recurrent superficial venous thrombosis	1 (2.6%)
DOAC, n (%)	
- Dabigatran	9 (23.6%)
- Rivaroxaban	5 (13.2%)
- Apixaban	22 (58.0%)
- Edoxaban	2 (5.2%)
Cytoreductive therapy at baseline, n (%)	
- Hydroxyurea	29 (76.4%)
- Busulfan	3 (7.9%)
- Ruxolitinib	1 (2.6%)
- none	4 (10.5%)
- other	1 (2.6%)
Cause of DOAC suspension, n (%)	
- haemorrhagic event associated with renal failure	7 (18.4%)
- intolerance (cutaneous rash)	2 (5.2%)
- other	1 (2.6%)
- other	4 (10.5%)
Haemorrhagic events (HE), n (%)	
- epistaxis	5 (13.1%)
- gastrointestinal bleeding	2 (5.2%)
- other	3 (7.9%)
Median follow-up from diagnosis, months (range)	77 (8-337)
Median follow-up from DOAC start, months (range)	22 (1-66)
Status at follow-up, n (%)	
- alive	29 (76.4%)
- dead	6 (15.8%)
- lost to follow-up	3 (7.9%)

MPN: Myeloproliferative neoplasms; DOAC: Direct oral anticoagulants

At baseline, 34 (89.5%) pts were under cytoreductive therapy, mainly represented by hydroxyurea (29); 9/10 pts treated with DOAC for VTE already received cytoreduction. However, after a median follow-up of 22 (1-66) months from baseline, no pts had recurrent TE under DOAC. 7 (18.4%) pts permanently stopped DOAC after a median time of 21 (1-66) months. The causes of suspension were: HE with associated renal failure (2), cutaneous rash (1) and other (4). Overall, we recorded no TE and 5 HE (13.1%), only one clinically relevant. 3/5 pts restarted DOAC after HE, without recurrent bleeding. There were no statistically significant differences in terms of the main clinical and biological features between pts who experienced HE or not. At time of analysis, 6 pts (15.8%) died, none for reasons attributable to DOAC use. In conclusion, our preliminary results suggest that DOAC are safe and efficient in MPN pts. A matched case-control study is starting in our institution in order to compare efficacy and safety of DOAC vs VKA in MPN setting.

C069

FAMILIAL OCCURRENCE OF SYSTEMIC AND CUTANEOUS MASTOCYTOSIS IN AN ADULT MULTICENTER SERIES: A REPORT OF 22 CLUSTERED CASES

I. Tanasi¹, M. Bonifacio¹, M. Pizzolato², F.I. Grifoni³, M. Sciumè³, C. Elena^{4,5}, P. Benvenuti^{4,5}, F. Mannelli^{6,7}, R. Parente⁸, D. Schena², L. Scaffidi¹, P. Bonadonna⁹, C. Papayannidis¹⁰, M. Rondoni¹¹, M. Criscuolo¹², A.M. Vannucchi^{6,7}, M. Triggiani⁸, G. Martinelli¹³, M. Krampera¹, R. Zanotti¹

¹Department of Medicine, Section of Hematology, University of Verona; ²Department of Medicine, Section of Dermatology, Azienda Ospedaliera Universitaria Integrata di Verona; ³Hematology Unit, Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico; ⁴Department of Molecular Medicine, University of Pavia; ⁵Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo; ⁶CRIMM Center for Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliera Universitaria Careggi; ⁷Department of Experimental and Clinical Medicine, University of Florence; ⁸Department of Medicine, Division of Allergy and Clinical Immunology, University of Salerno; ⁹Allergy Unit, Azienda Ospedaliera Universitaria Integrata di Verona; ¹⁰Department of Experimental, Diagnostic and Specialty Medicine, Hematology/Oncology 'L. e A. Seragnoli', University of Bologna; ¹¹Hematology Unit, Ospedale Santa Maria delle Croci; ¹²Department of Radiological, Radiotherapeutic and Hematological Sciences, Fondazione Policlinico Universitario A. Gemelli IRCCS; ¹³Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Italy

Background: Mastocytosis refers to a group of clonal disorders characterized by abnormal proliferation of mast cells infiltrating various tissues. The clonal nature of mastocytosis has been largely demonstrated by the identification of gain-of-function mutations in exon 17 causing constitutive activation of the c-KIT protooncogene. In the large majority of patients with systemic disease a somatic aspartate-to-valine substitution in codon 816 (D816V) of the KIT gene is demonstrable. Typically, mastocytosis is considered a non-hereditary disease, although clustered cases were reported in pediatric series with an estimated frequency of 8-10%, in the absence or presence of c-KIT mutations, generally located outside the exon 17. Prevalence data about familial cases in adults are lacking. To our knowledge, somatic D816V KIT mutation was reported only in two adult clusters and in two pediatric familial cases.

Aims: In this retrospective, observational, multicenter study we sought: a) to estimate the prevalence of familial disease in a series of 1541 patients with mastocytosis referred in adulthood (>16 years old) to 8 Italian Institutions, and b) to describe the clinical and molecular characteristics of clustered cases.

Methods: Diagnosis of mastocytosis was made according to the 2016 World Health Organization criteria. All patients were asked for the occurrence of the same disease in relatives. Clinical and molecular data were recorded in all patients and in the affected relatives, when available.

Results: Familial occurrence of mastocytosis was reported in 22 patients (1.4% of the whole cohort). Clinical and molecular characteristics are reported in Table 1. Index cases were 12 males and 10 females, median age at diagnosis was 43 years (range 16-65 years). Diagnosis in index patients was systemic mastocytosis (SM) (n=15), mastocytosis in the skin (MIS) (n=3), and maculopapular cutaneous mastocytosis (MPCM) (n=4). All but one SM patients harbored D816V KIT mutation.

Each index case reported a single affected relative: they were 14 males and 8 females, with median age at diagnosis of 20 years (range 0-70 years). Diagnosis in relatives was SM (n=7), MIS (n=3), MCPM (n=6) and Mastocytoma (n=6). The relationship between index and relatives was mainly "parent and child" (41%) and "siblings" (27%). Notably, 4 SM patients had one child who developed single or multiple mastocytoma; in other two families index and/or relatives developed an associated hematological neoplasia.

Table 1. Clinical and molecular features of patients with familial mastocytosis.

Pat. N°	Sex	Diagnosis (WHO 2016)	Age at diagnosis (years)	Age at symptoms' onset (years)	KIT mutation (BM or PB)	Relative's degree	Diagnosis	Age at diagnosis (years)	KIT mutation
1	F	ISM	55	17	D816V (BM)	Uncle	ISM	70	NA
2	M	MIS	21	3	ND	Father	MIS	20	NA
3	M	ISM	65	54	D816V (BM)	Nephew	Mastocytoma	Birth	NA
4	F	SM-AHN (MPN)	63	53	D816V (PB)	Sister	SM-AHN (CML)	NA	NA
5	F	ISM	43	38	D816V (BM)	Brother	SM-AHN (PV)	43	D816V (PB)
6	M	ISM	61	41	D816V (BM)	Cousin	MIS	40	NA
7	M	BMM	70	70	D816V (BM)	Cousin	MPCM	41	WT (BM)
8	F	MPCM	18	6	WT (BM)	Brother	MPCM	NA	NA
9	M	BMM	33	32	D816V (BM)	Son	Mastocytoma	0.5	NA
10	M	BMM	49	49	D816V (BM)	Daughter	Mastocytoma	4	NA
11	M	ISM	48	31	D816V (BM/PB)	Son	ISM	44	D816V (BM)
12	M	ISM	48	17	D816V (BM)	Mother	MIS*	NA	NA
13	F	BMM	62	54	D816V (BM)	Son	BMM	38	D816V (BM)
14	F	ISM	44	37	D816V (BM/PB)	Daughter	Mastocytoma	1	NA
15	M	ISM	60	24	WT (BM)	Son	ISM	22	NA
16	F	ISM	37	31	D816V (BM)	Brother	MPCM	23	WT (BM)
17	M	MPCM	18	15	WT (BM)	Brother	MPCM	9	WT (BM)
18	M	ISM	37	35	D816V (BM)	Niece	ISM	25	WT (BM)
19	M	MPCM	16	2	WT (BM)	Sister	Mastocytoma	5	NA
20	F	MPCM	43	6	WT (BM)	Daughter	Mastocytoma	1	NA
21	F	MIS	26	24	WT (PB)	Son	MPCM	2	NA
22	F	MIS	30	6	ND	Daughter	MPCM	4	NA

MPCM, maculo-papular cutaneous mastocytosis; MIS, mastocytosis in the skin; BMM, bone marrow mastocytosis; ISM, indolent systemic mastocytosis; SM-AHN, systemic mastocytosis with associated hematological neoplasia; MPN, myeloproliferative neoplasm; CML, chronic myeloid leukemia; PV, polycythemia vera; BM, bone marrow; PB, peripheral blood; WT, wild-type; ND, not done; NA, information not available

C070

COMORBIDITY AND BODY MASS INDEX IN PATIENTS WITH POLYCYTHEMIA VERA: A PV-NET STUDY

G. Benevolo, E. Elli, F. Cavazzini, A. Tieghi, A. D'Addio, M. Bergamaschi, N. Polverelli, G.A. Palumbo, C. Di Pietro, M. Breccia, G. Auteri, E. Sabattini, E. Diral, E. Beggiato, D. Bartoletti, K. Codeluppi, I. Carosino, A. Cuneo, F. Lanza, F. Di Raimondo, R. Lemoli, M. Cavo, N. Vianelli, R. Latagliata, F. Palandri

Division of Hematology Città della Salute e della Scienza Hospital; Hematology Division San Gerardo Hospital ASST Monza; Division of Hematology University of Ferrara; Department of Hematology Azienda USL - IRCCS di Reggio Emilia; Section of Hematology & SCT Unit Hospital of Ravenna; Clinic of Hematology Department of Internal Medicine (DiMI) IRCCS AOU San Martino-IST; Unit of Blood Diseases and Stem Cells Transplantation, Department of Clinical and Experimental Sciences University of Brescia ASST Spedali Civili of Brescia; Department of Scienze Mediche Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia" University of Catania; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital; Division of Cellular Biotechnologies and Hematology University Sapienza; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital; Unit of Haemolymphopathology Department of Haematology and Oncology Sant'Orsola-Malpighi University Hospital Bologna; Hematology Division San Gerardo Hospital ASST Monza; Division of Hematology Città della Salute e della Scienza Hospital; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital; Department of Hematology Azienda USL -

IRCCS di Reggio Emilia; Division of Cellular Biotechnologies and Hematology University Sapienza; Division of Hematology University of Ferrara; Section of Hematology & SCT Unit Hospital of Ravenna; Department of Scienze Mediche Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia" University of Catania; Clinic of Hematology Department of Internal Medicine (DiMI) IRCCS AOU San Martino-IST; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital; Division of Cellular Biotechnologies and Hematology University Sapienza; Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital

In different cancer types, including myelofibrosis (MF), high Charlson Comorbidity Index (CCI) and abnormal body mass index (BMI) were shown to increase mortality. However, no similar data are available on patients (pts) with Polycythemia Vera (PV). Here, we assessed the impact of CCI and BMI on PV complications, drug tolerability and outcome on the basis of real-life data. A network called "PV-NET" started in January 2019 and now includes 11 European Hematology Centers with the aim to collect clinical/laboratory data at diagnosis and during follow-up of 2016 WHO-defined PV pts within a multicentre observational clinical study. Overall survival (OS) was calculated by Kaplan-Meier method from PV diagnosis to last contact or death (log-rank p). Cumulative incidences of events were conducted with Fine & Gray model, with death as a competing risk. A total of 447 with 2016 WHO-defined PV were collected. Median follow-up was 4.7 yrs (0.5-33) (total observation: 2593 pt-yrs). Baseline characteristics were: median age: 62.7 yrs (18.3-88.3); females: 45.4%; median WBC/PLT ($\times 10^9/L$): 10/445; median hemoglobin (g/dl)/hematocrit (%): 18.4/55.2 (males); 17.5/54 (females); high thrombotic risk: 65.3%. Age-adjusted CCI was 0 (17.2%), 1 (19%), 2 (25.3%), and ≥ 3 (38.5%). Median BMI was 24 (17.4-36.3); 43.2% were overweight ($BMI \geq 25$). At diagnosis or during follow-up, 57 all-grades thrombosis, 20 bleedings, 34 infections and 41 2nd neoplasia were recorded. 9 pts progressed to blast phase (PB) and 24 to MF; 28 pts died. Incidence rates $\times 100$ pt-yrs of events were: 1.3 (thromboses), 0.6 (bleedings), 1.2 (infections), 1.7 (2nd neoplasia) and 0.96 (MF and BP). Pts with $CCI \geq 3$ and with $BMI < 22$ (1st quartile) had a significantly higher rate of all-grades thrombosis ($p=0.003$) and 2nd neoplasia ($p=0.001$) respectively. Both CCI and BMI were significantly associated with OS (Figure 1). Pts received phlebotomies (PHL) (89.3%), hydroxyurea (HU) (80.5%), interferon (IFN) (8.7%), and busulfan (3.4%). Pts with $CCI \geq 3$ were significantly less treated with IFN ($p < 0.001$). Overall, 1.6%, 11.1%, and 38.5% of pts had grade ≥ 2 toxicity and/or stopped therapy because of intolerance during PHL, HU, and IFN, respectively. HU dose was not influenced by CCI/BMI; nonetheless, $BMI < 22$ significantly predicted a higher rate of HU ($p=0.004$) and IFN ($p=0.01$) intolerance. CCI and BMI are rarely assessed in PV but may influence treatment decision and outcome.

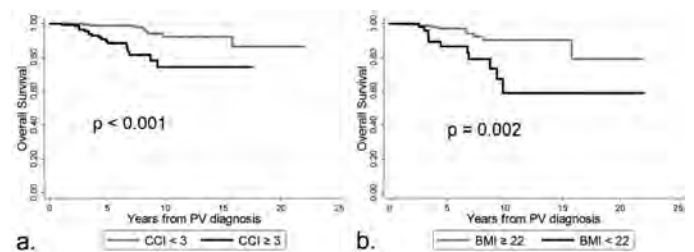


Figure 1. Overall survival according to Charlson Comorbidity Index (CCI) (a) and Body Mass Index (BMI) (b).

Myelodysplastic Syndromes

C071

CORRELATIONS BETWEEN MUTATIONAL AND IMMUNOPHENOTYPIC PROFILES IN THE DIAGNOSIS OF MDS

E. Fabiani, L. Maurillo, C. Gurnari, C. Attrotto, G. Falconi, R. Palmieri, E. Buzzatti, F. Esposito, E. De Bellis, F. Buccisano, A. Venditti, W. Arcese, F. Lo Coco, M.T. Voso

Dipartimento di Biomedicina e Prevenzione, Università di Tor Vergata, Italy

Introduction: The diagnosis of myelodysplastic syndromes (MDS) is based on the presence of peripheral blood (PB) cytopenia, bone marrow (BM) dysplasia and of karyotype abnormalities. Flow-cytometry (FC) and FISH are recommended in doubtful cases. The mutational profile by NGS may add information in cases with inconclusive results by standard analyses.

Aims: The aim of our work was to evaluate the usefulness of adding NGS in the diagnostic algorithm of patients with a suspected MDS, including evaluation of the Ogata score and expression of LIM (lineage infidelity markers, including CD7, CD56, CD2, CD5 and CD25) on CD34+ cells.

Patients and methods: At the time of diagnosis, we studied BM samples harvested from 46 patients with MDS and 17 healthy controls using the Ogata cytofluorimetric score, and LIM expression. A subgroup of 24 MDS patients was also studied by NGS using a custom panel of 94 genes known to be involved in myeloid neoplasms.

Results: The diagnosis of MDS was confirmed by an Ogata score ≥ 2 in 33/46 patients (72%), whereas LIM analysis identified 40/46 patients positive for at least one infidelity marker (87%), with CD7 as the most expressed (41%). Thus, the addition of LIM analysis to Ogata score supported the diagnosis of MDS in 8 of 13 patients with an Ogata score < 2 , increasing the sensitivity to 89%. NGS screening performed in 24 patients identified at least one mutation in 23 out of 24 samples (96%), with a median number of 3.7 mutation/patient. The most frequently mutated genes were: DNMT3A, SF3B1, SLX4 and SRSF2 (5/24, 20,8% of patients). We found a statistically significant correlation between a high Ogata score (≥ 2) and > 4 mutations/patient ($p=0.003$). In addition, all 6 patients with an FC score equal to 0 were found to carry at least one somatic mutation in critical genes. Looking at associations between the mutational status and IPSS-R (very high/high/intermediate vs low/very low), we found a correlation between the number of mutations in three of the major pathways involved in MDS pathogenesis (DNA methylation, histone modification and splicing machinery, figure 1) and IPSS-R grouping (median 2.5 mut in IPSS-R high, vs 1.1 mut in IPSS-R low, $p=0.0073$). Of note, SRSF2 mutations were significantly more frequent in patients with an Ogata score ≥ 2 (5/13 vs 0/11, $p=0.0411$).

Conclusions: NGS combined with LIM analysis and with the Ogata score may improve diagnostic sensitivity in MDS.

UPN	Ogata score	Methylation			Acetylation		Splicing			Mutations (n)	IPSS-R
		DNMT3A	TET2	IDH2	ASXL1	BCOR	BCORL1	SF3B1	SRSF2		
E747	0								1	High	
E658	3								2	High	
E755	1								1	Intermediate	
E404	3	2							3	Intermediate	
E650	4				2				4	Intermediate	
E657	4								3	Intermediate	
E762	3								3	Intermediate	
E751	0								2	low	
E752	0								1	low	
E748	1								2	low	
E753	1								1	low	
E616	2								2	low	
E620	2								0	low	
E624	2								0	low	
E655	2								0	low	
E595	3								0	low	
E622	3								1	low	
E647	3								3	low	
E749	0								1	very low	
E750	0								1	very low	
E759	0								1	very low	
E754	1								2	very low	
E758	1								0	very low	
E649	2								2	very low	

Distribution of mutations identified by next generation sequencing in MDS patients according to Ogata score and IPSS-R.

Figure 1.

C072

IDENTIFICATION OF A NOVEL MUTATION PREDISPOSING TO FAMILIAL AML AND MDS SYNDROME BY A NGS APPROACH

S. Bernardi¹, C. Zanaglio¹, E. Dereli Eke¹, F. Cattina², C. Foroni¹, M. Farina², S. Masneri³, B. Rambaldi², A. Turra², N. Polverelli², E. Morello², L. Gandolfi², T. Zollner², E. Accorsi Buttini², M. Malagola², D. Russo²

¹Unit of Blood Diseases and Stem Cells Transplantation- Laboratorio CREA Centro di Ricerca Emato-oncologica AIL- Department of Clinical and Experimental Sciences- University of Brescia- ASST Spedali Civili di Brescia; ²Unit of Blood Diseases and Stem Cells Transplantation-Department of Clinical and Experimental Sciences- University of Brescia- ASST Spedali Civili di Brescia; ³University of Brescia-Department of Molecular and Translational Medicine, Brescia, Italy

Introduction: Recently, revised WHO classification (2016) has recognized familial AML and MDS (FAMS) as a useful model to investigate predisposing genetic mutations. A pivotal importance is given to the identification of patients with germline mutations in genes involved in the familial predisposition to neoplasia. Next Generation Sequencing panels are a well known tool to investigate these cases suggesting they are more frequent than those previously recognized. Even though well-established causative gene mutations are recently discovered, about 80% cases remain unexplained. This suggest that other inherited mutations could predispose to MDS/AML. New deep sequencing approaches would help to the identification of more cases, genes, as well as novel syndromes. Our aim is to look for predisposing mutations in patients and relatives affected by AML and MDS with familial history of myeloid malignancies.

Methods: At present, 12 AML/MDS patients have been enrolled in a multicentric prospective study (Clinical trial.gov NCT03058588). Leukemic (bone marrow) and germline (buccal swab) DNA were analyzed by NGS gene panel approach based on a 28 genes associated to myeloid leukemogenesis, including the 9 genes associated to FAMS (RUNX1, GATA2, ETV6, TERT, TERC, SRP72, ANKRD26, DDX41, CEBPA). NGS libraries were performed by a Nimblegen (Roche) custom panel based on gene capture strategy and the sequencing was performed by MiSeq (Illumina).

Results: Ten patients did not reveal any germline mutations, one presented a germline mutation on RUNX1 and one revealed a new mutation on ETV6. Particularly, this mutation c.*514C>T in 3'UTR of ETV6, with VAF of 50% on tumor DNA, has never been described before. ETV6 variant was confirmed by Sanger sequencing on the germline DNA in heterozygosis. The same variant in heterozygosis was also confirmed on 2 affected relatives still alive. In silico analysis, performed on PolymiRST Database, revealed that c.*514C>T in 3'UTR of ETV6 results in a gain of miRNA binding site (hsa-miR-4717-3p and hsa-miR-942-3p) that seems to repress the gene.

Conclusions: The down-regulation of ETV6 is associate to an alteration of cell growth and hematopoiesis. This specific miRNA interference have been already described in solid and hematologic tumors. Due to these evidences, NGS approach can help to the identification of new mutations involved in FAMS predisposition.

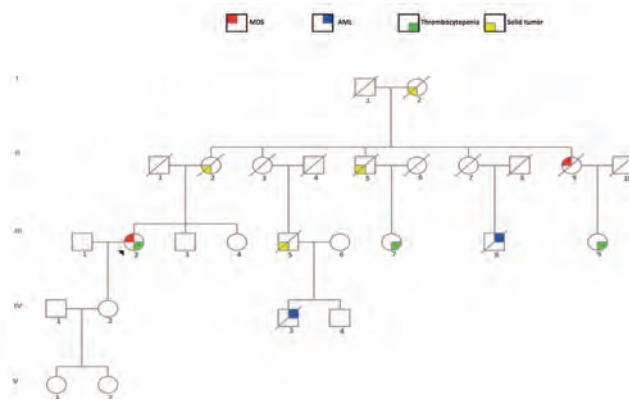


Figure 1. Case index pedigree (black arrow indicates the case index; MDS=Myelodysplasticsyndrome; AML=Acute myeloid leukemia).

C073**DANAZOL: AN "OLD-FASHIONED" DRUG FOR A CURRENT PROBLEM IN LOWER-RISK MYELODYSPLASTIC SYNDROMES**

M. Riva¹, L. Crucitti¹, E. Ravano¹, M. Nichelatti¹, G. Reda², R. Cassin², C. Frangi¹, R. Cairoli¹, A. Molteni³

¹ASST Grande Ospedale Metropolitano Niguarda; ²Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; ³ASST Cremona, Italy

Severe thrombocytopenia in lower-risk MDS is an uncommon event, but very significant about prognosis. No specific therapy in this setting are currently available: hypometilating agents are not licensed in Europe, and a trial testing Eltrombopag is ongoing (Oliva 2017). Few data were reported about danazol, an attenuated androgen, that seems to have some effectiveness in this still unmet need. We retrospectively reviewed 35 thrombocytopenic pts (30 MDS and 5 MDS/MPN) treated with danazol. The initial and maximal dose was 600 mg/day, modulated by response and toxicity. The response was evaluated according to the revision of IWG criteria (Platzbecker 2018). Two pts treated for less than 3 months (mo) were not included in the analysis. At baseline, the platelet (PLT) count was lower than $20 \times 10^9/\text{mL}$ in 11 pts, the median was $23 \times 10^9/\text{mL}$. The response rate was 63,6% (21 pts on 33 evaluable). Median time to response was 3.5mo (range 0.3–12.4); the average response time was 5.09mo. In the first year of treatment, the PLT count changed in a significant way (F test after repeated measures ANOVA: $p < 0.001$) Figure 1. Pairwise comparisons of PLT count according to Bonferroni showed a significant difference for: baseline (b.) vs 3mo ($p=0.0013$), b. vs 6mo ($p=0.0255$), b. vs 9mo ($p=0.0047$) and b. vs 12mo ($p=0.0014$). The median and average duration of the response were respectively 12,5 and 32,5mo. Within the 21 responders, only 6 (28%) lost the response (the median and average duration of response were respectively 5.8 and 12.9mo); the median PFS was not reached after 24mo. The probability to maintain the response after 50mo was assessed at 58,2% (C.I. 24,1% to 81,4%). The OS showed a significant difference (logrank test: $p=0.0064$) between responders and non-responders. Adverse events recorded were as follows: 2 cases of Grade3 (1 liver toxicity and 1 renal failure) with need to stop drug; 10 cases of Grade1-2 (increase in transaminases in 4 pts and in serum creatinine in 6 pts) with need to reduce danazol to 400 mg/day; 3 cases of reversible cutaneous rash; 1 case of amenorrhea (the only fertile woman in the series); 1 case of weight loss and 1 of weight gain. Even if the mechanism of action is unclear, this series confirms the efficacy of danazol and safety to improve PLT count in MDS pts with severe thrombocytopenia. The response may not be immediate, but reachable after 3-6 mo of treatment. A responsive patient has a good probability to maintain a long-lasting response.

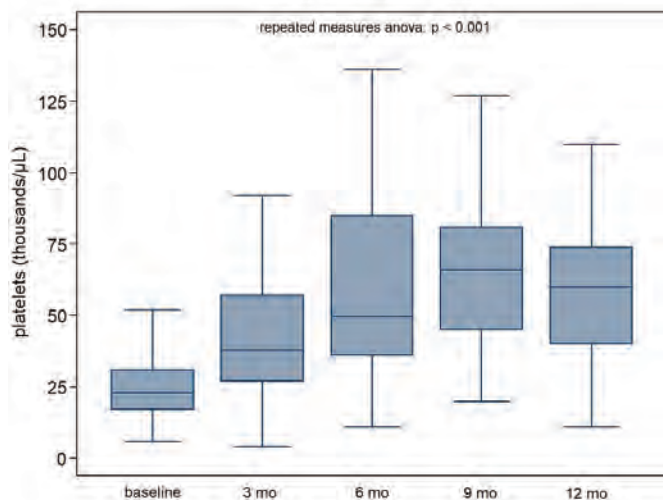


Figure 1.

C074**INTRACELLULAR RE-LOCALIZATION OF NUCLEOSIDE METABOLIZING ENZYMES CORRELATES WITH SENSITIVITY TO AZACITIDINE.**

E. Masala,¹ A. Brogi,¹ D. Nosi,² V. Santini¹

¹MDS Unit, Department of Experimental and Clinical Medicine, University of Florence; ²Department of Experimental and Clinical Medicine, Section of Anatomy and Histology, University of Florence, Italy

We evaluated the role of nucleoside metabolizing enzymes in modulation of cellular response to azacitidine. Azacitidine sensitive SKM1 (SKM1-S) and resistant (SKM1-R) cell lines were analyzed for expression of UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2 by quantitative PCR, in parallel with 51 cases of IPSS high risk MDS. We evaluated by NGS the most frequently mutated genes in MDS in 42 different cases of HR-MDS patients before azacitidine treatment. UCK1 and UCK2 expression was blunted by siRNAs in SKM1 sensitive cells to determine their role in *in vitro* sensitivity to azacitidine. UCK1 and UCK2 silencing was obtained with specific siRNAs (OriGene Technologies, MD, USA); cells were exposed to azacitidine 0,1 and 1 M. After assessment of effective gene silencing, apoptosis was evaluated by Annexin V, and percentage of 5-methylcytosine evaluated by ELISA assay (Global DNA Methylation LINE-1 kit ActiveMotif, CA, USA). UCK1 and UCK2 protein expression and localization was analyzed by confocal laser scanning microscope using anti-UCK1 (1:200; ThermoFisher Scientific, USA) and anti-UCK2 (1:200; ThermoFisher Scientific, USA) antibodies. Labeled cells were observed under a Bio-Rad MRC1024 ES confocal laser scanning microscope (CLSM; Bio-Rad). SKM1-R cells did not express UCK1, UCK2, hENT1, hCNT3, RRM1 and RRM2. A reduction of apoptosis was observed in UCK1 and UCK2-silenced SKM1-S after azacitidine treatment. Azacitidine induced DNA hypomethylation was reduced by defective expression of UCK1 and UCK2. The confocal laser analysis confirmed that SKM1-R express very low levels of UCK-1 and UCK-2 proteins. UCK1 and UCK2 localization was observed in the cytoplasm of both cell lines at basal conditions. Exclusively in SKM1-S azacitidine *in vitro* exposure could re-localize UCK1 and UCK2 proteins in the nucleus. Gene expression of all metabolizing enzymes in MDS primary cells was not significantly correlated with clinical response to azacitidine and cellular localization of UCK1 and UCK2 proteins is under evaluation. SFR3B1, TET2, TP53 and ASXL1 resulted as most frequently mutated genes in our cohort; mutations do not correlate with response. UCK1, UCK2 and the corresponding proteins are absent in azacitidine-resistant cell line SKM1-R. Their silencing induced significantly decreased azacitidine effects. SKM1-S cells, when exposed to the drug, re-localized UCK1 and UCK2 into the nucleus. This event seems to be directly correlated with cellular response to the drug.

C075**CLINICAL FEATURES AND PROGNOSTIC SIGNIFICANCE OF RECIPROCAL BALANCED TRANSLOCATION IN A COHORT OF 79 PATIENTS WITH MYELODYSPLASTIC SYNDROMES**

S. Mohamed, S. Nigro, A. Campagna, A.L. Piccioni, I. Carosino, S. Rosati, M. Ansuinelli, E. Scalzulli, M. Porrazzo, M. Breccia, A. Aloe Spiriti, R. Latagliata, R. Foà, M. Mancini

Dipartimento di Medicina Traslazionale e di Precisione sezione di Ematologia Università "Sapienza"; Unità di Ematologia Azienda Ospedaliera Sant'Andrea Università "Sapienza"; Unità di Ematologia Azienda Ospedaliera Sandro Pertini

Chromosomal abnormalities are very common in de novo myelodysplastic syndromes (MDS) and are represented by losses or gains of genetic material. On the contrary, reciprocal balanced translocations (RBT) are rare and their prognostic value is uncertain. In order to highlight clinical features and survival of MDS patients with RBT, we retrospectively analyzed a cohort of 79 patients diagnosed between 7/1987 and 2/2018, enlisted in the GROM-L registry, characterized by having a RBT at the cytogenetic analysis. Complete peripheral blood and bone marrow features were recorded for all eligible patients, with follow-up data and date of leukemic evolution. IPSS and IPSS-R were retrospectively assigned. Cytogenetic analysis was performed at local specialized laboratories in each participating centers. Based on karyotype, the entire cohort was divided into three cytogenetic subgroups: 24 patients with an isolated RBT (30%), 23 patients with a RBT associated to another chromosomal abnormality (29%) and 32 patients with a RBT in the

context of a complex karyotype (41%). The clinical features of the entire cohort as well as of these cytogenetic subgroups are reported in Table 1. The most frequently involved chromosomes were: chromosome 1 in 18 patients (23%), chromosome 3 in 22 patients (28%), chromosome 5 in 32 patients (41%), chromosome 7 in 29 patients (37%), chromosome 11 in 7 patients (9%), chromosome 12 in 13 patients (16%) and chromosome 17 in 5 patients (6%). During the follow-up, 23 patients (29%) evolved into acute myeloid leukemia (AML), with a median time to evolution of 9.3 months (range 4.2-23.8), without differences according to the different cytogenetic subgroups (p=0.137). The 5-year overall survival (OS) of the entire cohort was 36.9% (CI 95% 24.9-48.9). According to the cytogenetic subgroups, the 5-year OS was 50% (CI 95% 27.7-72.3) in patients with an isolated RBT, 52.6% (CI 95% 29.6-75.6) in patients with a double abnormality including a RBT and 12.9% (CI 95% 0-28.5) in patients with a complex karyotype including a RBT (p<0.001). According to our data, RBT's presence in MDS characterizes patients with a significantly more aggressive clinical course and worse prognosis only when associated with a complex karyotype. MDS patients with isolated RBT or with RBT associated with a single additional abnormality show a more indolent course, with a median OS exceeding 50 months, thus falling into the intermediate-1 risk group of IPSS score classification.

Table 1. Patient's clinical features.

Characteristics	Total patient n=79	Isolated RBT n=24 (30%)	RBT associated with another abnormality n=23 (29%)	RBT in complex karyotype n=32 (41%)
Sex				
Male	46 (58%)	11 (46%)	13 (56%)	22 (69%)
Female	33 (42%)	13 (54%)	10 (44%)	10 (31%)
Age, years				
<60	15 (19%)	11 (46%)	7 (30%)	3 (9%)
≥60	64 (81%)	13 (54%)	16 (70%)	29 (91%)
Median years	69 (17-88)	72 (17-88)	66 (23-82)	73 (52-86)
WHO				
MDS unilineage	6 (8%)	1 (4%)	3 (12%)	2 (6%)
MDS multilineage	26 (33%)	8 (33%)	6 (26%)	12 (37%)
MDS with sideroblasts	2 (2%)	1 (4%)	0	1 (3%)
MDS EB1	14 (18%)	5 (21%)	5 (22%)	4 (13%)
MDS EB2	17 (22%)	5 (21%)	5 (22%)	7 (22%)
Syndrome Del5q	2 (2%)	0	2 (9%)	0
MDS U	12 (15%)	4 (17%)	2 (9%)	6 (19%)
IPSS				
Low	0	0	0	0
Interm-1	34 (43%)	14 (58%)	13 (57%)	7 (22%)
Interm-2	35 (44%)	6 (25%)	9 (39%)	20 (62%)
High	10 (13%)	4 (17%)	1 (4%)	5 (16%)
IPSS-R				
Very low	0	0	0	0
Low	14 (18%)	5 (21%)	6 (26%)	3 (9%)
Interm	21 (26%)	8 (33%)	8 (35%)	5 (16%)
High	22 (28%)	7 (29%)	7 (30%)	8 (25%)
Very high	22 (28%)	4 (17%)	2 (9%)	16 (50%)
Peripheral and Bone marrow				
Hb, g/dl, median	9.8 (6.3-15.5)	10 (6.6-13.2)	9.9 (6.4-12.8)	9.2 (6.3-15.5)
Plt, x10 ³ /mmc, median	124 (21-776)	226 (27-776)	216 (34-597)	69 (21-397)
ANC, x10 ³ /mmc, median	1.3 (0.1-12.5)	1.3 (0.4-5.1)	1.7 (0.2-9.7)	1.3 (0.1-12.5)
BM blast, %, median	4 (0-18)	5 (0-18)	4 (1-18)	4 (0-18)
Status				
Alive	21 (27%)	8 (33%)	9 (39%)	4 (13%)
Dead	49 (62%)	12 (50%)	12 (52%)	25 (78%)
Lost	9 (11%)	4 (17%)	2 (9%)	3 (9%)
AML evolution				
Yes	23 (29%)	8 (33%)	5 (22%)	9 (28%)
No	56 (70%)	16 (67%)	18 (78%)	23 (72%)

Allogeneic and Autologous Transplantation 2

C076

INTERLEUKIN-6 AS EARLY BIOMARKER FOR ACUTE GVHD AND SURVIVAL AFTER ALLOGENEIC TRANSPLANT WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE

R. Greco, F. Lorentino, R. Nitti, M.T. Lupo Stanghellini, F. Giglio, D. Clerici, E. Xue, L. Lazzari, S. Piemontese, S. Mastaglio, A. Assanelli, S. Marktel, C. Corti, M. Bernardi, F. Ciceri, J. Peccatori

Haematology and Bone Marrow Transplant Unit, IRCCS San Raffaele Scientific Institute, Milano, Italy

Although the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) has dramatically improved in the past decade, it is still compromised by transplant-related mortality (TRM), mainly caused by Graft-versus-Host Disease (GvHD). We conducted a prospective observational study to ascertain the potential of serum interleukin-6 (IL6) levels, measured before conditioning and 7 days after allo-HSCT, in predicting acute GvHD, TRM and survival after allo-HSCT with Post-Transplant Cyclophosphamide (PT-Cy), sirolimus and MMF as GvHD prophylaxis. We collected samples from 166 consecutive allo-HSCT patients. Most patients were affected by myeloid malignancies. Most patients (91%) received unmanipulated PBSC and myeloablative conditioning regimen (81%). Stem cell donors were unrelated (n=41), family haploidentical (n=89), HLA-identical sibling (n=36). Median follow-up on survivors was 469 days (range 69-1269). By ROC analysis, we identified a threshold of 2.5 pg/ml for pre-transplant IL6 and 16.5 pg/ml for post-transplant IL6. Rates of grades II-IV and III-IV acute GvHD were higher in patients with post-transplant IL6 levels higher than 16.5 pg/ml (47% vs 14%, p<0.001; 32% vs 3%, p<0.001, respectively). Instead, baseline IL6 levels higher than 2.5 pg/ml were associated with grade II-IV acute GvHD (36% vs 26%, p=0.03). We found a trend towards a worse TRM in patients presenting high post-transplant IL6 (36% vs 23%; p=0.06). Survival analysis confirmed a significantly decreased 2-year overall survival (OS) in patients with baseline IL6 levels higher than 2.5 pg/ml (38% vs 79%; p<0.001) and/or post-transplant IL6 concentrations higher than 16.5 pg/ml (47% vs 83%; p<0.001). Both univariate and multivariate analyses confirmed the ability of baseline IL6 levels to predict OS (HR 4.3; p<0.01) and grade II-IV acute GvHD (HR 1.8; p=0.04), and of post-transplant IL6 to identify patients with worse OS (HR 3.3; p<0.01) and higher risk of grade II-IV (HR 5; p<0.01) and grade III-IV acute GvHD (HR 10.2; p<0.01). In multivariate analysis, both baseline (HR 6.7; p<0.01) and post-transplant IL6 (HR 3.5; p=0.02) predicted higher TRM. In this prospective observational study, measurement of plasma IL6 resulted a valuable biomarker in predicting the risk of acute GvHD and TRM, providing a window for additional prophylactic or preemptive strategies, and potentially improving the final outcome of allo-HSCT.

C077

IMMUNE RECONSTITUTION - BASED SCORE AT DIAGNOSIS OF CGVHD PREDICTS GVHD SEVERITY AND OVERALL-SURVIVAL: A NOVEL PROGNOSTICATION TOOL FOR GVHD TREATMENT TAILORING

M.T. Lupo-Stanghellini¹, F. Serpenti¹, R. Milani², C. Messina¹, R. Greco¹, S. Giralanda¹, D. Clerici¹, F. Giglio¹, C. Liberatore¹, G. Casirati¹, F. Farina¹, S. Mastaglio¹, E. Guggiari¹, F. Lunghi¹, S. Marktel¹, M.G. Carrabba¹, M. Bernardi¹, C. Bonini^{3,4}, A. Assanelli¹, J. Peccatori¹, C. Corti¹, F. Ciceri^{1,3}, F. Lorentino¹

¹Hematology and Bone Marrow Transplantation Unit; ²Immunohematology and Transfusion Medicine Unit; ³University Vita-Salute San Raffaele; ⁴Experimental Hematology Unit, San Raffaele Scientific Institute

Introduction: Allogeneic stem cell transplantation survivors are at a relevant risk of developing chronic GvHD (cGvHD), which importantly affects quality of life and increases morbidity and mortality. We have previously demonstrated the role of immune reconstitution (IR) as a predictive biomarker of occurrence of cGvHD. The aim of this study was to evaluate the prognostication power of IR at cGvHD onset.

Methods: We analyzed data from 424 adult pts consecutively undergoing 1st allogeneic transplant between Jan-2011 and Dec-2016 at our Institution: 151 pts developed cGvHD (median follow-up 4 y). Pts were

divided into a test cohort (111 pts) and a validation cohort (40 pts). We built a Cox multivariate models for OS (overall survival) in pts with cGvHD of any severity. Variables included in the models were: pts age, R-DRI score, type of donor, GvHD prophylaxis, IR values (CD4, CD19, NK, IgA, IgM) at cGvHD diagnosis, history of prior aGvHD, Karnofsky PS, Plt <100.000/L, ALC<1000/L, Eos <500/L. Once we identified the variables independently predicting OS by multivariate analysis, we elaborated a formula for a prognostic risk index by using the coefficients derived from the model. Each pt was then assigned a score and we defined 3 groups of OS risk (low, intermediate and high) by dividing the score into 3 classes using the first and third quartiles. Finally, to evaluate predictive performance of the IR-score, we calculated the receiver operating characteristics (ROC) curve via the area under the curve (AUC), to summarize the IR-score ability to correctly classify events and non-events.

Results: Our multivariate model defined the variables independently predicting OS at cGvHD onset: CD4+ >233/ L, NK <115/ L, IgM <0.45g/L, Karnofsky PS <80%. Final score was calculated as follows: 2,4 (if CD4 >233/ L) + 2,1 (if NK <115/ L) + 2,1 (if IgM <0,45g/L) + 4,3 (if Karnofsky <80). Low risk pts were defined as having a score ≤2.4, intermediate risk >2,4 and ≤4.5, high risk >4.5. The 3y-OS and Transplant Related Mortality (TRM) for both cohorts are reported in Table 1. The ROC curve analysis supports the validity of the IR-score: OS AUC 85.5% - TRM 76,4% with 95% confidence intervals higher than 50%. Furthermore IR-score was able to stratify across NIH-severity classification.

Conclusions: IR-score at diagnosis of cGvHD predicts GvHD severity and overall-survival. IR-score could be adopted to identify patients at high risk and modulate cGvHD treatments accordingly.

Table 1. 3-years Overall Survival and Transplant Related Mortality according to IR-Score stratification.

	Test Cohort		Validation Cohort	
	3y CI of TRM	3y OS	3y CI of TRM	3y OS
Low	4%	96%	0%	92%
Intermediate	11%	76%	11%	78%
High	29%	27%	37%	58%
p	0,007	<0,001	0,03	0,003

C078

UNMANIPULATED HAPLOIDENTICAL MARROW TRANSPLANTATION WITH A MODIFIED POST-TRANSPLANT CYCLOPHOSPHAMIDE (PT-CY) REGIMEN: AN UPDATE OF THE GENOVA-ROME GEMELLI EXPERIENCE

S. Giammarco¹, A. Bacigalupo¹, A.M. Raiola², A. Dominiotto², C. Di Grazia², S. Bregante², M.T. Van Lint², P. Chiusolo¹, L. Laurenti¹, F. Sorà¹, S. Sica¹, E. Angelucci²

¹Università Cattolica del Sacro Cuore; Fondazione Policlinico Universitario A. Gemelli-IRCCS, Istituto di Ematologia, Roma; ²IRCCS AOU San Martino IST, Istituto di Ematologia, Genova, Italy

The aim of the study is to update the outcome of our HAPLO program on 444 patients grafted between 2011 and 2017, in two transplant Units (Genova and Rome). Pts were selected for HAPLO grafts in the absence of a suitable HLA matched related or unrelated donor. Pts median age was 52 years (14-74), and 106 pts were over 60 years of age. Remission status was as follows: CR1 (n=171), CR2 (n=112) and advanced disease (n=161). Median donor age was 34 years (18-67). The diagnosis was AML (n=154), ALL (n=87), MDS (n=83), myelofibrosis (n=47), non Hodgkin lymphoma (n=31) other (n=44). We used 2 myeloablative conditioning regimens, one chemotherapy based (n=346) including Thiotepa, Busulfan, Fudarabine (TBF) and one radiation based (n=99) with full dose radiation (999-1200 rads) (TBI) and fludarabine. The TBF regimen was used with full dose Busulfan 3.2 mg/kgx3, or 3.2 mg/kg x2, for patients over 60 years of age. Median age for the TBF regimen was 55 years (18-74), whereas for the TBI it was 35 years (14-64). GvHD prophylaxis was CsA 2 mg/kg i.v. starting day 0, MMF 2 gr/day p.o. starting day+1 to day+30, PT-CY 50 mg/kg day +3 and day+5. When possible CsA was tapered starting day +100 and discontinued day +180. All patients received unmanipulated marrow as a stem

cell source. Graft rejection was 0% for patients receiving TBI, 2.7% for TBF (BU3 days) and 6.7% for TBF (BU2 days). Fourteen patients received a second HAPLO graft with the Baltimore regimen, and 11 achieved trilineage recovery. Death due to rejection was overall 0.75%.

The CI of acute GvHD II-IV was 28% and of aGvHD grade III-IV 3%. The CI of moderate severe chronic GvHD was 18%. At one year post transplant 88% of patients were off CsA and 83% were off steroids. The average Karnofsky score was 97%. Chronic GvHD was scored as absent (68,7%) minimal (24.8%), moderate (4.8%) and severe (1.4%). Chimerism was scored as full chimera, in 96% of patients. NRM at 4 years, was 16% for remission patients and 22% for patients with advanced disease (p=0.1). Relapse was 20%, 27%, 43% for patients in CR1, CR2, advanced disease (p<0.0001). Actuarial 4 year survival was 72%, 54, 35% for patients in CR1, CR2, advanced disease. Survival was comparable for remission patients receiving either TBF (BU3)(n=111, 72%) or TBF (BU2) (n=54, 64%), despite a significant age difference (44 vs 61 years). We confirm very encouraging outcome of a HAPLO program using myeloablative conditioning, a modified PT-CY day+3+5, and CsA starting on day0. Engraftment, GvHD and disease control have been consistent across different age groups and diagnoses.

C079

TREATMENT OF STEROID RESISTANT ACUTE GRAFT VERSUS HOST DISEASE WITH AN ANTI-CD26 MONOCLONAL ANTIBODY- BEGELOMAB

E. Metafuni¹, F. Gualandi², A.M. Raiola², R. Varaldo², C. Di Grazia², E. Angelucci², E. Benedetti³, F. Locatelli⁴, A. Risitano⁵, M. Musso⁶, F. Zallio⁷, F. Ciceri⁸, P. Chiusolo¹, A. Rambaldi⁹, F. Bonifazi¹⁰, M. Parma¹¹, M. Martino¹², F. Onida¹³, A.P. Iori¹⁴, C. Selleri¹⁵, C. Borghero¹⁶, A. Bertaina⁴, M.T. Van Lint², A. Bacigalupo¹

¹Fondazione Policlinico Universitario Agostino Gemelli-IRCCS; ²Divisione Ematologia e Trapianto Midollo IRCCS AOU San Martino IST; ³Istituto di Ematologia Università di Pisa; ⁴Istituto di Pediatria Ospedale Bambin Gesù; ⁵Istituto di Ematologia Università Federico II; ⁶Divisione di Ematologia Ospedale la Maddalena; ⁷Divisione di Ematologia Ospedale S Biagio; ⁸Divisione Ematologia Ospedale San Raffaele; ⁹Divisione Ematologia Ospedale Papa Giovanni XXII; ¹⁰Istituto Seragnoli Policlinico Sant'Orsola; ¹¹Istituto di Ematologia Ospedale S Gherardo; ¹²Divisione di Ematologia Ospedale Bianchi Melacrinò Morelli; ¹³Istituto di Ematologia Policlinico Università di Milano; ¹⁴Istituto di Ematologia Policlinico Umberto I; ¹⁵Divisione di Ematologia Ospedale S Giovanni di Dio; ¹⁶Divisione di Ematologia Ospedale San Bortolo, Italy

We are reporting the outcome of 69 patients with steroid refractory acute graft versus host disease (SR-GvHD), treated an anti-CD26 monoclonal antibody (BegelomabR): 28 patients were enrolled in two pilot studies, whereas 41 patient were treated on a multicenter follow up compassionate use of the antibody. The median age of the patients was respectively 42 and 44 years. At the time of anti-CD26 treatment, GvHD was overall recorded as grade II in 8 patients, grade III in 33 and grade IV in 28 patients. In the pilot studies patients had failed 1 line of treatment, whereas in the follow up compassionate use, patients had failed one line (n=18), two lines (n=11), three lines (n=11) or 4 lines of treatment (n=1). There were no adverse events attributable to the antibody. Day 28 response was recorded in 75% and 63% in the pilot studies and follow up patients. Response in grade II GvHD was evaluable only in the pilot studies (57%); response in grade III GvHD was recorded in 80% and 83% patients in the two groups; response in grade IV GvHD was recorded 66% and 56% of patients in the two groups. Overall there were 60% responses for skin and liver stage 3-4, and 70% responses for gut stage 3-4 GvHD. The cumulative incidence of non relapse mortality (NRM) at 6 months was 28% and 38%. For day 28 responders, this figure was 19% and 22%, for non responders it was 57% and 66% in the two groups. The overall survival at 1 year was 50% for the pilot studies and 33% for the follow up patients. In conclusion, Begelomab induces a high remission rate on day+28 in patients with SR-GvHD, including a significant proportion of patients with severe gut and liver GvHD.

C080

LONG TERM OUTCOME OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS: THE ROLE OF MINIMAL RESIDUAL DISEASE

S. Paolini, L. Romani, E. Ottaviani, G. Marconi, C. Papayannidis, S. Parisi, C. Sartor, J. Nanni, G. Cristiano, M.C. Abbenante, A. Talami, M. Olivi, S. Ragaini, L. Bandini, C. Venturi, M. Raffini, V. Robustelli, G. Martinelli, N. Testoni, C. Baldazzi, M. Cavo, A. Curti

Istituto di Ematologia "L. e A. Seràgnoli" Dipartimento di Medicina Specialistica Diagnostica e Sperimentale Università di Bologna, IRCCS "Casa Sollievo Della Sofferenza", Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST)

Background: The role of autologous stem cell transplantation (ASCT) as consolidation therapy in acute myeloid leukemia (AML) remains unclear and the evaluation of minimal residual disease (MRD) could better identify patients who benefit from it. **Aims:** We report our single center experience to evaluate overall survival (OS) and disease free survival (DFS) of AML patients who consecutively received ASCT and to retrospectively analyze the correlation between MRD status pre ASCT and OS in patients with molecularly detectable MRD.

Methods: From 1998 to 2018, 65 adult patients with de novo (61/65) or secondary (4/65) AML underwent ASCT at Seràgnoli Hematology Institute. Median age was 50 (range 16-68 years). Only 7 patients had adverse risk according to ELN2017 classification. 24 patients (MRD marker group) had any of the following detectable leukemia-specific molecular markers at diagnosis evaluated through qPCR: 9 NPM1, 5 RUNX1/RUNX1T1, 10 CBFβ/MYH11. All received intensive induction chemotherapy.

Results: 62 out of 65 patients obtained complete remission (CR) after induction. The median number of consolidation cycles before ASCT was 2. All patients received myeloablative busulphan based-conditioning regimen. Before ASCT five patients were MRD+. With a median follow up of 10 years, median OS of the population was not reached and median DFS was 2470 days. OS at 3, 5 and 10 years were respectively 68%, 61% and 54%. Better outcome was observed in patients receiving more than one consolidation cycle in terms of DFS ($p=0.006$) and OS ($p=0.03$). In the MRD marker group, 2/2 NPM1 MRD+ relapsed and 3/3 CBFβ MRD+ (all with CBFβ/MHY11 fusion transcript) obtained molecular remission and are still alive in CR. Pre-ASCT MRD positivity do not impact prognosis in term of OS (10-yr OS 80% for MRD+, 88% for MRD-) or DFS (2-yr DFS 70% and 61% for MRD- and MRD+ respectively). Of note, NPM1 MRD- patients who underwent ASCT remained in CR for a long time after ASCT (median OS not reached and median DFS of 1156 in NPM1- and 249 NPM1+).

Summary/Conclusions: Our study confirm that ASCT is a feasible and effective strategy for adult AML patients, mainly in those with low-intermediate ELN2017 risk and treated with at least 2 post-remissional cycles and it can concede long term survival mainly to NPM1 AML patients MRD- before ASCT. The application of prospective evaluation of MRD through more sensitive molecular methods (qPCR) may better select the patients who could benefit from ASCT.

Non Hodgkin Lymphoma 2

C081

COMBINED RESULTS FROM THE PHASE 3 MAVORIC STUDY: PRIMARY SAFETY AND EFFICACY, WITH POST HOC ANALYSES ON EFFICACY BY PRIOR SYSTEMIC THERAPY, LONG TERM EXPOSURE OF MOGAMULIZUMAB (MOGA) IN PATIENTS WITH CUTANEOUS T-CELL LYMPHOMA (CTCL), AND EFFICACY, AND SAFETY IN EARLY STAGE MYCOSIS FUNGOIDES (MF) PATIENTS

P.L. Zinzani¹, Y. Kim², M. Bagot³, J. Scarisbrick⁴, L. Geskin⁵, P. Ortiz-Romero⁶ on behalf of MAVORIC investigators

¹Institute of Hematology "Seràgnoli" University of Bologna; ²Stanford University Stanford; ³Hôpital Saint-Louis Université Paris 7; ⁴University Hospital Birmingham; ⁵Columbia University Medical Center; ⁶Department of Dermatology University Complutense

The MAVORIC study compared MOGA efficacy with vorinostat (VORI) in patients (pts) with previously treated MF/Sézary syndrome (SS). Post hoc analyses examined MOGA treatment (tx) by prior systemic therapy (PST), long-term safety and a stage Ib/IIA subgroup analysis. MF/SS pts who had failed ≥ 1 PST were randomized to MOGA or VORI (186 each arm, table). Primary endpoint was progression-free survival (PFS). Confirmed Overall response rate (ORR) was based on global composite response score in 4 disease compartments achieved at 2 consecutive visits. Individual compartment responses were also assessed. Time to next tx (TTNT) was defined as time to any therapy excluding topical steroids/focal radiation tx. PFS, ORR and interaction with time from tx were assessed based on immune activity of last prior regimens and analysed with Cox proportional hazards and logistic regression models. ORR and safety were analysed by exposure group. To detect linear trends across exposure quartiles (determined post hoc), frequencies and continuous data were assessed using Chi-square test and analysis of variance respectively. MOGA significantly prolonged PFS and increase ORR compared to VORI. Confirmed ORRs in MOGA pts after 1, 3 or ≥ 6 prior therapies were 25%, 35% and 30% respectively. ORR and duration of response to MOGA did not differ by last PST. Logistic regression analyses showed immune activity impact of last PST and time from prior tx had no effect on MOGA PFS or ORR ($p>0.05$). In the 184 MOGA pts (2 pts withdrew), mean exposure time was 275 days (d; standard deviation: 292; range: 1-1617). The long-term exposure cut-off was >351 d. Of pts with a best response of stable disease, 35/80 had ≥ 171 d of tx. Tx-emergent adverse events (TEAEs) or serious AEs (SAEs) did not vary with increasing MOGA exposure (table). After >351 d, the most common tx-related TEAEs were drug eruption (9/45) and thrombocytopenia (5/45). MOGA efficacy by TTNT and ORR was observed in the subset of IB-IIA MF patients. MOGA significantly prolonged PFS compared to VORI. Post hoc analyses showed meaningful clinical benefit of MOGA regardless of type or immune class of prior therapy and in less advanced MF patients, with no increased safety risk with >1 year MOGA tx.

C082

EARLY PROGRESSION AS A PREDICTOR OF SURVIVAL IN MARGINAL ZONE LYMPHOMA: AN ANALYSIS OF THE FIL NF10 STUDY

I. Defrancesco¹, M. Merli², S. Rattotti³, V. Tarantino⁴, L. Marcheselli⁵, F. Cavallo⁶, M. Varettoni³, B. Bianchi², F. Merli⁷, A. Tedeschi⁸, M.G. Cabras⁹, F. Re¹⁰, C. Visco¹¹, M. Torresan Delamain¹², E. Cencini¹³, M. Spina¹⁴, S. Ferrero⁶, A. Ferrari⁷, M. Deodato⁸, D. Mannina¹⁵, O. Annibali¹⁶, G. Cimino¹⁷, L. Orsucci¹⁸, M. Frigeni¹, M. Cesaretti⁵, L. Arcaini³, S. Luminari⁷

¹Department of molecular medicine University of Pavia; ²Hematology University Hospital Ospedale di Circolo e Fondazione Macchi ASST Sette Laghi University of Insubria; ³Division of Hematology Fondazione IRCCS Policlinico San Matteo; ⁴Clinical and Experimental Medicina University of Modena and Reggio Emilia; ⁵Fondazione Italiana Linfomi; ⁶Hematology Division University of Torino; ⁷Hematology AUSL IRCCS; ⁸Department of Hematology Niguarda Cancer Center Niguarda Hospital; ⁹Division of Hematology Ospedale Oncologico Armando Businco; ¹⁰Division of Hematology Azienda Ospedaliero Universitaria; ¹¹Department of Medicine Section of Hematology University of Verona; ¹²Center of Hematology and Hemotherapy Department of Internal Medicine Faculty of

Medicine State University of Campinas; ¹³Department of Oncology Division of Hematology Azienda Ospedaliera Universitaria Senese; ¹⁴Division of Medical Oncology A National Cancer Institute; ¹⁵Department of Hematology Azienda Ospedaliera Papardo; ¹⁶Unit of Hematology Stem Cell Transplantation Transfusion Medicine and Cellular Therapy University Campus Bio-Medico; ¹⁷Department of Cellular Biotechnology and Hematology University La Sapienza; ¹⁸Division of Hematology 2 AOU Città della Salute e della Scienza

Introduction: Marginal Zone Lymphomas (MZL) is an Indolent B-cell Non-Hodgkin Lymphoma (INFL) and has a heterogeneous clinical behavior. Recently time to progression shorter than 24 months (POD24) was identified to stratify Overall Survival (OS) in follicular NHL. Here we examined the ability of POD24 to predict subsequent OS in a large international MZL cohort as part of the NF10 international multicenter observational study (NCT02904577) promoted by the Fondazione Italiana Linfomi (FIL).

Method: The NF10 Project is a prospective international registry of consecutive INFL patients with a histologically confirmed diagnosis of INFL. Patients had to be followed according to local institutional guidelines. POD24 was calculated only for patients requiring immediate therapy and was defined as experiencing lymphoma progression within 24 months from diagnosis. Subsequent OS was defined as time from POD24 or time from progression in patients without POD24.

Results: Of the 1325 patients enrolled in the NF10 study, we identified 321 with MZL for whom immediate therapy was planned right after lymphoma diagnosis. The median follow up was 43 months. Overall, POD24 was confirmed in 59 patients (18%). Three-year OS for patients with POD24 was 53%, with an HR of 19.5 (95%CI 8.4-45), compared with patients without POD24 (3-yr OS 95%). Anemia, thrombocytopenia, lymphopenia, and treatment without immunochemotherapy were independent predictors of POD24.

Conclusions: Assessment of POD24 stratifies subsequent outcome in MZL and should be considered as a surrogate for OS in clinical research and for patient management. POD24 data are currently being validated on the subset of MZL patients who were not initially treated with immunochemotherapy (watch and wait group). Data will be presented at the conference.

C083

IBRUTINIB COMPARED TO STANDARD CHEMOTHERAPY FOR CENTRAL NERVOUS SYSTEM RECURRENCE OF MANTLE CELL LYMPHOMA

C. Rusconi, D. Tucker, S. Bernard, C. Muzi, L. Crucitti, P.M. Stefani, M.C. Cox, G. Gini, A. Re, R. Sciarra, A.M. Liberati, L. Morello, A. Arcari, D. Mannina, O. Vitagliano, R. Sartori, A. Chiappella, M. Balzarotti, U. Vitolo, C. Thieblemont, S. Rule, C. Visco

Division of Hematology ASST Grande Ospedale Metropolitano Niguarda, Department of Haematology Royal Cornwall NHS Trust, Service d'onco-hématologie Hôpital Saint-Louis AP-HP, Division of Hematology ASST Grande Ospedale Metropolitano Niguarda, Division of Hematology ASST Grande Ospedale Metropolitano Niguarda, UOC Ematologia Dipartimento di Medicina Specialistica ULSS Marca Trevigiana, Haematology Unit Department of Clinical and Experimental Medicine La Sapienza University AO Sant'Andrea, Division of Hematology Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, U.O. Ematologia Spedali Civili di Brescia, Division of Hematology Fondazione IRCCS Policlinico San Matteo, Università di Perugia AO Santa Maria di Terni, Department of Medical Oncology and Hematology Humanitas Clinical and Research Center IRCCS, Hematology Unit Ospedale Guglielmo da Saliceto, UOC di Ematologia Azienda Ospedaliera Papardo, Division of Hematology Cardarelli Hospital, Hematology Department Castelfranco Veneto Hospital ULSS2 Marca Trevigiana, Hematology AO Città della Salute e della Scienza di Torino, Department of Medical Oncology and Hematology Humanitas Clinical and Research Center IRCCS, Hematology AO Città della Salute e della Scienza di Torino, Service d'onco-hématologie Hôpital Saint-Louis AP-HP, Plymouth University, Peninsula Schools of Medicine and Dentistry, Department of Medicine, Section of Hematology, University of Verona

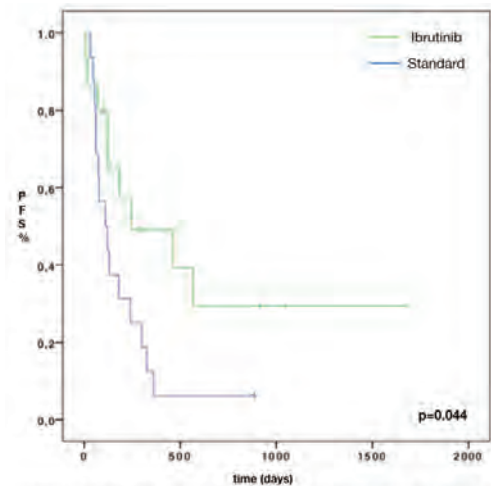
Background: Central nervous system (CNS) relapse of Mantle Cell Lymphoma (MCL) is a rare condition for which a standard of care has not been identified. Responses to conventional treatment for CNS-MCL are poor, with median survival of less than 6 months. Ibrutinib is approved for relapsed/refractory MCL; small series demonstrated its effi-

cacy on CNS localization thanks to the capability to cross the blood-brain barrier.

Methods: We retrospectively analyzed a multi-center series of consecutive patients (pts) with CNS relapse of systemic MCL with the aim to evaluate outcome of pts treated with ibrutinib compared to pts treated with a standard chemotherapy (CT) and previously described in the MANTLE-FIRST study. Overall survival (OS) and progression-free survival (PFS) were estimated from the time of initiation of therapy for CNS-MCL.

Results: The entire series consisted of 31 pts: 16 pts (52%) in the standard cohort (SC), who received conventional CT, and 15 pts (48%) in the ibrutinib cohort (IC). Median age was 54 years (range: 39-70) in SC and 60 years (range: 54-76) in IC. MIPI score was high in 46% pts in SC and in 73% of pts in IC. Upfront treatment for systemic MCL included high-dose cytarabine and autologous stem cell transplantation in 100% and 68% in the SC and in 80% and 33% in the IC. Median number of therapies for MCL before CNS recurrence was 1 in the SC and 2 (range: 1-3) in the IC. In the SC, treatment for CNS relapse consisted of rituximab plus high-dose methotrexate in 5 pts (33%), bendamustine in 6 pts (37%), ifosfamide in 2 pts (12%) and other regimens in 3 pts (18%). Pts in the IC received ibrutinib 560 mg p.o. daily. Intrathecal CT was added in 5 pts (31%) in the SC and 7 pts (47%) in the IC, respectively. Radiotherapy was delivered to 3 pts, all in the SC, in one case as consolidation and in 2 cases as salvage. With a median follow-up of 10.4 months, the 1-year PFS and OS of the entire study population are 24% and 46%. A statistically significant difference in 1-year PFS was observed in favor of ibrutinib versus standard CT (49% vs 6%, $p=0.044$). The difference in 1-year OS in favor of ibrutinib versus standard CT did not reach statistical significance (57% vs 37%, $p=0.097$).

Conclusions: In this study, ibrutinib monotherapy appears to be effective for CNS-MCL; with the usual limitations of a retrospective analysis, present data show a benefit in PFS for CNS-MCL pts treated with ibrutinib in comparison to standard chemoimmunotherapy.



Graph of progression free survival in patients treated with standard CT compared with patients treated with ibrutinib

Figure 1.

C084

INTRALESIONAL RITUXIMAB SUPPLEMENTED WITH AUTOLOGOUS SERUM IN RELAPSED CD20+ INDOLENT LYMPHOMAS OF THE CONJUNCTIVA: ACTIVITY AND SAFETY RESULTS OF THE "IRIS" TRIAL

M. Sassone¹, E. Miserocchi², S. Govi¹, M.E. Corti³, A. Giordano Resti², C. Cecchetti¹, T. Calimeri¹, S. Perrone¹, M. Ponzoni^{4,5}, G. Modorati², A.J.M. Ferreri¹

¹Lymphoma Unit, IRCCS San Raffaele Scientific Institute, Milano; ²Ophthalmology Unit, IRCCS San Raffaele Scientific Institute, Milano; ³Pharmacy Unit, IRCCS San Raffaele Scientific Institute, Milano; ⁴Pathology Unit, IRCCS San Raffaele Scientific Institute, Milano; ⁵Vita e Salute San Raffaele University, Milano, Italy

Background: Most lymphomas primarily arising in the conjunctiva ex-

hibit indolent behavior. The excellent prognosis of this lymphoma seems to suggest that less invasive and safer therapies should be preferred to conventional treatment. Topical and intralesional therapies is reported in only few pts treated with intralesional interferon. Herein, we report a monoinstitutional phase II trial addressing safety and activity of intralesional rituximab supplemented by autologous serum in pts with CD20+ indolent lymphomas of the conjunctiva (NCT01514344 – IRIS trial).

Methods: Adults with R/R CD20+ indolent lymphoma limited to the conjunctiva (mono- or bilateral disease), and measurable disease were registered and treated with intralesional injections of 10-20 mg of undiluted rituximab (1-2 mL) combined with local anesthetic. Pts received four weekly doses followed by six monthly injections. Objective response was assessed after weekly doses and at the end of the therapeutic program by photographs of the lesions. In non responder pts, 500 microL of autologous serum were added to rituximab. Safety was the primary endpoint; response rate and antitumor effect of the addition of autologous serum were secondary endpoints.

Results: 20 pts (median age 63) were registered; all pts had MZL, 4 pts had HBV/HCV infection, no pt had increased LDH serum levels. No interruptions or delay due to toxicity were registered only 3 pts experience mild local effects. 11 pts achieved tumor regression after the 4 weekly doses, whereas 9 pts required autologous serum. At the end of the whole treatment 10 pts were in CR and 2 in PR, with an ORR of 63% (95%CI: 42-84%). At a median follow-up of 3 years (range 7-73), 12 pts remain relapse-free; 2 pts experienced relapse in the contralateral eye, with a 3-year local disease control rate of 63 ±11%, and a 3-year PFS of 60 ±12%. Interestingly, 3 failed pts were retreated with intralesional rituximab and autologous serum achieving a further response that lasted 12, 25+ and 32+ months, respectively. All pts are alive.

Conclusions: Intralesional rituximab is a safe and active treatment in this setting of pts. The addition of autologous serum is associated with improved response in some cases. Retreatment of local relapses can result in a second long-lasting response.

CHOEP + stem cell transplant (SCT). Aims were to define the recommended dose of Ro when administered in combination with CHOEP and to evaluate safety. Patients aged 18-65 eligible to SCT, with advanced PTCLnos, angioimmunoblastic and ALKneg anaplastic lymphoma, were eligible. Treatment plan was: 6 Ro-CHOEP every 21 days (Ro day 1 and 8), followed by DHAP with stem cell harvest and SCT. Patients in complete response (CR) after induction proceeded to autoSCT, while those in partial response (PR), with an available HLA-matched donor, proceeded to alloSCT upfront. Dose allocation of Ro was based on the continual reassessment method; dose-limiting toxicity (DLT) was defined as any grade (g) 3-4 non-hematologic toxicity or a delay >15 days during the first 2 cycles. The maximum tolerated dose of Ro was defined as the dose that achieves a DLT in 33% of patients. From 2014 to 2017, 21 patients were enrolled into the phase Ib part. Median age was 57 years (IQR 53;61); 18 (86%) had stage III-IV and 8 (38%) IPI risk >2. The first triplet was treated with Ro at 12 mg/ms, and no DLTs were observed; the subsequent cohorts were treated with Ro at 14 mg/ms. Nine DLTs were reported in 7 patients [8 events of g3 (3 mucositis, 1 maculopapular rash, fatigue, fever, respiratory failure, typhilitis) and 1 event of g4 neutropenic fever], and prompted to define 14 mg/ms the recommended dose of Ro. Hematologic g3-4 toxicities in 117 courses of Ro-CHOEP were neutropenia in 38% and thrombocytopenia in 45%; g3-4 non-hematologic toxicities by patient were: arrhythmia in 1 (5%), gastrointestinal events in 3 (14%) and infections in 5 (24%); no toxic deaths. A median of 4.3 10 (IQR 3.4-5.71) peripheral blood CD34+ cells/Kg were collected during the harvest. At least 90% of the planned dose of doxorubicine, cyclophosphamide, etoposide, vincristine were administered in 87%, 86%, 83% and 89% of cycles, respectively. Median interval time between Ro-CHOEP was 21 (19-36) days. At the end of induction, overall response rate was 16/21 (76%, CR 71%). Biomarkers and biological analysis are ongoing. In conclusion, the phase Ib FIL-PTCL13 defined Ro 14 mg/ms on day 1 and day 8 as the recommended dose, when administered in combination with CHOEP, prior up-front SCT, in untreated young PTCLs, without unexpected toxicities. The phase II part of the study is ongoing.

C085

ROMIDEPSIN-CHOEP PLUS UP-FRONT STEM-CELL TRANSPLANTATION IN PERIPHERAL T-CELL LYMPHOMA (PTCL): RESULTS OF PHASE Ib PTCL13 STUDY OF THE FONDAZIONE ITALIANA LINFOMI

A. Chiappella¹, C. Carniti², A. Evangelista³, A. Re⁴, M.G. Cabras⁵, V. Stefoni⁶, C. Castellino⁷, A.G. Congiu⁸, A. Pinto⁹, M. Zanni¹⁰, C. Rusconi¹¹, A.L. Molinari¹², R. Ciancia¹³, L. Baldini¹⁴, L. Nassi¹⁵, F. Re¹⁶, M. Tani¹⁷, F. Ilariucci¹⁸, F. Cavallo¹⁹, A. Santoro²⁰, S. Volpetti²¹, A. Doderò²², S. Pileri²³, G. Ciccone²⁴, P. Corradini²⁵

¹Ematologia, AOU Città della Salute e della Scienza di Torino; ²Università degli Studi di Milano; ³SSD Epidemiologia Clinica e Valutativa, Clinical Trial Center, AOU Città della Salute e della Scienza di Torino e CPO – Piemonte; ⁴Ematologia, ASST Spedali Civili di Brescia; ⁵Ematologia, Ospedale Oncologico Armando Businco; ⁶Ematologia, Policlinico S.Orsola-Malpighi - Istituto di Ematologia "Seragnoli"; ⁷Ematologia e Trapianto di Midollo Osseo, AO Santa Croce e Carle; ⁸Ematologia; Ospedale Policlinico San Martino S.S.R.L. - IRCCS per l'Oncologia; ⁹Ematologia Oncologica, Istituto Nazionale Tumori - IRCCS Fondazione G. Pascali; ¹⁰Ematologia, AO SS. Antonio e Biagio e Cesare Arrigo; ¹¹Ematologia, ASST Grande Ospedale Metropolitano Niguarda; ¹²Ematologia, Ospedale degli Infermi; ¹³Oncoematologia, trapianti emopoietici e terapie cellulari, Centro di Riferimento Oncologico (CRO) IRCCS; ¹⁴Ematologia, Fondazione IRCCS Cà Granda OM Policlinico; ¹⁵Ematologia, AOU Maggiore della Carità di Novara; ¹⁶Ematologia e CTMO, AOU di Parma; ¹⁷Ematologia, Ospedale Santa Maria delle Croci; ¹⁸Ematologia, Azienda Ospedaliera Arcispedale Santa Maria Nuova – IRCCS; ¹⁹Ematologia, Università degli Studi, AOU Città della Salute e della Scienza di Torino; ²⁰Dipartimento di Oncologia Medica ed Ematologia, Humanitas Clinical and Research Hospital, Università Humanitas; ²¹Ematologia, AOU. S. Maria della Misericordia di Udine; ²²Ematologia, Fondazione IRCCS Istituto Nazionale dei Tumori; ²³Emopatologia, Istituto Europeo Oncologico IRCCS; ²⁴SSD Epidemiologia Clinica e Valutativa, Clinical Trial Center, AOU Città della Salute e della Scienza di Torino e CPO – Piemonte; ²⁵Ematologia, Fondazione IRCCS Istituto Nazionale dei Tumori and Università degli Studi di Milano, Italy

The treatment of PTCL is challenging. In the phase Ib FIL-PTCL13 (NCT02223208), we tested the combination of romidepsin (Ro) with

patients harbored somatic mutations that were detectable in plasma cfDNA. cfDNA genotyping correctly identified 62% of mutations (n=8/13) discovered in BM tumor PCs. Overall, the variant allele frequencies (VAF) in plasma samples correlated with those in tumor biopsies. Notably, the remaining mutations not discovered in cfDNA had a low representation (under the VAF threshold of 8%) in the purified BM PCs. In none of the cases with paired BM sample, cfDNA genotyping identified additional somatic mutations not detected in the purified BM PCs. Finally, cancer fraction estimated by ULP-WGS was on average 36.5% for BM samples (10 pts) and 2.6% for cfDNA samples (17 pts).

Our results provide the proof of principle that cfDNA genotyping is a feasible, non-invasive real-time approach that could identify biomarkers of progression in SMM patients.

C089

MAINTENANCE THERAPY WITH BORTEZOMIB/DEXAMETHASONE IN NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA PATIENTS CARRYING HIGH RISK FEATURES

K. Mancuso, P. Tacchetti, L. Pantani, S. Rocchi, I. Rizzello, I. Caratozzolo, G. De Cicco, A. Fusco, N. Testoni, C. Terragna, G. Marzocchi, M. Martello, E. Borsi, L. Dozza, E. Zamagni, M. Cavo

"Seràgnoli" Institute of Hematology Bologna University School of Medicine, Italy

Maintenance therapy with lenalidomide after autologous stem-cell transplantation (ASCT) is currently the standard of care for Newly Diagnosed Transplant Eligible Multiple Myeloma (NDTEMM) patients (pts). However, data on its efficacy in pts carrying high risk (HR) features are still conflicting. Some findings have suggested that post-ASCT treatment with bortezomib (V) represents a feasible and effective maintenance strategy in pts with HR cytogenetic abnormalities (CAs). We retrospectively analyzed 34 NDTEMM pts who received maintenance therapy, from March 2009 to December 2018, with V (1.3 mg/m² IV or SC from January 2011) and dexamethasone (d, 20 mg the day of and after V), every 2 weeks for up to 2 years. HR MM was defined as follows: 4 pts carried ISS stage III, 2 extramedullary disease, 1 plasmacell leukemia and 28 at least one CAs among del17p, t(4;14), t(14;16) or 1q21 gain, detected by FISH. Pts received a median of 4 cycles of induction with bortezomib/thalidomide/dexamethasone (VTD), followed by single (11 pts) or double ASCT (20 pts) and 2 additional VTD cycles as consolidation therapy (31 pts). Three pts could not proceed to ASCT and continued with 5 cycles of bortezomib-melphalan-prednisone. Vd was started after a median of 30 days from consolidation and prosecuted for a median of 24 months. At the time of analysis, 3 pts were still on maintenance, 18 (58%) completed the planned 2-year treatment period, while 13 had premature withdrawal due to either disease progression (12 pts, 39%) or (1 patient) severe toxicity (massive DVT). Five pts upgraded the depth of response during Vd. The most common grade ≥ 2 adverse event was peripheral neuropathy (PN), occurring in 32% of the population (10 pts grade 2 and 1 patient grade 3). Only 2 pts developed grade 2 PN for the first time during maintenance; among 9 pts with preexisting PN, 6 remained stable, 1 improved and 2 worsened. Eight pts required V dose reduction due to PN. At a median follow-up of 67 months, median PFS from the beginning of maintenance therapy was 39 months, with a 48% probability of being event-free at 48 months. Median OS was not yet achieved, with a 4-year estimate of 83%. Median time from end of maintenance to progression was 8.3 months. In conclusion, maintenance therapy with Vd represents a safe and efficacious option for long-term management of NDTEMM pts with HR features and may provide a useful alternative in case of severe contraindications to IMiDs or economical constraints.

C090

COMPARISON OF EFFICACY AND TOXICITY BETWEEN DARATUMUMAB AND POMALIDOMIDE IN MULTIPLE MYELOMA PATIENTS WITH ADVANCED RELAPSE

F. Colasante, C. Cigana, R. De Marchi, A. Sperotto, R. Stocchi, M. Cerno, S. Buttignol, D. Schiavo, R. Fanin, F. Patriarca

Clinica Ematologica di Udine, Italy

Since 5 new drugs have been recently licenced in Italy for the treatment of relapsed/refractory multiple myeloma (RRMM) patients (pts), the evaluation of their efficacy and toxicity in the real life and the identification of the sequencing providing the best chance of long term outcome are challenging. The aim of the study was to compare efficacy and safety of daratumumab monotherapy versus pomalidomide+dexamethasone in advanced RRMM pts, treated between June 2013 and October 2018. Criteria of inclusion was pretreatment with at least 2 lines including bortezomib and lenalidomide. Thirty-three pts received 42 treatments that include 26 pomalidomide+dexamethasone and 16 daratumumab. At the time of relapse, median age was 68 years (range 45-81), R-ISS 1, 2 and 3 was 26%, 31% and 43% respectively and 36% had unfavourable FISH cytogenetics. Daratumumab and pomalidomide groups had similar clinical features, except a significant greater proportion of pts with unfavourable cytogenetics in the daratumumab group compared to the pomalidomide group (50% vs 27%, p:0.03). The time to achieve at least a PR was comparable in the 2 groups: in fact, 50% of pts treated with daratumumab and 38% of those treated with pomalidomide reached at last PR after 2 months (p: 0.4). The maximum responses obtained after daratumumab showed no significant differences compared to pomalidomide: CR (7% vs 0%), VGPR (31% vs 23%), PR (25% vs 31%), SD/resistance (37% vs 46%) (p: 0.4). At median follow-up of 12 months (range 2-30), 44% of daratumumab treatments was still ongoing unlike 11% of pomalidomide+dexamethasone therapies (p:0.03). The main cause of discontinuation was disease progression, that was significantly higher in the pomalidomide group (78% vs 44%, p: 0.05). Haematological toxicity was more common in the pomalidomide group and included neutropenia III-IV (42% vs 0%, p:0.03) and thrombocytopenia I-II (23% vs 6%, p:0.04). Main extrahematologic toxicities were infections (in the urinary tract and in the lungs) and were 27% in the pomalidomide group and 19% in the daratumumab group (p: 0.5). One-year PFS was 52% in the daratumumab group vs 28% in the pomalidomide group (p: 0.09). One-year OS was similar in both groups (62% vs 55%, p: 0.3). In conclusion, we observed that efficacy and tolerability of both treatments was not inferior to those reported in the pivotal studies. A possible advantage of daratumumab in pts with high risk cytogenetics should be confirmed in larger studies.

Acute Myeloid Leukemia 2

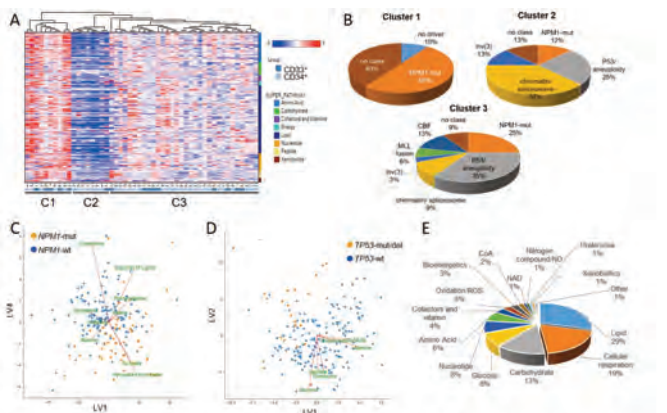
C091

INTRACELLULAR AND SYSTEMIC METABOLIC PROFILING OF ACUTE MYELOID LEUKEMIA IMPROVES GENOMIC CLASSIFICATION AND SUGGESTS NOVEL THERAPEUTIC TARGETS

G. Simonetti¹, A. Padella², C. Mengucci^{3,4}, E. Fonzi¹, G. Picone³, M. Pazzaglia², M. Perricone², M.C. Fontana², S. Bruno², M.T. Bochicchio¹, E. Franchini¹, J. Nanni², G. Marconi², I.F. do Valle⁴, R. De Tommaso², A. Ferrari¹, M. Ghetti¹, R. Napolitano¹, C. Papayannidis², C. Cerchione¹, E. Ottaviani², G. Castellani⁴, D. Remondini⁴, F. Capozzi³, G. Martinelli¹

¹Istituto Scientifico Romagnolo per lo Studio e Cura dei Tumori (IRST) IRCCS; ²Department of Experimental, Diagnostic and Specialty Medicine, "Seràgnoli" Institute of Hematology, University of Bologna; ³Department of Agricultural and Food Sciences, University of Bologna; ⁴Department of Physics and Astronomy, University of Bologna, Italy

Targeting cell metabolism is a promising therapeutic strategy in acute myeloid leukemia (AML). However, few studies identified specific metabolic vulnerabilities due to the lack of a comprehensive characterization of AML cell metabolism. The study aimed to stratify AML patients based on their metabolic landscape and integrate their genomic and metabolic profiles. Genomic data were obtained by whole exome sequencing (n=165) or targeted NGS (n=18). Of them, 119 AML (and 145 healthy donors) were evaluated for serum and urine metabolites by nuclear magnetic resonance (NMR). Intracellular metabolites of AML bone marrow cells (35 CD34+ and 15 CD33+CD14- samples) and controls (21 cord blood CD34+ and 21 peripheral blood CD33+ samples) were analyzed by mass spectrometry.



(A) Unsupervised hierarchical clustering of intracellular metabolite profiles (C: clusters). (B) Molecular classification of the three clusters (according to Papaemmanuil et al. NEJM 2016). (C) BiPlot of the first two PCA components, showing metabolite level contribution to the observed dispersion between NPM1-mut and wt AML. (D) BiPlot on PLS-DA reduced space for TP53-wt and TP53-mut/del AML. Metabolites are plotted along their maximum variance direction in the PLS-DA score space. (E) Frequency of mutations according to metabolic superpathways in 165 AML cases.

Figure 1.

Unsupervised hierarchical clustering of intracellular metabolites clearly defined 3 clusters (C). C1 showed high metabolite concentration and enrichment of NPM1-mutated (mut) AML, C2 low metabolite levels and high frequency of cases with altered chromatin/splicing genes; C3 was enriched for TP53-mut/aneuploid AML, with a more complex metabolic profile (p=0.029). By training a Gaussian Process classifier on NMR data, we obtained separation of C1 and C3 also at serum level, which was confirmed on the NMR test set. In particular, NPM1-mut AML displayed abundance of tricarboxylic cycle byproducts and reduced glutamine and creatinine. At intracellular level, NPM1-mut AML were characterized by increased intermediates of purine and pyrimidine metabolism, in line with an active biosynthetic activity. Moreover, unsupervised NMR data analysis identified a clear separation (0:7 accuracy) between TP53-wt and mut/deleted AML, the latter showing reduced threonine, alanine, glucose, lactate and glutamine serum concentration. At genomic level, 88% of patients carried at least one mutation in a metabolism-related gene including enzymes and metabolic regulators, with lipid metabolism, cellular respiration, metabolism of

carbohydrates, glucose and nucleotides being the pathways most frequently targeted by mutations both in our cohort and the TCGA dataset. The integration of genomic and metabolic profiles provides a novel refined AML classification and suggest subtype-specific metabolic targets, including nucleotide metabolic pathways and bioenergetics in subgroups of NPM1-mut and TP53-mut/del AML, respectively.

Supported by: EHA research fellowship award, AIRC, FP7-NGS-PTL, Fondazione del Monte.

C092

ABSTRACT WITHDRAWN

C093

OTTIMIZZAZIONE DELLA TERAPIA CON TKI NEL PAZIENTE ANZIANO CON LMC-PH+ IN RISPOSTA MOLECOLARE STABILE MR3.0 O MR4.0: RISULTATI A UN ANNO DELLO STUDIO DI FASE III OPTKIMA, MULTICENTRICO E RANDOMIZZATO

M. Malagola, F. Efficace, N. Polverelli, F. Cottone, E. Abruzzese, A. Iurlo, C. Bucelli, F. Stagno, M. Bonifacio, M. D'Adda, M. Lunghi, M. Crugnola, F. Lunghi, F. Castagnetti, G. Rosti, M.L. Ferrari, M. Bergamaschi, G. Binotto, R. Sancetta, M.R. Coppi, V. Gai, G. Rege Cambrin, A. Romano, M. Tiribelli, S. Russo, L. Aprile, A.P. Falcone, S. Rupoli, A. Russo Rossi, E. Usala, B. Martino, L. Gandolfi, S. Bernardi, C. Zanaglio, M. Farina, M. Baccarani, D. Russo

Chair of Hematology, Dept of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili di Brescia, Health Outcomes Research Unit, Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA), GIMEMA Data Center and Health Outcomes Research Unit, Hematology, S Eugenio Hospital, Tor Vergata University, Hematology Division, Foundation IRCCS Ca' Granda - Maggiore Policlinico, Department of Hematology, University of Catania, Department of Medicine, Section of Hematology, University of Verona, Division of Hematology, ASST-Spedali Civili di Brescia, Division of Hematology, Department of Translation Medicine, University of Eastern Piedmont, Hematology Unit, Maggiore Hospital University of Parma, Hematology and Bone Marrow Transplantation (BMT) Unit, San Raffaele Scientific Institute, Department of Experimental, Diagnostic and Specialty Medicine, Institute of Hematology and Medical Oncology "L. & A. Seràgnoli", University of Bologna, Hematology and Bone Marrow Transplant Unit, ASST Papa Giovanni XXIII, Clinical Hematology, Ospedale Policlinico S. Martino, Istituto di Ricovero e Cura a Carattere Scientifico, Hematology and Clinical Immunology, Department of Medicine, Padua School of Medicine, Hematology Unit, Dell'Angelo Hospital, Haematology and BMT Unit "Antonio Perrino" Hospital, Hematology Division, Azienda Ospedaliera Santi Antonio e Biagio e Cesare Arrigo, Hematology Division, Ospedale Mauriziano, Istituto Nazionale Tumori Regina Elena - IFO, Division of Hematology and BMT, Department of Medical Area, University of Udine, Division of Hematology, Dipartimento di Patologia Umana dell'Adulto e dell'Età Evolutiva, Policlinico G. Martino, University of Messina, Hematology Unit, Department of Medicine, Surgery and Neuroscience, Azienda Ospedaliera Universitaria Senese, University of Siena, Hematology Unit, IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Hematology Clinical Unit, Ancona Hospital, Hematology and Transplants Unit, University of Bari, Hematology Unit, Ospedale Oncologico A. Businco, Hematology Unit Bianchi Melacrinò Morelli Hospital

The therapeutic strategy of CML in the TKIs era aims to improve the management of the disease and quality of life (QoL). To this purpose, in July 2015, we started a prospective multicentric randomized trial in which patients older than 60 years in stable MR3.0 or MR4.0 molecular response were randomized to receive a FIXED intermittent TKIs regimen (one month ON and one month OFF) (Russo D, Blood 2013; Russo D, BCJ 2015), versus a PROGRESSIVE intermittent TKIs regimen (one month ON and one month OFF for the 1st year; one month ON and two months OFF for the 2nd year; one month ON and three months OFF from the 3rd year) (OPTkIMA study, ClinicalTrials.gov: NCT02326311). Molecular monitoring was performed according to the 2015 ELN guidelines. In case of confirmed MR3.0 loss, patients were planned to resume TKIs daily. QoL was evaluated at baseline, every three months during the first year and regularly thereafter, using the EORTC QLQ-C30 and its CML module (QLQ-CML24) and the

EORTC Elderly Module (QLQ-ELD14). Up to December 2018, 186 patients have been enrolled by 26 Italian Hematological Centers and 166 patients (89%) completed the first year of OPTkIMA study by intention to treat. Focusing on these latter patients, their median age was 71 years (range 60-89), 129 (77%) were receiving imatinib and 76 (46%) have been randomized in the PROGRESSIVE arm. 46 patients assigned to the PROGRESSIVE arm (61%) have entered the second year of therapy. 47/166 patients (28%) went out of the study during the first year. 39/166 cases (23%) lost MR3.0 (Table 1). Thus the probability of loosing the MR3.0 while on OPTkIMA was 19% at one year (Figure 1). All the 39 patients resumed TKIs continuously and all obtained at least the MR3.0 response, within 6 months. The intermittent treatment was well tolerated, with 7 serious adverse events, none of which treatment-related. The top three most prevalent baseline symptoms (QLQ-C30) were fatigue (80%), pain (51%) and sleep disturbance (48%). With regard to QoL disease-specific domains (QLQ-CML24), no statistically significant changes were noted during the first year of treatment. Further QoL analyses are currently ongoing. According to this first interim report, a policy of intermittent TKIs administration in elderly patients is safe and well tolerated. The probability of MR3.0 loss while on OPTkIMA at 1 year was 19%, comparable to the 20% of the previously published INTERIM trial.

Table 1. Patients and causes of OPTkIMA discontinuation in the 1st year of intermittent treatment one month ON and one month OFF.

	TOT	IC withdrawn	Second Cancer	Loss of MR3.0
3 rd Month	14	3	1	10
6 th Month	21	1	1	19
9 th Month	4	0	1	3
12 th Month	8	0	1	7
TOT	47/166 (28%)	4/166 (2%)	4/166 (2%)	39/166 (23%)

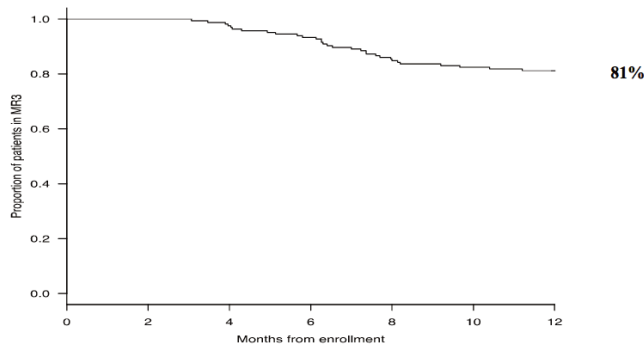


Figure 1. Probability of loosing MR3,0 at 1 year of OPTkIMA.

ular risk. CD200 and bcl2 were evaluated by multi-parametric flow cytometry, with high intensity of expression defined by a MFI >11 and >17, respectively. CD200 and bcl2 were overexpressed in 172 (59%) and 136 (45%) cases, respectively. CD200 and bcl2 co-overexpression (double-positive, DP) was found in 93/291 (32%) patients, while 77 (26%) were double negative (DN) and 120 (41%) expressed only CD200 or bcl2. DP cases were more frequent among secondary AML (43% vs 27%, p=0.008), CD34+ AML (39% vs 24%, p=0.006) and CD56- patients (38% vs 20%, p=0.002). Overall, 274 patients were evaluable for response to induction therapy, and 175 (64%) attained a complete remission (CR). CR rate was lower in CD200+ (60% vs 75%, p=0.007); co-expression of CD200 and bcl2 did not worsen CR probability, but DN patients have higher CR rate compared to all other groups (77% vs 59% p=0.01). Relapse occurred in 60/175 patients, while 115/175 remained in CR, with a 3-year DFS of 59%. CD200 and bcl2, alone or in association, did not influenced DFS. Overall 174 patients (60%) have died, with a 3-years OS of 36%. DP patients had a 3-year OS of 23%, compared to 35% in patients with isolated CD200 or bcl2 expression and 54% in DN (p=0.004). In multivariate analysis (MVA) statistical significance was found for age ≥55, CD34 positivity, high WBC count and CD200/bcl2 DP. Combining the four variables resulting from MVA, we designed a score predicting very different OS probability: 3-year OS was 91% in patients without risk factors compared to 51%, 29%, 13% and 0% in those with 1, 2, 3 or 4 risk factors, respectively (p<0.00001) (Figure 1).

In conclusion, CD200 and bcl2 concomitant expression was associated with lower OS probability, along with known prognostic factors such as age, WBC count and CD34+. These data may foster the use of bcl2 inhibitors and anti-CD200 antibodies in DP AML patients.

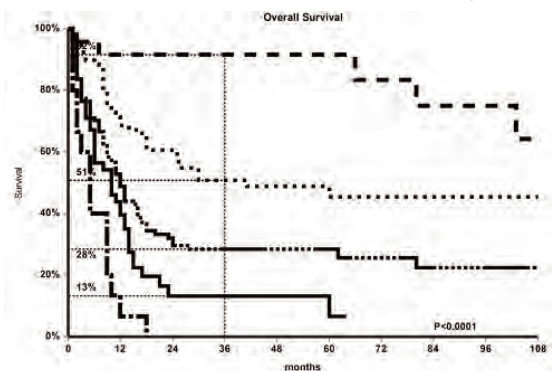


Figure 1.

C094

CONCOMITANT EXPRESSION OF CD200 AND BCL2 IS ASSOCIATED WITH REDUCE SURVIVAL IN ACUTE MYELOID LEUKEMIA PATIENTS

M. Tiribelli¹, A. Geromin¹, D. Raspadori², A. Michelutti¹, M. Cavallin¹, S. Sirianni², S. Di Giusto¹, E. Toffoletti¹, R. Fanin¹, M. Bocchia², D. Damiani¹

¹Division of Hematology and Bone Marrow Transplantation, Department of Medical Area, University of Udine; ²Division of Hematology, University of Siena, Italy

CD200 overexpression is associated with poor prognosis acute myeloid leukemia (AML) and inhibition of bcl2-mediated apoptosis has a role in myeloid malignancies. Despite a frequent association of bcl2+ and CD200 overexpression in AML has been observed, no data on the role of concomitant expression of CD200 and bcl2 on outcomes have been reported. We analyzed 291 AML patients treated at our Institutions. Median age was 60 (range: 18-84) yrs, and 183 (63%) patients were >55 yrs; 91 patients (27%) had secondary AML, 109 (37%) had WBC ≥30x10⁹/L and 82 (28%) had an unfavorable cytogenetic/molec-

C095

MEASURABLE RESIDUAL DISEASE BY MULTIPARAMETRIC FLOW-CYTOMETRY IS A RELIABLE TOOL FOR RISK-STRATIFICATION OF FLT3-MUTATED AML PATIENTS.

R. Palmieri¹, L. Maurillo¹, M.I. Del Principe¹, A. Di Veroli², G. Paterno¹, E. De Bellis¹, R. Cerretti³, G. De Angelis³, B. Mariotti³, T. Ottone¹, M. Divona¹, S. Lavorgna¹, M. Irno Consalvo¹, C. Conti¹, C. Attrotto¹, D. Fraboni¹, M.T. Voso¹, S. Amadori¹, F. Lo Coco¹, W. Arcese³, A. Venditti¹, F. Buccisano¹

¹Ematologia Dipartimento di Biomedicina e Prevenzione Università di Roma "Tor Vergata"; ²UOC Ematologia Ospedale Bel Colle; ³Ematologia Dipartimento di Biomedicina e Prevenzione Università di Roma "Tor Vergata", Rome Transplant Network

Background: Mutations of the gene encoding Fms Related Tyrosine Kinase 3 (FLT3), both at the tyrosine kinase domain (TKD) and the juxta-membrane level (ITD), represent the most common lesions found in Acute Myeloid Leukemia (AML), identifying patients (pts) with poor prognosis. FLT3 mutations, due to their instability, are considered an unreliable tool for measurable residual disease (MRD) monitoring. Instead, multiparametric flow cytometry (MFC) may be a reliable tool to monitor MRD in this molecular subset.

Aims: The aim of the study was to investigate the feasibility of MRD assessment by MFC in FLT3 mutated (FLT3+) AML and to assess if MRD persistence may affect overall (OS) and disease-free survival (DFS) in this molecular subset in comparison with FLT3 wildtype(FLT3wt)

ones.

Methods: We retrospectively analyzed a series of 39 pts with de novo FLT3+ AML (34/39, 87% ITD, 2/39, 5% TKD, 3/39, 8% ITD/TKD). We compared MRD status and clinical outcome with a matched group of FLT3wt AML (n=128). Both groups were treated according to intensive EORTC/GIMEMA protocols. MRD was measured by MFC at the post-consolidation (PC) timepoint. Pts were defined as MRD-negative (MRD-), when obtaining a residual leukemic cells (RLCs) count below 3.5×10^{-4} (0.035%).

Results: Overall median age was 47 (range 17-72). In the FLT3+ group, 22/37 (59%) pts carried a NPM1 mutation vs 32/124 (26%) of FLT3wt ones ($p < 0.001$). Moreover, FLT3 mutation was associated with a WBC count $> 50 \times 10^9/L$ (20/39, 51%) vs 30/128 (23%) of those FLT3wt ($p < 0.001$). PC strategy was different in the 2 subsets with 16/39 (41%) FLT3+ pts submitted to allogenic stem cells transplantation (ASCT) as compared to 34/128 (27%) FLT3wt ones ($p = 0.01$). At PC, MRD- rate was significantly lower in FLT3+ pts. (4/39, 10%) as compared to those FLT3wt (40/128, 31%) ($p = 0.009$). In particular, 32/35 (91%) FLT3+ pts were above the ELN threshold of positivity (0.1%) and 11/35 (31%) above 1%. For FLT3+ pts, OS was longer for MRD- pts as compared to MRD+ ones (67% vs 31%, $p = 0.9$). As for as DFS, we observed the same difference for MRD- pts vs MRD+ ones (67% vs 29%, $p = 0.8$). Negative impact of MRD was proportional to the amount of RLCs at PC (Figure 1) ($p = 0.045$). For FLT3+ pts, ASCT was the only strategy allowing a prolonged OS as compared to autologous SCT or standard chemotherapy (4-years OS 63% vs 0% vs 15%, $p = 0.019$, respectively).

Conclusions: MRD as measured by MFC is a biomarker of quality of response, influencing long term outcome also in FLT3+ pts.

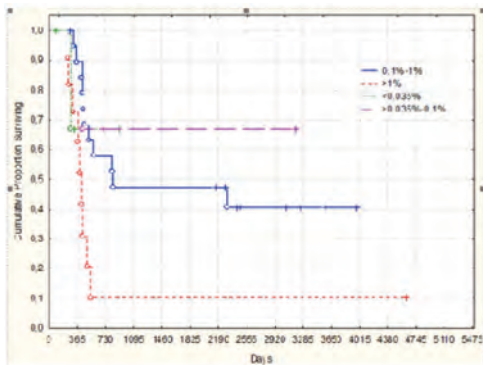


Figure 1.

Quality of Life, Pain Therapy and Home Care, Supportive Care

C096

RESULTS OF A REGIONAL PROJECT OF HOME-BASED CONTINUITY OF CARE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

C. Cartoni, I. Carmosino, G. Palumbo, G. Annechini, G.A. Brunetti, A. Andriani, S. Amadori, M. Bartolini, G. Cimino, P. De Fabritiis, C. Girmenia, T. Mazzini, M. Montanaro, A. Tendas, R. Foà

Hematology, Department of Translational and Precision Medicine, Sapienza University, Policlinico Umberto; Hematology, Fabrizio Spaziani Hospital, Frosinone; Associazione Italiana contro le Leucemie, Linfomi e Mieloma (AIL); Hematology, San Giovanni - Addolorata Hospital; Hematology, Department of Translation and Precision Medicine, Sapienza University of Rome, S. Maria Goretti Hospital; Hematology Division, S. Eugenio Hospital, Tor Vergata University; Hematology, Belcolle Hospital

Background: The Lazio Region and the Italian Association against leukemia, lymphoma and myeloma (AIL) have funded a specific program of home care with the aim of improving the quality of life of frail patients with hematological diseases and of optimizing the use of hospitalizations. The interventions, the intensity and the costs of care, are reported.

Patients and methods: One-year, real-life observational analysis at 7 hematology centers. Patients were part of 3 prognostic groups: i) advanced/terminal phase (AT), in need of palliative therapies or end-of life care; ii) chronic phase (CP) of an incurable disease, with a life expectancy > 6 months, in need of life-prolonging therapies and early palliative care; iii) active phase (AP) of curable disease, in need of supportive care or anti-cancer treatment. No. of interventions, No. hospital beds equivalents and intensity care index (ICI): number of actual days of care/number of total days of home care duration, were analyzed respectively. The mean cost of a single cycle of care was calculated, excluding drugs, blood units and laboratory tests.

Results: Of the 680 patients managed, 361 (53%) were affected by AML/MDS/MPN, 176 (26%) multiple myeloma, 143 (21%) by NHL. They received 875 cycles of domiciliary assistance, 225 (26%) in AT of disease, 217 (25%) in CP, 433 (49%) in AP, with a mean number of 1.3 cycle of care/by patient. In a year, patients received 12,145 nurse visits, 4,947 medical visits, 3,553 transfusions, 991 anti-neoplastic drug infusions. Overall, 19,225 actual days of care were provided, corresponding to 58.5 hospital bed equivalents. The median duration of cycle of assistance and the intensity of care varied according to the phase of the disease - 63 days in AT, 58 days in AP, 153 days in CP, and an ICI value of 0.44, 0.27 and 0.14, respectively - and to the hematology center. The mean cost of cycle of care was 2,453 €, for a mean duration of 91 days.

Conclusions: This program, carried out at 7 hematology centers, allowed a high number of frail or not self-sufficient patients to be assisted at home, instead of being subjected to an inappropriate hospitalization. This multiprofessional approach has guaranteed the required levels of intervention and the continuity of management by the referral hematology center. Disease phase influenced the duration and the intensity of the home care; costs of the home care were in agreement with the standard levels reported.

C097

INTERNATIONAL VALIDATION OF A NEW EORTC QLQ-C30 SCORE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: ANALYSIS OF 2134 PATIENTS ENROLLED IN GIMEMA TRIALS

K. Sommer¹, F. Cottone¹, J. Giesinger², P. Fazi¹, M. Vignetti¹, F. Efficace¹, On behalf of the GIMEMA WP Quality of Life

¹Italian Group for Adult Hematologic Diseases (GIMEMA), Data Center and Health Outcomes Research Unit; ²Medical University of Innsbruck, University Hospital Innsbruck, Psychiatry II

The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) is a widely used cancer-specific patient-reported outcome measure in assessing

health-related quality of life across 15 health domains. Since this wide range of scales poses analytical challenges due to multiple testing, a recent study validated a single QLQ-C30 summary score (SS) in patients with solid tumors. The current study investigated the validity of the SS in patients with hematological malignancies. Univariate linear regression analyses were performed to investigate the ability of the SS, compared to the original QLQ-C30, to discriminate between patients groups differing on known characteristics, i.e. ECOG performance status (0-1 vs 2-3), comorbidity (yes vs no), red blood cells transfusion dependency (TD, yes vs no, patients with myelodysplastic syndromes only), age (≤ 60 vs > 60 years) and sex. Linear mixed models were used to examine responsiveness to change of the SS, comparing baseline versus post-induction scores in patients with acute myeloid leukemia. The performance of the SS was compared to those of each original QLQ-C30 scale by Cohen's effect size (ES). The SS outperformed 12 of the QLQ-C30 scales for ECOG (ES=0.66) and 11 scales for comorbidity (ES=0.17). Comparing groups by age and sex, the SS outperformed 13 (ES=0.17) and 12 (ES=0.25) scales respectively. With regard to the disease-specific comparisons, the SS (ES=0.21) showed better discrimination than eight QLQ-C30 scales for TD and the SS was more sensitive to change (ES=0.41) than 10 of the QLQ-C30 scales when comparing baseline versus post-induction scores. The current findings provide support for the validity of the EORTC QLQ-C30 summary score as an endpoint in patients with hematological malignancies. Specifically, the findings endorse its use as a potential new study endpoint in this population when symptom or other health domain specific hypotheses are not available or when mixed hematologic cancer populations are being studied.

C098

IMPACT OF COMORBIDITY ON HEALTH-RELATED QUALITY OF LIFE PROFILE IN LONG-TERM SURVIVORS OF ACUTE PROMYELOCYTIC LEUKEMIA

F. Sparano¹, M. Breccia², G. Avvisati³, F. Cottone⁴, T. Intermesoli⁴, E. Borlenghi⁵, P. Carluccio⁶, F. Rodeghiero⁷, F. Fabbiano⁸, M. Luppi⁹, C. Romani¹⁰, M. Sborgia¹¹, S. D'Arda¹², B. Martino¹³, N. Cantore¹⁴, M. Crugnola¹⁵, G. Nadali¹⁶, S. Amadori¹, M. Vignetti¹, F. Lo Coco¹⁷, F. Efficace¹, On behalf of the GIMEMA WP Quality of Life¹

¹Italian Group for Adult Hematologic Diseases (GIMEMA); ²"Sapienza" University of Rome; ³University Campus Bio-Medico; ⁴University of Milan; ⁵ASST Spedali Civili; ⁶University of Bari; ⁷San Bortolo Hospital; ⁸Ospedali Riuniti Villa Sofia - Cervello; ⁹Azienda Ospedaliero-Universitaria Policlinico University of Modena and Reggio Emilia; ¹⁰Ospedale Armando Businco; ¹¹Department of Hematology-Bone Marrow Transplant Center; ¹²Città della Salute e della Scienza Hospital; ¹³Azienda Ospedaliera Bianchi Melacchino Morelli; ¹⁴San G. Moscati Hospital; ¹⁵Parma University; ¹⁶University of Verona; ¹⁷University of Rome Tor Vergata

As a result of the outstanding progress in the treatment of acute promyelocytic leukemia (APL), the number of APL long-term survivors has substantially increased over the last decades. Therefore, it is of growing importance to investigate their health-related quality of life (HRQOL) outcomes, to better address possible unmet needs. The primary objective of this work is to describe, in long-term APL survivors, the prevalence of symptoms and functional limitations by number of comorbidities. The secondary objective is to investigate factors potentially associated with their overall quality of life. The analyses were based on 244 APL survivors recruited in two large multicenter GIMEMA trials. Key symptoms, functional limitations, and overall QoL/health status (QL) were measured by the EORTC QLQ-C30 questionnaire. A higher score reflects either a worse or a better HRQOL outcome, respectively in symptom and functional/QL scales. For each scale, we assessed the mean score by the number of recently developed comorbidities (i.e. 0, 1, >1 comorbidities), using Wilcoxon-Mann-Whitney test to assess statistical significance. Univariate and multivariate linear regression (MLR) analyses were used to investigate factors associated with self-reported QL. The median age at diagnosis was 39 years, with a median follow-up from diagnosis of 14 years. Patients with at least one comorbidity reported statistically significant worse scores for all EORTC QLQ-C30 scales, except for nausea, constipation and diarrhoea. For example, survivors with 0 comorbidities reported a mean score of 87, 96.1 and 99.6 respectively for QL, physical and role functioning scales. For the same scales, those with >1 comorbidities re-

ported respectively a mean score of 69.7, 84.7 and 86. The largest differences in symptom scale were those for pain (2.2 vs 19.2), dyspnea (4.6 vs 17.8), and fatigue (9.4 vs 26.8), respectively for patients with 0 vs >1 comorbidities. MLR confirmed the results from univariate analysis, suggesting that having a greater social support ($p<.001$), being male ($p=.011$) and absence of comorbidities ($p<.001$) are independently associated with better QL. Our findings provide evidence that long-term APL survivors reporting more than one comorbidity deserve special attention. In addition, we provide new evidence on independent factors predicting quality of life outcomes in this population.

C099

IMPAIRMENT OF QUALITY OF LIFE IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES AND HIGH SERUM FERRITIN CONCENTRATION

G. Caocci, P. Niscola, G. Gaidano, F. Cottone, G.A. Palumbo, M. Breccia, A. Ricco, L. Fianchi, C. Frairia, V. Santini, M. Bergamaschi, I. Capodanno, N. Di Renzo, G. La Nasa, M. Vignetti, F. Efficace. On behalf of the GIMEMA WP Quality of Life

Department of Medical Sciences and Public Health University of Cagliari Businco Hospital, Haematology Unit and Pathology Department S. Eugenio Hospital Rome, Division of Hematology, Department of Translational Medicine University of Eastern Piedmont, Health Outcomes Research Unit GIMEMA Data Center and Health Outcomes Research Unit, Division of Hematology AOU 'Policlinico-V.Emanuele', Division of Cellular Biotechnologies and Hematology University Sapienza, Haematology and Transplants Unit University of Bari, Department of Hematology Università Cattolica Sacro Cuore, Haematology II AOU Città della Salute e della Scienza San Giovanni Battista Molinette Hospital, Functional Unit of Haematology Azienda Ospedaliero Universitaria Careggi University of Florence, Clinica Ematologica IRCCS AOU San Martino IST, Hematology Unit Arcispedale Santa Maria Nuova, Istituto di Ricovero e Cura a Carattere Scientifico, Hematology and Stem Cell Transplant Fazzi Hospital, Department of Medical Sciences and Public Health University of Cagliari Businco Hospital, Health Outcomes Research Unit GIMEMA Data Center and Health Outcomes Research Unit, Health Outcomes Research Unit GIMEMA Data Center and Health Outcomes Research Unit

Introduction: Patients with lower risk myelodysplastic syndromes (LR-MDS) frequently develop iron overload, secondary to ineffective erythropoiesis and transfusion dependency. Iron overload is monitored by serum ferritin level and can lead to organ impairment, especially heart and liver. The primary objective of this study was to investigate the relationship between health-related quality of life (HRQoL) and serum ferritin levels in patients with LR-MDS.

Methods: Analysis was based on newly diagnosed LR-MDS patients who were recruited in a large observational study involving 37 centers. All patients were classified according to the International Prognostic Scoring System (IPSS) in low and intermediate-1 risk. At baseline, two groups of patients were identified, based on a serum ferritin level of ≥ 1000 ng/mL, considered as cut-off of severe iron overload. All patients completed the well-validated EORTC QLQ-C30 questionnaire. For each scale, clinically meaningful differences were evaluated based on published evidence-based international guidelines.

Results: The median age of enrolled LR-MDS patients was 72 years (range 32-94); 58% of them were males. Out of 382 patients available for the analysis, 353 reported serum ferritin value below 1.000 ng/ml (median 309, range 4-979) and 29 above the cut-off (median 1.906, range 1.039-5.827). Transfusion dependency and presence of ≥ 2 comorbidities were significantly higher in the ferritin ≥ 1000 group (41.4% vs 11.1%; $p<0.001$ and 58.6% vs 29.6%; $p=0.005$, respectively). HRQoL scales and prevalence of symptoms scores by ferritin level group and the respective mean score differences, are reported in Table. Patients with ferritin ≥ 1000 ng/mL reported worse outcomes (i.e., lower mean scores) across all functioning scales. In particular, the largest mean difference was found for emotional functioning, being: 65.5 and 74.6 for those with ferritin ≥ 1000 ng/mL and for those below this threshold respectively. Similarly, with regard to symptom burden, those patients with higher ferritin levels had clinically relevant worse (i.e., higher mean scores) severity of fatigue, pain, insomnia, dyspnea and appetite loss. Further details are reported in table.

Conclusions: Patients with LR-MDS and serum ferritin values ≥ 1000 ng/mL reported a substantial burden of symptoms and functional im-

pairments. The role of iron chelation in improving HRQoL in patients with iron overload should be investigated.

Table 1.

Patient-Reported QoL	Ferritin <1000 ng/mL Values: mean (SD) (n=353)	Ferritin ≥1000 ng/mL Values: mean (SD) (n=29)	Mean Differences
Functional scales			
Physical Functioning	75.09 (21.35)	68.51 (23.36)	*6,58
Role Functioning	76.49 (28.22)	70.11 (30.98)	*6,38
Emotional Functioning	74.58 (21.20)	65.48 (25.23)	*9,1
Cognitive Functioning	82.29 (20.73)	79.17 (21.09)	*3,12
Social Functioning	84.75 (22.98)	75.60 (27.40)	*9,15
Global health status / QoL	60.53 (22.41)	54.17 (23.19)	*6,36
Symptoms			
Fatigue	35.32 (25.85)	40.61 (26.44)	*5,29
Nausea / Vomiting	4.39 (10.38)	5.75 (10.23)	1,36
Pain	17.00 (23.78)	25.29 (22.55)	*8,29
Dyspnoea	24.72 (28.10)	35.63 (33.25)	*10,91
Insomnia	25.50 (28.62)	29.89 (34.90)	*4,39
Appetite loss	11.80 (23.22)	27.59 (32.21)	*15,79
Constipation	14.68 (24.18)	19.05 (27.86)	4,37
Diarrhoea	8.03 (18.16)	7.14 (18.94)	-0,89

* Clinically relevant differences.

C100

PHYSICAL ACTIVITY AND HEALTH-RELATED QUALITY OF LIFE OUTCOMES IN MULTIPLE MYELOMA SURVIVORS: RESULTS FROM THE PROFILES REGISTRY

M. Servadio¹, F. Cottone¹, K. Sommer¹, S. Oerlemans², L. van de Poll-Franse^{2,3,4}, F. Efficace¹, On behalf of the GIMEMA WP Quality of Life¹

¹Italian Group for Adult Hematologic Diseases (GIMEMA); ²Netherlands Comprehensive Cancer Organisation; ³Center of Research on Psychology in Somatic Diseases (CoRPS); ⁴The Netherlands Cancer Institute

The objective of this study was to investigate whether physical activity (PA) is associated with health-related quality of life (HRQOL) outcomes in multiple myeloma (MM) survivors.

We used data from the Patient Reported Outcomes Following Initial treatment and Long-term Evaluation of Survivorship (PROFILES) registry. Patients (n=175) were invited to complete the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 and EORTC Multiple Myeloma (QLQ-MY20) questionnaires. Sixty-four percent of patients (n=112/175) completed the questionnaires and time since diagnosis was more than 3 years for 61% of patients. Information regarding PA assessed by the European Prospective Investigation into Cancer (EPIC) Physical Activity questionnaire. Patients were classified into two groups: physically active and not physically active patients. We selected as primary HRQOL outcomes all the scales of the EORTC QLQ-MY20 questionnaire (i.e. future perspective, body image, disease symptoms and side effects of treatment) and two scales of the EORTC QLQ-C30 questionnaire (i.e. fatigue and global health status/HRQOL). The remaining scales of the EORTC QLQ-C30 were evaluated as secondary HRQOL outcomes. We performed univariable and multivariable linear regression models to evaluate possible associations between PA and HRQOL outcomes. Clinically meaningful differences were also examined. Multivariable regression models showed that regular PA was independently associated with higher global HRQOL score (R²=0.14, p=0.001), lower fatigue (R²=0.22, p=0.002) and fewer side effects of treatments (R²=0.19, p=0.001). In addition, clinically relevant differences were found for global health status/HRQOL (Δ =19.21), fatigue (Δ = -21.53) and side effects of treatment (Δ = -3.62) scales between physically active and not physically active patients. Exploratory analyses performed on the secondary HRQOL outcomes confirmed statistically significant better outcomes for physically active patients.

While no causal relationship can be established at this stage, current findings contribute to a better understanding of the relationship between PA and disease specific HRQOL aspects in MM survivors. Future prospective studies are warranted to further elucidate on the beneficial effects of PA on HRQOL outcomes of MM survivors.

POSTERS

Non-Oncological Hematology

P001

HAEMOGLOBIN CONTENT OF RED BLOOD CELLS IS DIFFERENT IN POLYCYTHEMIA VERA (PV) AND IDIOPATHIC ERYTHROCYTOSIS (IE)

G. Biagetti, G. Bogoni, E. Cosi, I. Bertozzi, M.L. Randi

Clinica Medica 1 - Azienda Ospedaliera di Padova - DIMED, Italy

Background: In PV patients the major risk are thrombosis and patients with IE have a thrombotic risk mainly in arterial vessels. Half patients with IE carry HFE polymorphisms and haemoglobin (Hb), mean cell haemoglobin (MCH) and mean cell haemoglobin content (MCHC) are known to be higher than normal in patients with iron overload due to higher bioavailability of iron. We hypothesize that such parameters could be different in IE and PV and they may be useful in evaluating the risk of IE patients.

Patients and methods: We evaluated FBC at diagnosis of 93 patients with PV carrying JAK2V617F or JAK2 ex12 mutation and of 78 patients with IE, without JAK2 or EPOR, VHL, PHD2, HIF-1 genes mutations. Statistical analysis was performed using the t Student test.

Results: Results are shown in Table 1.

Discussion and Conclusions: While Hb and HT are higher than normal, MCV, MCH, MCHC are normal in both PV and IE. However, the latter are significantly higher in IE patients. Our data suggest that, while in PV patients a high number of RBC is produced by the neoplastic medulla, in IE there is an increased production of haemoglobin that spreads in a relatively normal RBC number. The presence of an excess of iron may create an oxidant environment and, in the presence of high MCHC, RBC are not typically discoid and display reduced deformability. We could then postulate that, while in PV the thrombotic risk is due to the high number of circulating neoplastic cells, in IE the arterial thrombotic risk could be given by higher concentration of haemoglobin that reduces deformability of cells. This observation must be confirmed by larger studies.

Table 1.

	NV	IE n= 78	PV n= 93	p
M/F	-	61/17	52/41	0.003
Age at diagnosis (years)	-	54 ± 16	64 ± 14	<0.0001
RBC (x10 ¹² /L)	4.50 - 5.90	5.74 ± 0.35	6.25 ± 1.01	<0.0001
Hb (g/L)	140 - 175	174 ± 9	176 ± 18	0.3
Ht (%)	0.41 - 0.50	51 ± 2	54 ± 6	0.0001
MCH (pg)	26 - 33	30.4 ± 1.8	28.7 ± 3.3	<0.0001
MCHC (g/L)	320 - 360	339 ± 13	327 ± 13	<0.0001
MCV (fl)	80 - 96	89.8 ± 5.0	87.9 ± 7.1	0.05

P002

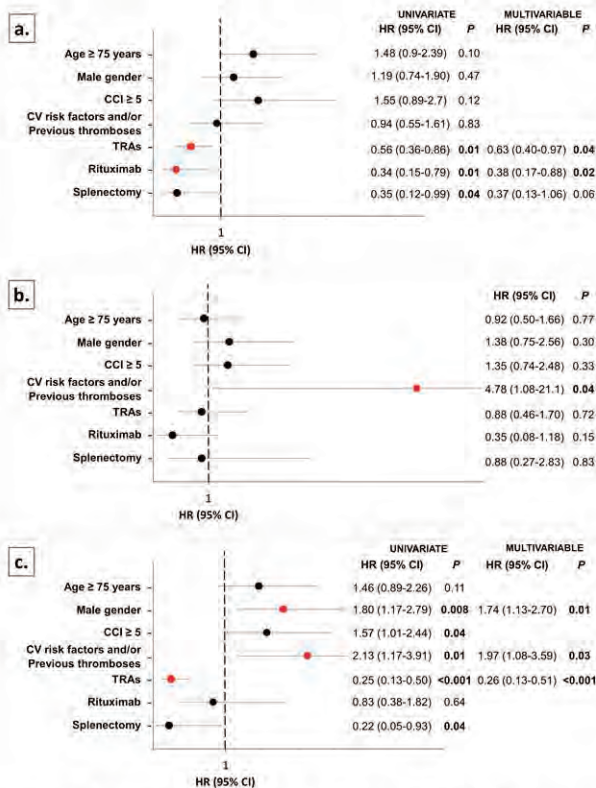
IMMUNE THROMBOCYTOPENIA IN THE ELDERLY. A MULTICENTER ITALIAN STUDY ON 524 PATIENTS

F. Palandri, C. Santoro, M. Carpenedo, S. Cantoni, W. Barcellini, G. Carli, V. Carrai, E. Rossi, E. Rivolti, A. Lucchesi, F. Rotondo, E. Baldacci, G. Auteri, E. Sutto, C. Di Pietro, L. Catani, D. Bartoletti, V. De Stefano, M. Ruggeri, M.G. Mazzucconi, M. Cavo, F. Rodeghiero, N. Vianelli

Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Hematology Division University of Sapienza, Division of Hematology Ospedale San Gerardo ASST Monza, Division of Hematology ASST Niguarda Ca' Granda Hospital, Hematology Unit Fondazione IRCCS Ca' Granda Hospital, Hematology Project Foundation and Department of Hematology San Bortolo Hospital, Hematology Department Careggi University Hospital, Institute of Hematology Catholic University School of Medicine Fondazione Policlinico Universitario A. Gemelli IRCCS, Department of Hematology Azienda USL IRCCS, Hematology Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Hematology Unit Infermi Hospital, Hematology Division

University of Sapienza, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Institute of Hematology Catholic University School of Medicine Fondazione Policlinico Universitario A. Gemelli IRCCS, Hematology Project Foundation and Department of Hematology San Bortolo Hospital, Hematology Division University of Sapienza, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital, Hematology Project Foundation and Department of Hematology San Bortolo Hospital, Institute of Hematology "L. and A. Seràgnoli" Sant'Orsola-Malpighi University Hospital

Primary Immune thrombocytopenia (ITP) in the elderly is a major clinical challenge which is increasingly frequent due to global ageing population. To describe baseline ITP features, disease management, and outcome, a centralized electronic database was established in 11 Italian Hematology Centers. Clinical/laboratory data on 451 elderly pts treated from 2000 onwards were retrospectively collected. All pts were observed for ≥1 yr (total observation: 2704 pt-yrs). Baseline characteristics were: median age: 71.1 yr (60-91.5); females: 57.7%; median platelet (PLT): 16x10⁹/l (1-99); pts with hemorrhages: 237 (53.9%, grade ≥3: 7.3%). Patients were sub-grouped into two categories: elderly (60-74 yr, n. 258) and very elderly pts (VEP) (≥75 yr, n. 193). VEP had same PLT count/bleedings at diagnosis, but presented more frequently cardiovascular risk factors (CVRFs) and higher Charlson Comorbidity Index (CCI). Front-line therapy included prednisone (PDN, 82.9%), dexamethasone (DEX, 14.6%), thrombopoietin-receptor agonists (TRAs, 1.3%), and oral immunosuppressive agents (IAs, 1.1%). Compared to pts <75 yrs, VEP received significantly less frequently PDN starting dose ≥1 mg/kg/d (59.6% vs 72.5%, p=0.01) and DEX 40 mg/d (44% vs 85.4%, p<0.001). A 2nd- and 3rd-line approach was required in 290 (64.3%) and 158 (35%) pts, regardless of age. 2nd-line, rituximab (RTX, p=0.02) and splenectomy (p=0.03) were more used in pts <75 yrs, while VEP were more treated with IA (p=0.02) or TRAs (p=0.01). Splenectomy was never used in VEP 3rd-line.



Univariate and multivariable recurrent events Andersen-Gill analysis of baseline factors and treatments associated with bleeding- (a), thrombosis- (b) and infection-free (c) survivals.

Figure 1.

Overall, 88.3%, 87.2% and 92.4% of evaluable pts had a response to 1st, 2nd and 3rd-line therapies, respectively. Response and relapse rates were not influenced by age. 48 thromboses, 178 bleedings and 115 infections were observed during follow-up. Annualized incidence rates per 100 pt-yr complications were: 1.7 (thromboses, grade ≥ 3 : 0.8), 4.5 (bleedings, grade ≥ 3 : 1.5), and 3.9 (infections, grade ≥ 3 : 0.6). The probability of all-grades hemorrhages was significantly reduced by TRAs/RTX use, while CVRFs significantly predicted thromboses and infections. TRAs correlated with lower probability of infections (Figure 1). Among CVRFs/CCI, diabetes was significantly associated with ITP complications. Overall, 72 pts died; by competitive risks analysis for disease-related deaths, survival was comparable in the two age groups. Careful evaluations of comorbid conditions and implementation of age-adapted treatment strategies are required in the elderly.

P003

RESPONSE RATE AND RESPONSE DURATION AFTER DISCONTINUATION OF TREATMENT WITH THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RAS) IN PATIENTS AFFECTED BY PRIMARY IMMUNE THROMBOCYTOPENIA (PITP): RETROSPECTIVE STUDY. PRELIMINARY RESULTS. GIMEMA PROTOCOL ITP0714

M.G. Mazzucconi, C. Santoro, F. Paoloni, M. Carpenedo, V. De Stefano, S. Cantoni, N. Vianelli, M. Iodice, E. Baldacci, F. Zaja, A. Bosi, P. Schinco, P. Fazi, F. Rodeghiero; on behalf of the GIMEMA Working Party "Anemie e Piastrinopenie"

Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I; Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I; Data Center-GIMEMA Foundation - Franco Mandelli Onlus; ASST di Monza, Ospedale Sant'Eugenio, Unità Operativa Ematologia e CTA; Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Area Ematologica; SC Ematologia, ASST Grande Ospedale Metropolitano Niguarda; AOU di Bologna, Policlinico S. Orsola Malpighi, UOC Ematologia; Data Center-GIMEMA Foundation - Franco Mandelli Onlus; Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Sapienza Università, Policlinico Umberto I; ASUI di Udine, Presidio Ospedaliero Santa Maria Della Misericordia, Ematologia; SOD Ematologia, AOU Careggi; AOU Città della Salute e della Scienza, Ospedale S. Giovanni Battista Molinette - SC Ematologia - Università degli Studi di Torino; Data Center-GIMEMA Foundation - Franco Mandelli Onlus; AULSS 8 Berica - Ospedale di Vicenza - UOC Ematologia, Italy

Background: Eltrombopag (El) and Romiplostim (Rom) (TPO-RAs) represent second/third-line agents for pITP therapy with high success rate, but long-term treatment is generally required, because platelet (plt) level return to baseline within 2 weeks after discontinuation. However, literature data indicate the possibility of maintaining response after stop of both drugs.

Study design and Aim: retrospective, observational, multicentre study evaluating sustained response rate after discontinuation of TPO-RAs, in persistent or chronic adult pITP patients (pts) (>18y) who failed one or more therapy lines, including splenectomy. Definitions: Response (R): plt count $\geq 30 \times 10^9/L$ and at least 2-fold increase baseline count and no bleeding; Complete Response (CR): plt count $\geq 100 \times 10^9/L$ without bleeding, both in absence of concomitant medications and rescue therapy in the preceding 4 weeks; Sustained R (SR) and Sustained CR (SCR): responses assessed as the first plt count, at more than 4 weeks after discontinuation of TPO-RAs, that satisfy the response definitions.

Results: 148 eligible pts were recruited in 19 GIMEMA Centres from 2015 to 2016: F 93, M55, median age at TPO-RAs start, 58y (17-83); 86 pts (58.50%) received Rom, 61 (41.50%) El: no concomitant therapies in 51 pts, while corticosteroids, IVIg and other medications were administered in 88, 4 and 4 pts, respectively; pts had received a median of 4 (1-8) previous therapy lines before TPO-RAs, 50 (33.78%) had been splenectomised. Reasons of TPO-RAs start were: no response to splenectomy (8.70%), relapse after splenectomy (27.54%), contraindication to splenectomy and failure of one or more therapy lines (30.43%), refusal of splenectomy and failure of one or more therapy lines (23.19%), no eligibility to splenectomy (10.14%). At start, median plt count was $16 \times 10^9/L$ (1-134). Responses evaluation: 69 pts (49.64%) achieved a CR, (Rom 42.68%, El 59.65%), 41 a R (29.50%), (Rom 32.93%, El 24.56%) 29 (20.86%) were unresponsive (Rom 24.39%, El 15.79%). Ninety pts suspended TPO-RAs (Rom 58, El 32), 34/90 (39.53%) in CR or R. Out of 31 evaluable pts, 29 obtained SR or SCR,

(Rom 22/24; El 7/7) for a median time duration of 135.4 weeks (range 4.7-317.0). In summary, out of 148 pts treated with TPO-RAs, 29 (19.6%) reached a SR or SCR maintained for a median time of 2.6 years, according to the published data. Our results highlight that TPO-RAs therapy can be successfully suspended in a subset of pITP pts.

P004

ABSTRACT WITHDRAWN

P005

EFFICACY, SAFE, SUSTAINED RESPONSE OF TPO-MIMETICS IN NEWLY DIAGNOSED, PERSISTENT, CHRONIC ITP PATIENTS: A SINGLE CENTER EXPERIENCE

E. Rivolti, K. Codeluppi, F. Massaro, F. Soci, F. Merli

Hematology UNIT AUSL IRCCS Reggio Emilia, Italy

Background: The TPO-RAs romiplostim (R) and eltrombopag (E) represent a highly effective choice of treatment in chronic refractory/relapsed primary autoimmune thrombocytopenia (ITP) patients, but also in newly diagnosed and persistent ITP patients. In this setting there is no consensus about optimal treatment.

Aim: To observe the efficacy, the safety, the rate of sustained response of TPO-RAs in patients with chronic, persistent and newly diagnosed ITP in our center.

Patients and Methods: We retrospectively evaluated 28 patients (11 F; 17 M) treated with TPO-RAs from June 2010 to March 2019. The median age at the start of TPO RAs was 61 years. 21 patients were treated with E, 7 patients received E and R (5 patients switched from E to R, 2 from R to E), 10 patients were splenectomized, 18 patients were allocated to chronic ITP, 4 to persistent ITP, 6 to newly diagnosed ITP. The median follow up from the start of TPO-RAs was 24 (range 1-105) months, the median baseline platelet count was $19 \times 10^9/L$ (range 3-45), the median of previous lines of therapy was 2. The median time of treatment was 8 months (range 1-76) for E treatments and 4 months (range 1-21) for R treatments.

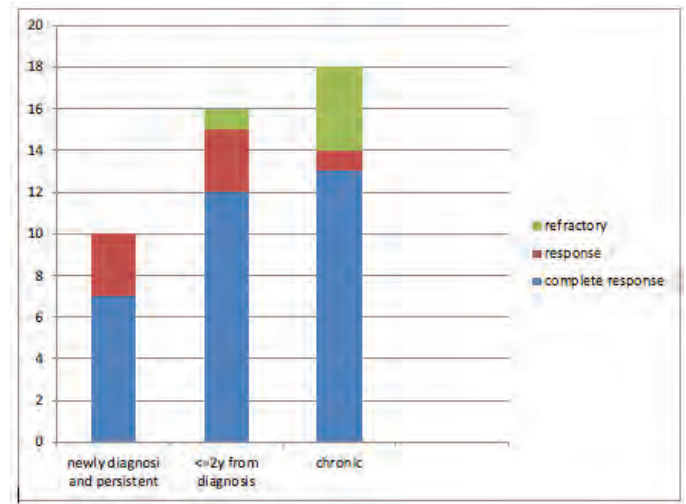


Figure 1.

Results: In E treatments we observed 19 complete response (CR), 3 response (R) with a response rate of 78.5%, while 6 patients (21%) were no responders. 19 (67%) stopped the treatment after a median time of 5 months: 3 (10.7%) for loss of response, 5 (18%) for no response, 6 (21%) patients for stable response, 1 (3.5%) for an adverse event, 4 (14%) patients died for other causes. In R group 2 patients achieved a CR, one patient achieved a R with an overall response rate of 43%, 3 patients were no responders, 6 patients discontinued the treatment: 4 for no response, 1 for adverse event, 1 for loss of response. The response rate to TPO-RAs was 100% in newly diagnosed and persistent patients, 93% in ITP patients with a time from diagnosis to treatment with TPO-RAs ≤ 2 years (16 patients), 72% in chronic ITP patients. The rate of discontinuation for stable response was 40% in newly di-

agnosed and persistent ITP patients. 6 patients(21%) experienced grade 3-4 adverse events: 1 pulmonary embolism, 1acute myocardial infarction, 1 deep vein thrombosis, 2 headache.

Conclusions: In our cohort of ITP patient the TPO- RAs use was more effective in early stage of disease (newly diagnosed , persistent ,but also <2 years from diagnosis) than in chronic stage of disease.

P006

IRON-CHELATION WITH FILM-COATED TABLET AFTER DISPERSIBLE TABLET FORMULATION OF DEFERASIROX IN A "REAL LIFE" APULIAN COHORT OF PATIENTS WITH THALASSEMIA

N. Sgherza¹, A. Quarta¹, A. Pasanisi², M.P. Solfrizzi¹, M. Serra³, R. Renni⁴, A. Vitucci⁵, C. Dapirile⁶, A. Palma⁵, R. Frappampina⁵, A. Giannotta¹, D. Visceglie⁶, G. Specchia⁵, D. Pastore¹

¹U.O.C. di Ematologia – Centro Trapianti di Midollo Osseo – Centro della Microcitemia - P.O. "A.Perrino"; ²Dipartimento di Scienze Mediche Chirurgiche e Sperimentali - Università di Sassari; ³DH Talassemia - U.O. Medicina Interna - Osp. "V. Fazzi"; ⁴DH Talassemia-U.O. Medicina Interna - Osp. Civile "F. Ferrari"; ⁵U.O. Ematologia con Trapianto -Azienda Ospedaliero-Universitaria - Policlinico di Bari; ⁶Servizio di Immunematologia e Medicina Trasfusionale – Day Service Talassemici -Osp. "Di Venere", Italy

Iron-chelation treatment has a crucial role in clinical management of patients (pts) with transfusion-dependent thalassemia (TDT). The once-daily oral deferasirox (DFX) dispersible tablet (DT) formulation, available since 2007, offered an improved option over parenteral deferoxamine and thrice-daily oral defiprone, providing greater compliance and patient satisfaction with good efficacy and safety. An improved film-coated tablet (FCT) formulation of DFX has therefore been developed offering a simpler and more convenient mode of oral administration, and potentially improving gastrointestinal tolerability. We report a retrospective multicentric Apulian "real-life" experience of switching from DT to FCT DFX in 113 subjects (64 males; 49 females) with TDT (major: 100 pts;intermedia: 13 pts). Median age was 43 years (range 20–61). 63 pts (56%) were without spleen. We compared last year of treatment of DT DFX (pts receiving other iron-chelators (deferoxamine, deferiprone), before starting FCT DFX were excluded) at a median dose of 24 mg/kg/day (range 10-40) with a median time of treatment of FCT DFX of 540 days (range 128-620) at a median dose of 15.4 mg/kg/day (range 7-27). Pts were strictly monitored, according to current guidelines, particularly with respect to renal, hepatic, ocular and auditory functions. Comparing pts receiving DT versus FCT no statistically significant differences were found in terms of median pre-transfusion hemoglobin level (9,5 g/dl vs 9,5 g/dl; P value: 0.2131), median serum ferritin (580 ng/l vs 508 ng/ml; P value: 0.9473), median transfusion interval (16 days vs 18 days; P value: 0.1109) and adverse events. Focusing our attention on interval between transfusions, it was stable for 96 pts (85%) while 17 subjects (15%), showed a median improvement of transfusion interval of 5 days (range 3-12) statistically significant (P value: 0.0135). Despite longer transfusion interval, median pre-transfusion Hgb in pts receiving FCT DFX (9.7 g/dl) was not lower than DT DFX (9,5 g/dl) (P value: 0.2474). One explanatory factor of the observed difference could be due to better adherence, satisfaction, and palatability of FCT DFX. Longer follow-up in a larger series of pts is warranted, but our experience shows a subset of pts with an improvement of interval between transfusions receiving FCT DFX. Our efforts are now oriented to determine peculiar biological and clinical features of this subset of pts.

P007

THROMBOPOIETIN-RECEPTOR-AGONISTS (TPO-RA) IN IMMUNE THROMBOCYTOPENIA (ITP): HIGH EFFICACY BUT AT INCREASED RISK OF THROMBOEMBOLIC EVENTS

R. Daffini, M. D'Adda, D. Gramegna, A. Passi, E. Borlenghi, M. Farina, E. Cerqui, C. Bottelli, S. Ferrari, G. Rossi

Hematology ASST Spedali Civili di Brescia, Italy

The reported incidence of thromboembolic events (TE) in ITP patients (pts) is higher than in ITP-free pts(163 events/10,000 ITP patient-years, Sarpatwari 2010; 430 events/10,000 ITP patient-years, Enger 2010). While TPO-RA don't increase the incidence of TE in ITP clinical trials-Wang 2016, Arai 2018-limited information is available in the real-

life setting. We analyzed all ITP pts receiving TPO-RA at our center, focusing on efficacy and on TE. Among 332 ITP pts, 26, median age 71 (28-85), received TPO-RA a median of 68 months (1-683) after diagnosis. They had failed at least 3 prior ITP medications. Eltrombopag (E) was used in 24 pts; Romiplostim (R) in 10 pts; 8 pts switched from E to R for failure (7) or thrombosis (1). Platelet (plt) median count at TPO-RA treatment start was 12.000/mm³ (2000-38000). Response (Plt>50.000/cmm) after 1 month was obtained in 13/24 (54%) pts receiving E, in 3/10 (30%) pts treated with R, and in 4/7 pts switched to R after E failure for an ORR of 88% (23/26). Median TPO-RA treatment duration is 17+ months (1-118+). At last follow-up 16 pts are still on E, 10 on R; their median plt count is 83000 (9000-336000). Treatment was stopped after failure (3) and in 4 responding pts: TE (2) splenectomy (1), stable remission (1).

Table 1.

Variables	TE free pts	TE pts	P value
Age at TPO-RA beginning	Median age 71 (28-85)	Median age 67 (46-82)	0.430
At least 1 CV risk factor (hypertension, diabetes, obesity, smoke, dyslipidemia)	15/19 pts	7/7 pts	0.479
Previous thrombosis	5/19 pts with previous TE	2/7 pts with previous TE	0.908
TPO-treatment duration	Median duration months 14,5 (1-58)	Median duration months 31 (1-115)	0.099
TPO Median dosage:	With E median dosage 50 mg/die (25-75) With R median dosage 6 mcg/week (4-8)	With E median dosage 50 mg/die (12,5-75) With E both pts had 6 mcg/week as dosage	0,925
Gender (M/F)	11 M, 8 F	6 M, 1 F	0.391
Concomitant medications (yes/no)	15/19 pts with concomitant medication	5/7 pts with concomitant medication	0.686
N° of previous medications	6 pts 3 rd line therapy, 13 pts > 3 rd line therapy	2 pts 3 rd line therapy, 5 pts >3 rd line therapy	0.883
Plts count (median value)	69000/cmm (9000-199000)	100000/cmm (28000-826000)	0.020
Splenectomy	3/19 pts	5/7 pts	0,4

One responding pt died of pneumonia while on E. During TPO-RA, 7/26 pts experienced one TE: acute coronary event (3), femoral arterial thrombosis (1),multiple cerebral thrombosis (1), pulmonary embolism (1) and basilic vein thrombosis (1). The events occurred a median of 2 months (1-113) after TPO-RA start. Median plt count was 100.000/cmm (28.000-826.000); only 1 pt had thrombocytosis. Overall, the incidence of TE was 960 per 10,000 patient-years at risk, twice the expected rate in ITP and 4 to 9 times the rate of the general population. Among variables analyzed (Table 1), only median plt count at thrombotic episode compared to median plt count during TPO-RA (100.000 vs 69.000 -p 0.02) significantly differed in pts with- or without thrombosis.

The study confirms the very high response rate of ITP pts to TPO-RA (88%) but it also shows an excess of TE compared to ITP pts not treated with TPO-RA, occurring early after treatment start and mainly in arteries (5/7, 71%). Risk factor was a relatively higher plt count.

P008

ELTROMBOPAG TREATMENT FOR APLASTICA ANEMIA REFRACTORY TO IMMUNOSUPPRESSIVE TREATMENT: RETROSPECTIVE STUDY OF 6 PATIENTS FROM A SINGLE INSTITUTION

C. Finelli, S. Parisi, S. Paolini, A. De Vivo, F. Bonifazi, P. Ricci, C. Sartor, M. Fogli, S. Fatica, M. Cavo

Institute of Hematology, S.Orsola-Malpighi University Hospital, Italy

Introduction: Eltrombopag (ELT) , an oral thrombopoietin-receptor agonist, has shown efficacy in acquired severe Aplastic Anemia (SAA) either in the refractory setting or in the first-line therapy, in addition to standard immunosuppressive treatment (IST). However the therapeutic efficacy and safety of ELT for SAA in the real-world setting still need to be explored, especially with regards to long-term outcome. Therefore we retrospectively analyzed our single-center experience on ELT treatment in SAA patients refractory to IST.

Methods: From June 2016, in our Institution, 6 pts (4 males), median age: 66.5 (40-85) yrs, were treated with ELT because of a diagnosis of SAA refractory to previous IST. The following response criteria were used: complete response (CR): Hb >10 g/dL, neutrophil count >1.5x10⁶/ml and platelet count >100x10⁶/ml; partial response (PR): transfusion-independence; minimal response (MR): improvement in one or more lineage not fulfilling the criteria of PR.

Results: At the start of ELT, 4 pts showed SAA, and 2 pts a very severe Aplastic Anemia (VSAA); previous IST treatment was: ATG + cyclosporine (CYA) in 4 pts (one of them had received 2 courses of ATG), CYA + prednisone in 1 pt, and CYA alone in 1 pt. The median time between the start of first-line therapy and the start of ELT was 4 (0.5-62) months. In 1 pt CYA had to be stopped soon because of toxicity. The patient who had previously received 2 courses of ATG started ELT 7 months after second line treatment. 4 pts were treated with ELT + CYA, and 2 pts with ELT alone (because of concomitant infection and previous toxicity of CYA, respectively). Maximum daily dose of ELT was 150 mg in 5 pts, and 100 mg in 1 pt. Best response to ELT was CR in 1 pt (initial PR after 4 months, CR after 6 months), PR in 1 pt (after 2 months), MR in 3 pts (after 1, 8 and 16 months, respectively), while 1 pt showed no response and died for infection 2 months after the start of ELT. All the 5 responder pts are still maintaining the response (median duration of response: 6, range 1-17 months); 1 pt, who has stopped ELT after 8 months because of CR, is still maintaining response after 13 months from discontinuation, under CYA alone. A grade 1 ELT-related transient liver toxicity was observed in 1 pt.

Conclusions: In conclusion, in our experience ELT confirmed to be effective and safe in SAA patients refractory to IST, and some pts may show a first response only after > 6 months of treatment.

P009

LOW DOSAGES OF THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RAS) IN MAINTENANCE OF REFRACTORY/RELAPSED IMMUNE THROMBOCYTOPENIA (ITP)

D. De Novellis¹, C. Giordano¹, C. Mortaruolo¹, K. Ferrara¹, F. Chiurazzi¹, F. Pane¹

¹Departement of Clinical Medicine, Division of Hematology, University Federico II, Naples, Italy

Introduction: The TPO-RAs are platelet growth factors commonly used in the treatment of relapsed/refractory immune thrombocytopenia (ITP). They simulate the action of endogenous thrombopoietin (TPO) on megakaryocytes above all promoting differentiation. These drugs offer a good response rate but might require indefinite use. We investigated the effect of maintenance therapy with lower dosages on 16 patients who had a complete and stable response to full dosages.

Methods: Sixteen patients affected by chronic refractory/relapsed were treated with TPO-RAs (romiplostim or eltrombopag) for a median of 9 months (range 6-12) and they achieved platelets stability (always >50x10⁹/L) Therefore the frequency of administration was extended and the dosages were slowly reduced up to suspension. When the tapering was excessive, an important reduction of platelets was registered, so we proposed an intermediate doses of "maintenance", lower than the therapeutic one but effective to stable the platelets count. Our end point was overall platelet response (defined as durable platelet count >50x10⁹/L) during low doses. The secondary end point was the frequency of side effects when the dosages were decreased.

Table 1.

PATIENT	SPLENECTOMY	TPO	DOSAGE	MONTHLY DOSE INTENSITY CONSIDERING LEON: 0.3µg/kg/7 GG FOR ROMIPILOSTIM AND 175 MG/W FOR ELTROMBOPAG	RESPONSE DURATION (MONTHS)	SIDE EFFECTS IN FULL DOSES	SIDE EFFECTS IN LOW DOSES	RELAPSE	LAST PLATELETS COUNT DURING LOW DOSES
C.C.	SI	ROMIPILOSTIM	0.3µg/kg/14GG	50.0%	50	CEFALEA	NO	NO	150000
LA.	SI	ROMIPILOSTIM	0.3µg/kg/14GG	50.0%	50	CEFALEA	NO	YES	
N.A.	NO	ROMIPILOSTIM	0.3µg/kg/14GG	50.0%	65	NO	NO	NO	300000
R.S.	SI	ROMIPILOSTIM	0.3µg/kg/14GG	50.0%	48	NO	NO	YES	
M.G.	NO	ROMIPILOSTIM	0.3µg/kg/14GG	50.0%	49	NO	NO	NO	510000
E.A.	SI	ELTROMBOPAG	75 MG/W	43%	18	NO	NO	NO	141000
E.G.	NO	ELTROMBOPAG	75 MG/W	43%	8	CEFALEA	CEFALEA	NO	125000
G.E.	SI	ELTROMBOPAG	75 MG/W	43%	45	NO	NO	NO	275000
I.C.	SI	ELTROMBOPAG	50 MG/W	43%	24	CEFALEA	NO	NO	388000
L.S.	SI	ELTROMBOPAG	75 MG/W	43%	25	CEFALEA	CEFALEA	NO	390000
L.A.	NO	ELTROMBOPAG	50 MG/W	28%	46	NO	NO	NO	97000
M.A.	NO	ELTROMBOPAG	75 MG/W	43%	54.5	NO	NO	YES	
R.A.	NO	ELTROMBOPAG	75 MG/W	43%	24	NO	NO	NO	300000
R.D.	SI	ELTROMBOPAG	75 MG/W	43%	7	NO	NO	NO	168000
R.P.	NO	ELTROMBOPAG	50 MG/W	28%	30	NO	NO	NO	123000
S.M.	SI	ELTROMBOPAG	75 MG/W	43%	40	NO	NO	NO	344000

Results: Median age population was 53 years (range 30-81) composed by 11 females and 5 males. All patients were treated with corticosteroid therapy as first line (prednisone 1 mg/kg for a month) and 9/16 received splenectomy as second line. Five out of 16 patients treated with Romiplostim and 11/16 with Eltrombopag. Headache was present in 5/16

patients during the therapy and in 2/16 in the maintenance period. The overall response rate was 81.25%. Relapse (identified as a drop of platelets below 30x10⁹/L) rate was 18.75%. Headache incidence was reduced when lower doses (0.31% vs 0.12%) were implemented.

Discussion: It's not completely clear how the TPO-RAs can preserve their effect when used at reduced doses; it has been proposed in literature that an immune regulatory effect on cytokines with low doses succeed in stabilizing the platelet counts. They were safely tolerated with reasonable relapse rates. In a chronic pathology, as ITP, it's possible to reduce the dosages to improve global life quality with a good impact on the side effects and on the sanitary cost, preserving the platelets stability. These data, which have to be confirmed in a larger population, suggest a different setting for TPO-RAs used as maintenance and not only as therapy of relapsed/refractory ITP.

P010

NO DIFFERENCE IN TIME TO RESPONSE TO THROMBOPOIETIN RECEPTOR AGONISTS IN SPLENECTOMIZED AND NON SPLENECTOMIZED CHRONIC ITP PATIENTS

C. Mortaruolo, K. Ferrara, D. De Novellis, F. Chiurazzi, F. Pane

Department of Surgery and Clinical Medicine, Division of Hematology, University Federico II, Naples, Italy

Introduction: The thrombopoietin receptor agonists (TPO RAs), Eltrombopag and Romiplostim, are approved to treat chronic ITP in patients who failed treatments of first line (glucocorticoids or i.v. immunoglobulin). However their use in acute or persistent ITP is frequent in clinical practice and first line use is under debate. The aim of our study was to evaluate if splenectomy improves the time to response in patients receiving treatment with TPO RAs.

Materials and Methods: In our retrospective study we analyzed 42 patients (10 males; 32 females), with a median age 53 years, range: 19-87 years, affected by chronic ITP non responsive to first line glucocorticoids. Patients were divided in two groups: group A, splenectomized patients (n=13) relapsed after surgery, and group B, non splenectomized ones (n=29); both groups were treated with TPO RAs. Patients underwent weekly monitoring for the first 4 weeks and then monthly; no patient discontinued treatment due to adverse events.

Results: Patients in group A were treated with TPO RAs (5 Romiplostim, 8 Eltrombopag) after a median time of 44 months (r. 2-193) after splenectomy due to disease relapse. All patients had response (R, platelets >50000/L) or complete response (CR, platelets >100000/L) after a median time of 1 month from TPO starting. Group B patients were treated with TPO RAs as second line therapy (17 Romiplostim, 12 Eltrombopag) after failing first line treatment with glucocorticoids. All patients experienced R or CR after a median time of 1 month too. Comparing the two groups no statistically difference was observed in plt counts before treatment with TPO RAs (group A: m. 15000/L, group B: m. 14500/L), median time to response (1 months for both groups), median duration of response (group A: 32 months, r. 10-107; group B: 29 months, r. 2-65) and median dosage of TPO RAs (2 µg/Kg for Romiplostim and 50 mg/die for Eltrombopag for both groups).

Conclusions: Registrational trials on Eltrombopag and Romiplostim demonstrated an overlapping in percentages of response between splenectomized and non splenectomized patients. In addition to this our data show that splenectomy does not influence time to obtain R or CR and response duration in patients receiving treatment with TPO RAs.

P011

SINGLE CENTER EXPERIENCE ON 36 PATIENTS WITH AGE OVER 60 CONSECUTIVELY DIAGNOSED WITH SEVERE APLASTIC ANEMIA

E. Accorsi Buttini, E. Cerqui, C. Cattaneo, E. Borenghi, A. Passi, C. Crippa, G. Rossi

ASST Spedali Civili, Brescia, Italy

Aplastic anemia (AA) in elderly patients is a challenging problem particularly when severe (SAA). Treatment in pediatric/adult patients is well standardized, but few data exist in elderly SAA pts where the best treatment approach is still debated, particularly regarding the use of immunosuppressive therapy with ATG, its dosage and toxicity. Reported response rates, often in small series, range from 7 to 62% (Kao SY, 2008 RR 42%; Tichelli A 1999 RR 62%, Killick S RR 7%). Aim of study is to

contribute our experience on 36 patients aged ≥ 60 , consecutively diagnosed with SAA at a single center and to evaluate the outcome of immunosuppressive vs low intensity/supportive therapy and to provide a benchmark given the recent addition of eltrombopag to the therapeutic armamentarium of as a treatment option for AA. The diagnosis of AA was made according to standard criteria (International Agranulocytosis and Aplastic Anemia Study Group, 1987) and the severity of disease was defined by Cammitta criteria (1986). All consecutive pts diagnosed with SAA at our Center between Januar 1987 and Januar 2019 were analyzed.

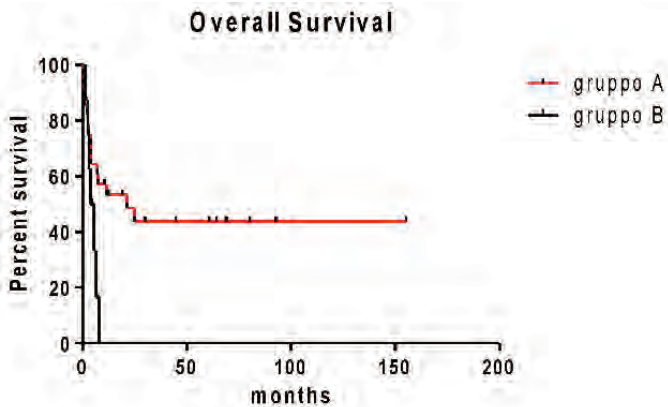


Figure 1.

Treatment was immunosuppressive therapy (horse or rabbit ATG with or without CSA) or treatments without ATG including supportive therapy, GCSF, CSA, androgens or steroids. The choice of treatment was based on clinical evaluation of fitness and comorbidities. Of 36 pts retrieved, 25 were SAA, 11 very SAA. Median marrow cellularity was 5%. ATG + CSPA (group A) was given to 28 (78%) while 8 pts received supportive therapy, G CSF, CSA and PDN only (groupB). In group A median age was 71, M/F 10/18. Ten of these pts received a reduced dose of ATG (35%). ORR was 50% (14pts). Ten pts died within three months of infection (7), bleeding (2) and acute renal failure(1), 5 did not respond and died of infection after 4-8 months. Of the remaining 13 pts, 7 are in complete remission, 5 in partial response and 1 is alive with disease. Survival at 4-years is 44%, with no significant differences according to ATG dosage. In group B median age was 79, M/F was 2/6. 7 pts died: infection (4), cancer (1), heart failure(2). The overall survival of the two groups differed significantly (P 0.03) (Figure 1). This study confirms that SAA in elderly pts has a fatal prognosis which can be reversed by immunosuppressive therapy. ATG+CSA is feasible and can cure >40%, even at reduced doses. These results represent a benchmark for evaluating the potential role of eltrombopag in elderly SAA.

Non Hodgkin Lymphoma 1

P012

PET/CT-DRIVEN BIOPSY FOR THE DIAGNOSIS OF LYMPHOMA

A. Broccoli¹, C. Nanni², A. Cappelli³, F. Bacci⁴, A. Gasbarrini⁵, E. Tabacchi², C. Piovani⁵, L. Argani¹, E. Sabbatini⁴, R. Golfieri³, S. Fanti², P.L. Zinzani¹

¹Institute of Hematology "Lorenzo e Ariosto Seràgnoli" University of Bologna; ²Nuclear Medicine Sant'Orsola-Malpighi Hospital; ³Radiology Unit Sant'Orsola-Malpighi Hospital; ⁴Hemopathology Unit Sant'Orsola-Malpighi Hospital; ⁵Oncological and Degenerative Spine Surgery Institute of Orthopedics "Rizzoli", Italy

Biopsy of affected tissue is required for lymphoma diagnosis and to plan treatment. Open incisional biopsy is traditionally the method of choice, with an accuracy of approximately 100%. Nevertheless, it requires hospitalization, availability of an operating room, sometimes general anesthesia and is associated with several drawbacks (morbidity, surgical complications, tumor contamination of surrounding tissues). The development of ultrasound and computed tomography (CT)-guided biopsies has almost overcome these disadvantages. However, a variable proportion of non-diagnostic procedures is reported, leading to an accuracy of 50-80%. Fluorodeoxyglucose (FDG) positron emission tomography (PET)/CT can be potentially used to drive biopsy to the most metabolically active area within a lymph node or extranodal masses which sometimes show no morphologically detectable changes on CT scan. The aim of the trial was to assess the diagnostic accuracy of a PET-driven needle biopsy in patients with suspect active lymphoma. One hundred patients with suspect lymphoma at onset or relapse have been enrolled between 2016 and 2018, provided they show FDG-avid findings. Patients were excluded if pregnant, breastfeeding or in case fine-needle PET/CT-guided biopsy was contraindicated. The trial was supported by the Italian Association for Cancer Research (Progetto AIRC IG 2015 Id 17781). Ninety-seven patients were eligible for biopsy, and overall 99 procedures have been performed: 3 (3.0%) were interrupted because of pain, but were successfully repeated in 2 cases with the same method. Median SUVmax of target lesions was 10.7 (1.6-67.9). Insufficient samples were obtained in 8.3% of cases (8/96 successful procedures), whereas in all other instances the tissue was considered adequate to formulate a diagnosis (diagnostic accuracy of 91.7%) and to guide the following clinical decision (Table 1). Among diagnostic specimens, the target was a lymph node in 60 cases and an extranodal site in 36 (bone in 23 cases, soft tissue in 7, liver in 4, kidney and adrenals in 1). Mean sample length was 10 mm (standard deviation ± 6 mm). The mean amount of pathologic infiltrate in collected samples was 70% ($\pm 34\%$) and the mean proportion of fibrosis/bone was 30% ($\pm 30\%$). No severe adverse events were reported during or after each procedure. In conclusion, patients can benefit from a minimally invasive procedure which allows a timely and accurate diagnosis of lymphoma at onset or relapse.

Table 1.

Failed procedures (interruption of the procedure)	3/99 (0.3%)
Non-diagnostic specimens (inadequate sample)	8/96 (8.3%)
Diagnostic specimens	88/96 (91.7%)
- Lymphoma	63
- Chronic lymphocytic leukemia	1
- Acute lymphoblastic leukemia	1
- Other (non-hematological/non-oncological)	15
- Solid tumor or metastasis	8

P013**90Y-IBRITUMOMAB TIUXETAN IN PATIENTS WITH EXTRA-NODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA) - THE ZENO STUDY**

M. Carella, B. Casadei, C. Pellegrini, V. Stefoni, G. Lolli, A. Broccoli, L. Nanni, M. Marangon, A. Morigi, N. Fabbri, G. Gabrielli, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

Currently, no standard treatments are available for extra-nodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma), which appears to be a radiosensitive tumor. 90Y-ibritumomab tiuxetan (YIT) delivers targeted radiotherapy in specific MALT localizations, avoiding the exposure of other tissues and consequent organ toxicities. The aim of this phase II trial was to evaluate the efficacy of a single course of YIT in generating clinical responses in patients with MALT lymphoma in first line or with relapsed/refractory disease. The radioimmunotherapy treatment plan consisted of an initial infusion of rituximab on day 1, then repeated on day 8, immediately followed by a weight-based dose of YIT. Seventeen patients were enrolled and 16 were evaluable for efficacy analysis as 1 patient refused to undergo YIT due to a drug infusion reaction after the first administration of rituximab. Four out of 16 patients were previously untreated. For the other 12 patients, the median duration of the last response was 13 months. All but 2 (IE, intraorbital) presented with a stage IV disease. At 3 months after YIT 10 complete responses (CR, 62.5%), 5 partial responses (PR) and 1 stable disease were observed, leading to an overall response rate of 94%. With a median follow up of 1 year, only 4 patients relapsed (3 from CR and 1 PR). Seven patients are in continuous CR with a median duration of response of 18 months. Adverse events after YIT were only hematologic and transient. The severity of hematologic toxicities (expressed as the lowest, i.e. nadir, concentration of granulocytes, platelets, and hemoglobin reached after radioimmunotherapy) are reported in the table. Grade 3 to 4 thrombocytopenia and neutropenia occurred in 7 patients (43.8%). Four patients (25.0%) received G-CSF; no patients received transfusions. In conclusions, YIT represents a highly effective and tolerable treatment option for MALT lymphoma, both in first-line and in relapsed/refractory disease setting.

Table 1.

	Baseline	Nadir (range)	Days from Baseline to nadir
Absolute neutrophil count, cells/mm ³	3400 (1500-6000)	700 (210-2950)	36 (19-52)
Platelets, cells x 10 ³ /mm ³	216 (118-338)	169 (89-121)	33 (19-38)
Hemoglobin, g/dL	13.6 (9.9-16.2)	11.9 (8.8-13.0)	39 (19-56)

P014**BENDAMUSTINE AND RITUXIMAB FOR PATIENTS WITH FOLLICULAR LYMPHOMA: A REAL LIFE EXPERIENCE**

A. Morigi, B. Casadei, C. Pellegrini, V. Stefoni, G. Lolli, M. Carella, A. Broccoli, L. Nanni, M. Marangon, N. Fabbri, G. Gabrielli, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

Bendamustine plus rituximab (BR) is an effective and manageable treatment option for lymphoma patients. Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma in the Western

World. The natural history of FL which is characterized by continuous relapses, appears to have been favorably impacted by the introduction several years ago of rituximab. Bendamustine is an alkylating agent with multiple unique mechanisms of action and is shown to be active in FL. The aim of this retrospective and monocentric study is to analyze the effectiveness and safety of the use of BR regimen in FL patients in first line or with relapsed/refractory disease in daily clinical practice. The treatment schedule was rituximab at the dose of 375 mg/m² on day 1 of the cycle and bendamustine at the dose of 90 mg/m² on day 2 and 3, every 28 days for a maximum of 6 cycles. We analyzed 86 patients: 56 had BR as first line approach, whereas 30 had BR as subsequent line of therapy. Sixty-six patients underwent 6 cycles of BR regimen, 12 had early discontinuation due to toxicity, 5 due to progression disease (PD) and the others attempted for stem cell transplant after achieving a complete response (CR) at interim restaging. Final responses were: 71 CR (82.6%), 9 partial response (PR), 1 stable disease and 5 PD, leading to an overall response rate of 93%. Interestingly, 9 patients converted from PR to CR after the first 3 cycles. Toxicities, both hematological and extra-hematological, were rare and almost mild. Only 12 patients had hematologic side effects, drug related, of which 7 had grade ≥3 (5 neutropenia and 2 thrombocytopenia). Median progression free survival was reached at 51.7 months and median disease free survival at 45.3 months, respectively. Fifty-eight patients are in continuous CR after a median follow up of 19 months. At the latest available follow up, only 8 patients were deceased due to PD. No differences in response rate or in survivals were observed between patients who did or did not undergo BR in first line. In conclusion, the BR regimen is safe and effective in FL patients inducing prolonged disease control with minimal side effects at any point in the therapeutic algorithm.

P015**STUDY OF GENE POLYMORPHISMS AS PREDICTORS OF TREATMENT EFFICACY AND TOXICITY IN PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMAS RECEIVING BENDAMUSTINE AND RITUXIMAB: RESULTS OF THE 3-YEAR FOLLOW-UP STUDY**

E. Cencini¹, A. Sicuranza¹, A. Fabbri¹, I. Ferrigno¹, A. Santoni¹, L. Valotto¹, D. Raspadori¹, M. Bocchia¹

Unit of Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena, Italy

Introduction: Indolent non-Hodgkin lymphomas (iNHL) and mantle-cell lymphoma (MCL) have a heterogeneous behavior, impacted by biological and clinical parameters. Bendamustine is widely used in association with rituximab to treat iNHL and MCL. The variability in treatment efficacy and toxicity could be related to genetic factors of the host, such as germline single nucleotide polymorphisms (SNPs) in genes that affect pharmacodynamics and the components of microenvironment. Genetic variants in immune and inflammatory response genes (such as the ones coding for IL-10, IL-2 and IL-6) and in angiogenic factors (such as VEGF) could affect clinical outcome and side effects.

Methods: We would like to demonstrate a correlation between SNPs and long-term treatment outcome in iNHL and MCL patients receiving bendamustine and rituximab. All samples were genotyped for the IL-2 (rs2069762), IL-10 (rs1800890, rs10494879), VEGFA (rs3025039), IL-8 (rs4073), CFH (rs1065489) and MTHFR (rs1801131) SNPs by allelic discrimination assays using TaqMan SNP Genotyping Assays (Applied Biosystem) containing primers forward and reverse and allele specific MGB (Minor Groove Binder) probes. SNPs assays were executed on a Rotor Gene 3000 platform system (Corbette, Explera) and the analysis of genotyping were performed using the Rotor Gene Software.

Results: We have enrolled 100 patients and we report an interim of 79 iNHL and MCL patients with a follow-up of at least 6 months that received rituximab 375 mg/m² and bendamustine 90 mg/m² every 28 days both as first-line treatment (62/79) and as ≥ 2nd line regimen (17/79). Overall response rate was 97.4% (CR rate 70.8%). We did not report any correlation between SNPs, CR rate and PFS. However, we confirm an association between SNP in IL-2 (rs2069762) and skin rash (p=0.0001). After a median follow-up of 3 years, median PFS and OS were not reached. Fifteen patients experienced grade 1-2 late infections (7 respiratory tract, 1 urinary tract and 1 fungal infection; moreover, 6 VZV reactivation occurred) during the first or second year after treatment; 5 secondary malignancies were reported (3/5 were relapses of solid neoplasms treated before NHL). Both late infections and second-

ary malignancies were not related to investigated SNPs.

Conclusions: We confirm our previous results suggesting a role for cytokine SNPs in bendamustine-related short-term toxicity; while SNPs seem not related to long-term toxicity or secondary malignancies.

P016

THE TREATMENT OF PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A TWO DECADES MONOCENTRIC EXPERIENCE WITH 151 PATIENTS

L. Nanni, B. Casadei, C. Pellegrini, V. Stefoni, G. Lolli, M. Carella, A. Broccoli, M. Marangon, A. Morigi, N. Fabbri, G. Gabrielli, L. Argani, M. Cavo, P.L. Zinzani

Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

The prognosis of patients with primary mediastinal B-cell lymphoma (PMBCL) refractory to first-line or second-line therapy is poor. This lymphoma, in fact, is characterised by high rates of curability with standard anthracycline-containing chemoimmunotherapy regimens, but still displays a severe prognosis if adequate responses are not rapidly achieved or if the disease recurs. Radiotherapy has proved to be effective to consolidate responses after induction, but it may be safely avoided in certain cases, especially when a metabolic complete response is obtained, as to reduce the incidence of radiation-induced long-term sequelae. The unique biological features of PMBCL are offering suggestions for the development and application of innovative drugs in patients who do not respond to first-line regimens or relapse. The aim of this retrospective and monocentric study is to analyze our large historical series of PMBCL to investigate the effectiveness and safety of standard therapeutic approaches in both first and subsequent lines in daily clinical practice over the last 20 years. One hundred and fifty-one patients were diagnosed and treated at our Institute through the last twenty years. Median age at diagnosis was 34.4 years and 91 (60%) were females. First line for all patients consisted of a polychemotherapy regimen ± rituximab and/or radiotherapy. Final responses were 120 complete response (CR, 79.5%), 12 partial responses, 2 stable disease and 17 progression of disease. One hundred and four (86.7%) patients are in continuous CR after first-line approach with a median time of response of 5.5 years leading to a disease free survival of 82.6% at 20 years. Fifty-three patients proceeded with further line(s) of treatment regardless of whether they were in response or were refractory to the first line. Refractory patients showed low response rates and short disease control with classic salvage therapies. In conclusion, with this large historical series we confirmed that while most patients with PMBCL are cured with standard frontline therapy, about the 20% are diagnosed with relapsed/refractory disease, which has a poor prognosis with limited treatment options. New agents with mechanisms of action based on peculiar biologic features of the tumor (as anti-PD-1 monoclonal antibody and chimeric antigen receptor T cell therapy) are showing promising results in patients with recurrent disease.

P017

CYTOFLUORIMETRIC AND IMMUNOHISTOCHEMICAL COMPARISON FOR DETECTING BONE MARROW INFILTRATION IN NON-HODGKIN LYMPHOMAS: A STUDY OF 345 PATIENTS

T. Statuto, F. D'Auria, L. Valvano, O. Villani, G. Pietrantonio, G. Mansueti, G. D'Arena, G. Vita, V. Lalinga, L. Del Vecchio, P. Musto

Laboratory of Clinical Research and Advanced Diagnostics, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy; Hematology and Stem Cell Transplantation Unit, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy; Pathology Unit, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy; CEINGE Biotechnology Avanzate s.c.a.r.l., Federico II University, Naples, Italy; Department of Molecular Medicine and Medical Biotechnology (DMMBM), Federico II University, Naples, Italy; Hematology Department of Basilicata, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy

Introduction: Morphological and immuno-histochemical (IHC) analysis of trephine bone marrow biopsies (BMB) is routinely performed during staging of patients with non-Hodgkin Lymphoma (NHL). In this setting, it is not clear whether flow cytometry (FC) study of bone marrow aspirates (BMA) may increase sensitivity and specificity in detecting

neoplastic infiltration.

Methods: Aiming to evaluate the possible diagnostic value of FC on BMA, as compared with BMB, we retrospectively reviewed 345 bone marrow specimens of patients affected by B/T NHL in whom these investigations were carried out simultaneously. FC B-cell clonality was assessed by the standard kappa/lambda/CD19 test and FC T-cell clonality by TCR v Repertoire analysis. Aberrant immune-phenotypes of neoplastic subpopulations were also investigated. A selected panel of monoclonal antibodies was used both for FC and IHC. Concordance was defined as the presence of a positive (in terms of disease detection) or negative result with BMA/FC and BMB/IHC.

Results: In 295 of 345 specimens (85.5%), there was a concordance among the two investigations. A discordance was detected in 50 cases (14.5%): 34 of these patients (9.9 % of total population) were BMA/FC positive, whereas BMB was negative. In 16 of these 50 specimens (4.6% of total population), FC did not detect lymphoid infiltration on BMA although the BMB was considered positive. Among discordant samples with BMA/FC+BMB/IHC-, 12 corresponded to DLBCL (35.3%), 11 to MZL (32.4%), 4 to FL (11.8%), 3 to MCL (8.8%), 1 to AITL (2.9%) and 3 to T-LGL (8.8%). Of 16 cases with BMA/FC-BMB/IHC+, 9 corresponded to DLBCL (56.3%), 3 to MZL (18.6%), 1 to FL (6.3%), 1 to MCL (6.3%), 2 to AITL (12.5%). The percentage of neoplastic cells detected by FC on BMA in discordant cases ranged from 0.2% to 43.2% (median 1%). The mean value was 6.5% (SD ± 11).

Conclusion: In this large retrospective study, most samples showed concordance between morphological and phenotypic studies in BMA and BMB. However, our results also indicated that FC analysis of BMA was able to detect limited bone marrow infiltrations in about 9.9% of NHL, while in 4.6% of patients diagnosed as having bone marrow infiltration by BMB, such a localization was not confirmed by FC. The clinical outcome of these cases will be further investigated.

P018

RISK ASSESSMENT AND MANAGEMENT OF CNS INVOLVEMENT IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ITALIAN CENTERS: A NATIONWIDE SURVEY BY THE FONDAZIONE ITALIANA LINFOMI (FIL)

G. Gini, A. Di Rocco, M. Sampaolo, L. Nassi, A. Arcari, M.C. Tisi, G. Loseto, M. Gentile, O. Annibaldi, A.J.M. Ferreri, M. Balzarotti

Clinic of Hematology - Ospedali Riuniti, Department of Translational and Precision Medicine - Sapienza University, Clinic of Hematology - Ospedali Riuniti, Hematology - Department of Translational Medicine - University of Eastern Piedmont and AOU; Maggiore della Carità, Division of Hematology - Guglielmo da Saliceto Hospital, Department of Hematology and Cell Therapy - San Bortolo Hospital, Division of Hematology - IRCCS Ospedale Oncologico, Department of Onco-Hematology - Hematology Unit - A.O. of Cosenza, Unit of Hematology and Stem Cell Transplantation - University "Campus Bio-Medico" - IRCCS San Raffaele Scientific Institute, Hematology Unit - Humanitas Cancer Center

Background: Central nervous system (CNS) involvement is a rare but almost fatal complication of diffuse large B-cell lymphoma (DLBCL). This event is preventable with adequate prophylaxis. There is no consensus on the optimal CNS risk model and prophylaxis treatment.

Patients and Methods: A web-based survey, focused on algorithms used in routine practice to identify DLBCL patients at high CNS risk and to prevent CNS dissemination, was developed and conducted in 2018 after consultation with specialist clinicians and a review of published literature. We sent the questionnaire to all FIL centers. The questionnaire was divided in three parts: the first evaluated the centers' characteristics and the disease incidence; the second investigated the CNS risk assessment and the diagnostic method; the third the type of prophylaxis used in high-risk patients.

Results: Sixty-three out of 110 fulfilled the survey. 46% of centers treat >30 DLBCL/years (yrs) and 40% 11-30 cases/yrs. In the last 5 yrs, 6% of centers reported >10 DLBCL with CNS and systemic localization at the diagnosis, 30% reported 4-10 cases and 48% reported <4 cases. Regarding relapses, 5% of centers declared >10 CNS recurrences, 32% reported 4-10 relapses and 35% reported <4 relapse. Concerning the identification of meningeal/CNS disease, cerebrospinal fluid (CSF) collection through lumbar puncture was performed only in patients with CNS-IPi >4 in 21% of centers, whereas in 56% of centers this procedure was performed in patients with either CNS-IPi >4 or with high-risk ex-

tranodal site. Patients with double/triple hit lymphoma, with double/triple + ABC and double expresser lymphomas were considered at high CNS risk in 44%, 49% and 49% of centers, respectively. CNS disease status was assessed by CSF evaluation (physico-chemical, cytological exam and flow cytometry) and imaging (CT or MRI) in 79% and 73% of centers, respectively. CSF flow cytometry was not routinely performed in 16% of centers; 67% of participants consider CNS positivity also when detected exclusively by flow cytometry. CNS prophylaxis consisted of intrathecal Methotrexate (MTX) in 58% of centers and in HD-MTX (1.5 to 3 gr/sqm), with or without intrathecal prophylaxis, in 40% of centers.

Conclusions: This survey shows real-life practice in Italian hematological centers and highlights the substantial inter-center differences in the use of methods of diagnosis and prophylaxis of CNS involvement in DLBCL patients.

P019

PHASE II STUDY WITH CRIZOTINIB IN PATIENTS WITH ANAPLASTIC LYMPHOMA KINASE (ALK)-POSITIVE LYMPHOMA RELAPSED/REFRACTORY TO CHEMOTHERAPY

E. Bossi, F.A. Brioschi, C. Steidl, S. Baretta, S. Cantù, L. Verga, D. Fontana, G.G. Sharma, L. Mologni, L. Mussolin, R.G. Piazza, C. Gambacorti-Passerini

Facoltà di Medicina e Chirurgia Università degli Studi di Milano Bicocca, Clinica Ematologica Ospedale San Gerardo Monza, Dipartimento di Salute della Donna e del Bambino Università degli Studi di Padova, Istituto di Ricerca Pediatrica Città della Speranza Università degli Studi di Padova, Clinica di Oncoematologia Pediatrica Università degli Studi di Padova, Italy

Crizotinib, an inhibitor of anaplastic lymphoma kinase (ALK) and cros oncogene 1 (ROS1), is approved for treatment of patients with ALK-positive or ROS1-positive advanced non-small-cell lung cancer. However, ALK rearrangements, are also implicated in anaplastic large-cell lymphoma, mainly the fusion-protein NPM-ALK. ALK-positive lymphomas respond to chemotherapy, but relapses, which bear a poor prognosis, develop in more than 50% of patients. In this ongoing, single-center, single-arm, phase II study, Crizotinib was administered as monotherapy with a starting dose of 250 mg twice daily to 11 ALK+ lymphoma patients who had progression of disease after at least one line of cytotoxic therapy. Patients were enrolled at the Department of Haematology, San Gerardo Hospital, Monza (Italy) from April 2015 to October 2018. Median number of previous cytotoxic treatments was 3 (range 2-6). Median age at diagnosis was 32 years (range 18-58 years) and 5/11 patients were male. The overall response rate (ORR) was 7 of 11 (63.6%; 95% CI = 35% to 85%). All responding patients achieved complete remission and negativity for the NPM-ALK transcript by Real Time Polymerase Chain Reaction (RT-PCR). Median follow up is 10 months (range 2-48). Disease status at the latest follow up is as follows: 6 patients are in complete response under continuous crizotinib administration, 4 patients had progression of disease and died, 1 patient stopped the treatment and was in complete response after allogeneic bone marrow transplantation. All relapses developed within the first 3 months of therapy.

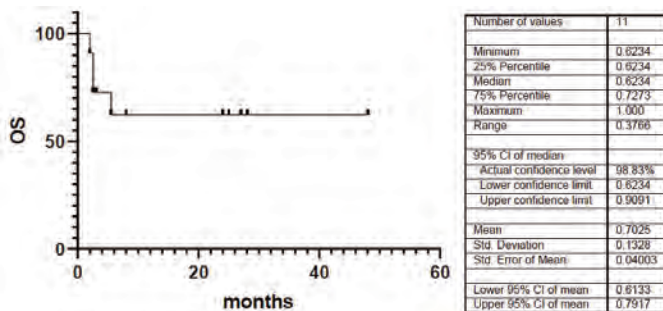


Figure 1.

The two-years Progression Free Survival (PFS) and Overall Survival (OS, see attached Figure 1) are 65% (95% CI = 53% to 77%) and 70% (CI = 61% to 79%), respectively. The most common treatment related

adverse events were diarrhea (90.9%), oedema lower legs (100%), vomiting (72.7%) and visual disorders (36.4%). All adverse events were Grade 1 or 2 and most of them were transient in nature. Crizotinib shows a good safety profile and carries out a potent antitumor activity with durable responses in advanced pretreated ALK+ lymphoma patients. Crizotinib should be made available for patients with relapsed/refractory ALK+ lymphoma.

P020

FAVOURABLE OUTCOME OF PRIMARY MEDIASTINAL B-CELL LYMPHOMA (PMBCL) PATIENTS TREATED WITH R-DAEPOCH IN COMPARISON WITH AN HISTORICAL COHORT

A. Guidetti, L. Devizzi, V. Marasco, L. Farina, G. Perrone, A. Doderò, P. Corradini

Divisione di Ematologia Fondazione IRCCS Istituto Nazionale dei Tumori, Dipartimento di Oncologia ed Emato-Oncologia Università degli Studi di Milano, Italy

Background: In PMBCL patients (pts) R-DAEPOCH conferred long lasting remissions without radiotherapy (RT) on mediastinum. In mostly young pts affected by PMBCL sparing the acute and late effects of RT is an important endpoint. We report clinical results of a consecutive cohort of PMBCL pts treated with R-DAEPOCH and a comparison with an historical PMBCL group.

Methods: Between 2010 and 2018, 41 PMBCL pts have been consecutively treated with 6 cycles of R-DAEPOCH at our Institution. Pts performed a baseline and end of treatment (EOT) CT and PET scan. R-DAEPOCH cohort has been compared with 32 pts treated before 2010 with Rituximab and anthracycline-based CT followed by RT (100%).

Results: Median age of 41 pts was 33 years (range, 18-63), 54% have high LDH levels and 71% bulky disease. Only one patient interrupted treatment for progressive disease, 7 pts (17%) only received RT at EOT. With the exception of an higher proportion of stage III/IV patients in the historical group (42% vs 17%), all other clinical characteristics were similar between the two cohorts. With R-DAEPOCH febrile neutropenia was observed in 12% of pts, G3/G4 mucositis in 17% and acral neuropathy in 24%, no late cardiac toxicity was observed. Three pts of the historical cohort developed a second neoplasm outside the RT field (after 1 month, 4 and 12 years respectively). Median follow-up was 36 (range, 5-112) and 116 months (range, 7 - 188) for R-DAEPOCH and the historical cohort, respectively. Among R-DAEPOCH pts 3-years PFS and OS were 84% and 91%. EOT PET was negative [Deauville score (DS)1-3] in 31 pts (76%) and positive (DS4) in 10 pts (24%). Pts with DS 4 at EOT PET had a poorer 3-years PFS, 44% vs 96% (p<0.0001) and OS, 63% vs 100% (p=0.0009) when compared to DS1-3 pts. Among DS4 pts, 4 received RT, 5 a salvage therapy, one no therapy after a second PET assessment revealing a decreased SUV value and 3 pts died for PD. In comparison to the historical cohort, R-DAEPOCH pts had a better 3-years PFS (84% vs 57%, p=0.02) whereas 3-years OS was not significantly different (91% vs 77%).

Conclusions: PMBCL pts treated with R-DAEPOCH have an excellent prognosis without RT when achieve PET negativity at EOT, whereas pts with DS4 seems to be at high risk of progression and should be strictly monitored and considered for RT or salvage therapy. The PFS advantage observed in R-DAEPOCH could be affected by differences in disease stage between groups and should be confirmed in a larger cohort of pts.

Myeloma and Monoclonal Gammopathies 1

P021

TARGETING TLR4 OVERCOMES BORTEZOMIB RESISTANCE IN MYELOMA CELLS

C. Giallongo, D. Tibullo, G. Camiolo, A. Barbato, F. Puglisi, D. Cambria, N. Parrinello, A. Romano, C. Conticello, G. Li Volti, G.A. Palumbo, F. Di Raimondo

Divisione di Ematologia, AOU Policlinico Vittorio Emanuele, Biometec, Dipartimento di Scienze Biomediche e Biotecnologiche, Università degli studi di Catania, Dipartimento di Chirurgia generale e specialità medico-chirurgiche-Hematology section, Dipartimento di Scienze Mediche, Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia", Università degli studi di Catania, Italy

Background: Multiple myeloma (MM) is a B-cell malignancy critically dependent for survival and proliferation on signals coming from its inflammatory microenvironment and toll-like receptors (TLR) may greatly contribute to inflammation. TLR are expressed on human MM cells and in particular TLR4 ligand promotes their proliferation and immune escape mechanisms. Since it has been demonstrated that the acquisition of proteasome inhibitors resistance involved increased mitochondrial respiration, we investigated the possible implication of TLR4 signaling with mitochondrial fitness as potential mechanism of drug resistance.

Results: The activation of TLR4 by LPS induced mitochondrial biogenesis by increasing PGC1, PRC and TFAM expression ($p < 0.01$) and mitochondrial mass in MM cell lines (U266, MM1S, OPM2, H929). TLR4 expression was increased after Bortezomib (BTZ) treatment ($p < 0.001$) and its signaling was functional as suggested by MyD88 up-regulation and MAPK activation. TLR4 protein was over-expressed in BTZ-resistant U266 (U266-R) cells compared to U266-S. Proteasome activity assay revealed that BTZ was still able to inhibit proteasome in U266-R in the same way as U266-S. Since U266-R showed higher mitochondrial mass and up-regulated TFAM, we investigated whether TLR4 was involved in resistance to BTZ going through mitochondrial fitness. U266-R cells were treated with either 15nM BTZ, 20 M TAK-242 or their combination. Combinatorial treatment overcame resistance inducing 55% of apoptosis. Compared to BTZ alone, TAK-242/BTZ treated cells showed higher and extended ROS levels, less mitochondrial mass and more depolarized mitochondria ($p < 0.001$). Moreover, BTZ alone induced over-expression of NDUFA6 and ND4 (subunits of mitochondrial respiratory complex I), OPA1 and MNF2 (important in fusion process); on the contrary their expression decreased after combination with TAK-242 ($p < 0.001$). Since increased ROS and mitochondrial depolarization activate mitophagy, we evaluated co-localization of the autophagosome marker LC3 with mitochondria (stained with mitotracker) using confocal microscopy. TAK-242/BTZ increased mitotracker/LC3 co-localization ($p < 0.01$) and down-regulated SQSTM1 sensitizing resistant cells to apoptosis through the disruption of mitochondrial aggregation and autophagic clearance of mitochondria.

Conclusions: Our data demonstrated that TLR4 inhibition in BTZ-resistant cells impaired mitochondrial fitness resulting in apoptosis and overcoming of drug resistance.

P022

BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE AND DOUBLE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: FINAL RESULTS OF THE PHASE 3 GIMEMA-MMY-3006 TRIAL

P. Tacchetti, E. Zamagni, F. Patriarca, M.T. Petrucci, L. Dozza, M. Galli, C. Crippa, F. Di Raimondo, L. Pantani, M. Offidani, S. Bringhen, F. Narni, V. Montefusco, R. Zambello, A. Spadano, C. Terragna, N. Pescosta, L. Baldini, G. Marzocchi, C. Cellini, D. Derudas, T. Caravita, M. Boccadoro, M. Cavo

Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Unità di Ematologia e Trapianto di Midollo Azienda Ospedaliero Universitaria S Maria Università di Udine, Dipartimento di Biotecnologie Cellulari e Ematologia Uni-

versità La Sapienza, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Divisione di Ematologia Ospedali Riuniti di Bergamo, Unità Operativa di Ematologia Spedali Civili di Brescia, Divisione di Ematologia AOU Policlinico Vittorio Emanuele Università di Catania, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Dipartimento di Ematologia Azienda Ospedaliero Universitaria Ospedali Riuniti di Ancona, Divisione di Ematologia Azienda Ospedaliero Universitaria Città della Salute e della Scienza Università di Torino, Dipartimento di Oncologia ed Ematologia Azienda Ospedaliero Universitaria Policlinico di Modena, Divisione di Ematologia Fondazione IRCCS Istituto Nazionale dei Tumori, Ematologia e Immunologia Clinica Azienda Ospedaliera di Padova, UO Ematologia Clinica Ospedale Civile Spirito Santo, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Divisione di Ematologia e Centro Trapianto Midollo Osseo Ospedale Regionale Generale, Fondazione IRCCS Cà Granda Unità di Ematologia Ospedale Maggiore Policlinico Università di Milano, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Unità di Ematologia Ospedale S Maria delle Croci, Struttura Complessa di Ematologia e Centro Trapianti di Cellule Staminali Emopoietiche Ospedale Oncologico A Businco, Ematologia Ospedale S Eugenio, Divisione di Ematologia Azienda Ospedaliero Universitaria Città della Salute e della Scienza Università di Torino, Istituto di Ematologia Seragnoli Azienda Ospedaliero Universitaria S Orsola Malpighi Università di Bologna, Italy

This final analysis of the phase 3 GIMEMA-MMY-3006 trial comparing bortezomib-thalidomide-dexamethasone (VTD) versus thalidomide-dexamethasone (TD) as induction therapy before, and consolidation after, double autologous stem cell transplantation for newly diagnosed multiple myeloma (MM), was performed to evaluate long-term outcomes at a median follow-up for surviving patients (pts) of 124.1 months. 474 pts were enrolled and randomly assigned to VTD (236) or TD (238) arms. 10-year progression free survival (PFS) and overall survival (OS) rates for the VTD and TD subgroups were 34% versus 17% (median, 60 versus 41 months, HR=0.62, $p < 0.001$) and 60% versus 46% (median not reached versus 110 months, HR=0.68, $p = 0.007$), respectively, representing a 38-32% reduction in the risk of progression and death with VTD. Randomization to VTD was an independent factor predicting for prolonged PFS (HR=0.60, $p < 0.001$) and OS (HR=0.66, $p = 0.010$). Among pts who received subsequent therapies, the 2nd PFS was similar following VTD or TD (median, 21 and 19 months). Conversely, PFS2 was longer for pts on VTD than on TD, with median values of 113 and 74 months (HR=0.64, $p < 0.001$), respectively. Multivariable analysis not including therapy identified high risk cytogenetic abnormalities [t(4;14) and/or del(17p)], ISS stage II/III, and failure to achieve complete response (CR), as leading factors adversely affecting survival. Low-risk (LR) (22%), intermediate-risk (IR) (39%) and high-risk (HiR) (39%) groups were identified by the presence of none, a single or at least two adverse variables, respectively. These three categories showed divergent PFS and OS curves within each treatment arm ($p < 0.001$). Based on conditional survival CS(tls) estimate for PFS, the probability of surviving 2 years further without progression improved progressively after 36 months (p value for trend=0.009). The 2-year conditional PFS became similar after 78 months for LR and IR groups (87% and 86%, respectively), but proved significantly lower in HiR (63%) ($p = 0.008$). The incidence of second primary malignancies per 100 person-years was 0.87 on VTD and 1.41 on TD. In conclusion, this final analysis of the GYMEMA MMY-3006 trial confirmed a persistent PFS benefit and revealed extended OS for pts in the VTD arm without new safety concerns. Cytogenetics, ISS stage and achievement of CR provided a prognostic model for survival. A PFS time of 78 months predicted for long-term survival outcomes in LR and IR groups.

P023

DARATUMUMAB IN HEAVILY PRETREATED, TRANSPLANT INELIGIBLE, AL AMYLOIDOSIS PATIENTS: A SINGLE CENTRE EXPERIENCE

M. Riva, G. Scapinello, L. Ammirati, F. Lessi, A. Branca, R. Zambello, F. Piazza, G. Semenzato, T. Berno

Department of Medicine, University of Padua, Hematology and Clinical Immunology Unit, Italy

Introduction: Immunoglobulin light chain (AL) amyloidosis is a heterogeneous disease, with extremely variable clinical picture and out-

come, especially in advanced organ involvement. Patients with relapsed/refractory disease after first line therapy represent an unmet clinical need. The high efficacy of immunotherapy could be a valuable rescue option.

Methods: We retrospectively analysed 10 AL amyloidosis patients, transplant ineligible, with cardiac, renal and multiorgan involvement, treated with Daratumumab in our Unit. Hematological and organ responses were assessed according to Amyloidosis Consensus Criteria. Patients characteristics are shown in Table 1. Six patients (60%) had cardiac and renal involvement. Among patients with cardiac involvement, patients with stage III and IV were 3 (30%) and 2 (20%); renal stage I and II were 6 (60%) and 4 (40%). 9 patients (90%) had lambda and 1 (10%) kappa light chain.

Table 1. Patients characteristics.

N=10	Median (range)	Frequency (%)
Age	63.2 (53-77)	
Male		6 (60%)
Female		4 (40%)
ECOG at start of therapy		
0-1		2 (20%)
≥2		8 (80%)
NYHA at start of therapy		
I-II		5 (50%)
III-IV		5 (50%)
Involved light chain		
- Kappa		1 (10%)
- Lambda		9 (90%)
Organ Involvement		
Heart		2 (20%)
Renal		1 (10%)
heart & Renal		6 (60%)
Soft tissue		1 (10%)
Karyotype		
- Normal		6 (60%)
- Complex		1 (10%)
- Missing		3 (30%)
FISH - Cytogenetics		
t(11;14)		1 (10%)
gain1q21		3 (30%)
Missing		6 (60%)
Duric Salmon Staging in pts with Multiple Myeloma and Amyloidosis		
1A		8 (80%)
3A		2 (20%)
Immunoparesis at diagnosis, n. (%)		10 (100%)
Prior IMiD and PI		10 (100%)
Best prior hematologic response		
CR		1 (10%)
PR		6 (60%)
No response		3 (30%)
Refractory to prior line of therapy		10 (100%)
dFLC at start of therapy with Dara, mg/dL	464 (28-2353)	
NTproBNP before therapy with Dara, ng/L	4375 (100-11845)	
Cardiac Troponin before therapy with Dara, ng/L	171.9 (5.5-517.5)	
Proteinuria before therapy with Dara	5.13 (0.13-16.07)	
Estimated GFR < 50		4 (40%)
Haematological response post Dara		
CR		4 (40%)
VGPR		1 (10%)
PR		5 (50%)
Organ Response post Dara		
No response		4 (40%)
Response		6 (60%)

The FISH analysis demonstrated t(11;14) and gain1q21 in 1 (10%) and 3 (30%) patients, respectively; cytogenetic was normal in 6 (60%) patients. Immunoparesis was observed at diagnosis in all of them. All patients had multiple myeloma and amyloidosis: 8 (80%) patients Duric Salmon stage 1A, 2 (20%) DS3A. Median plasma cell bone marrow infiltration was 35% at diagnosis. AL amyloidosis was proved by immunoelectron microscopy in 10 patients (8 abdominal fat pad biopsy, 1 endomyocardial biopsy and 1 skin biopsy). After bortezomib-based regimen and immunomodulatory therapy in all of them, we started Daratumumab in this setting. Best hematologic response was ≤VGPR to any prior therapy (1 VGPR, 5 PR and 4 no response by day 30; 1VGPR, 6 PR and 3 no response at the end of therapy). Daratumumab induced depth and rapid responses, resulted in 4 CR, 1 VGPR, 5 PR and organ response in 6 (60%) of them, with good tolerance, without rele-

vant adverse event (only one urosepsis). We observed only one death due to sudden cardiac arrest, in a patient who was in PR after Daratumumab.

Conclusions: In our experience, immunotherapy is safe and highly effective, also in patients with a more aggressive disease at diagnosis (advanced cardiac and renal involvement, and immunoparesis). These data could suggest that this group of patients may probably require the use of immunotherapy early in the disease course to prevent an irreversible organ involvement due to the direct proteotoxicity of amyloidogenic light chains.

P024

IMMUNE PROFILING OF PLASMA CELL DYSCRASIAS REVEALS AN EXTREME-T-CELL MODULATION IN TREATED MULTIPLE MYELOMA PATIENTS

G. Barilà, L. Pavan, S. Vedovato, T. Berno, A. Branca, A. Teramo, G. Calabretto, S. Manni, V. Trimarco, S. Carraro, M. Facco, F. Piazza, G. Semenzato, R. Zambello

Department of Medicine (DIMED), Hematology and Clinical Immunology section, Padua University School of Medicine, Padua, Italy

Multiple Myeloma (MM) is a hematological malignancy always preceded by a not malignant precursor state defined monoclonal gammopathy of undetermined significance (MGUS) and by a asymptomatic MM (smouldering MM, sMM). Immune dysfunction plays a key role in the pathogenesis of the disease, as demonstrated by the prognostic role of immunoparesis in the progression of MGUS and sMM into active MM (aMM). The aim of this study is to analyze the immune subsets distribution into plasma cells dyscrasias. A total amount of 895 bone marrow samples (170 MGUS, 188 sMM, 586 aMM) of 714 patients affected by plasma cells dyscrasias were studied at the different time point by flow cytometry for CD3, CD4, CD8, CD16, CD19, CD56, CD57, HLA-DR and GD antigens. By flow, CD3+ T cells, CD19+ B cells and CD3-/CD16+/CD56+ NK cells were initially studied in MGUS (n=170), sMM (n=188) and active MM (250 newly diagnosed and 286 treated) patients. In aMM cases, no significant differences were found in total T and NK cells percentages towards sMM (73.2 ± 12.5 vs 72.4 ± 10.3 , $p=0.6717$ and 14.8 ± 8.9 vs 14.1 ± 7.9 , $p=0.5676$) and MGUS cases (73.2 ± 12.5 vs 71.9 ± 9.0 , $p=0.3336$ and 14.8 ± 8.9 vs 13.3 ± 7.3 , $p=0.1205$) while B cells were lower, although significantly only towards MGUS (9.8 ± 9.1 vs 10.7 ± 5.9 , $p=0.2955$ and 9.8 ± 9.1 vs 12.3 ± 7.1 , $p=0.001$). A more detailed analysis of T cell subsets evidenced that aMM patients were characterized by lower percentages of CD4+ T cells (32.9 ± 12.9 vs 39.9 ± 9.5 , $p=0.0001$ and 32.91 ± 12.9 vs 38.4 ± 8.6 , $p=0.0001$) and higher percentages of CD8+ lymphocytes (45.1 ± 14.1 vs 38.8 ± 10.0 , $p=0.0001$ and 45.1 ± 14.1 vs 38.7 ± 9.9 , $p=0.0001$), CD8+/DR+ lymphocytes (9.7 ± 13.1 vs 4.1 ± 4.7 , $p=0.0001$ and 9.7 ± 13.1 vs 3.8 ± 4.5 , $p=0.0001$) and CD3+/CD57+ lymphocytes (18.9 ± 12.7 vs 14.5 ± 9.2 , $p=0.0001$ and 18.9 ± 12.7 vs 13.6 ± 8.8 , $p=0.0001$) while no significant differences were found in CD3+/GD+ cells (3.6 ± 4.0 vs 4.2 ± 3.6 , $p=0.2872$ and 3.6 ± 4.0 vs 4.2 ± 3.2 , $p=0.2638$). Interestingly, all these differences were mostly attributed to treated patients towards newly diagnosed MM (CD4+ cells = 27.0 ± 12.1 vs 39.5 ± 10.3 , $p<0.0001$, CD8+ cells = 50.5 ± 14.6 vs 39.0 ± 10.5 , $p<0.0001$, CD8+/DR+ lymphocytes = 14.1 ± 15.8 vs 4.5 ± 4.7 , $p<0.0001$ and CD3+/CD57+ lymphocytes = 21.7 ± 13.5 vs 15.8 ± 10.9 , $p<0.0001$). Our results demonstrated a profound T cell immune-modulation in active MM patients towards MGUS and sMM precursor states. Moreover, most of these changes are therapy-related, indicating that Myeloma treatment can shape the T cell compartment.

P025

SAFETY OF RAPID DARATUMUMAB INFUSION IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

E. Attardi, E. Antonioli, M. Staderini, M. Messeri, A. Buzzichelli, V. Nencini, A. Fani, A. Bosi

Haematology Unit, AOU Careggi, Florence, Italy

Daratumumab is a human CD38 monoclonal antibody approved for the treatment of patients with relapsed/refractory multiple myeloma (RRMM). Currently, it can be used as a monotherapy at second relapse and in combination with dexamethasone and bortezomib (Dara-VD) or lenalidomide (Dara-RD) at first relapse. Daratumumab displays an ex-

cellent safety profile. Mild-graded infusion-related reactions (IRRs) occur mostly during the first infusion. For this reason, recommended administration rates provide a timing of 6.5, 4.5 and 3.5 hours respectively for the first, second, and following infusions. In our practice, we observed a low rate of adverse reactions also in patients with advanced disease. This is a single-center study exploring the safety of a rapid daratumumab infusion. Inclusion criteria was having at least eight previous doses of daratumumab, administered per standard procedure. Previous IRR was not an exclusion criterion. We evaluated 20 consecutive patients (median age 65 years, M:F rate 1:1, MM IgG 45%, IgG 10%, IgA 15%, IgA 10%, micromolecular 10%, micromolecular 5%) affected by RRMM. Six patients (30%) were treated with daratumumab single agent, 3 patients (15%) with Dara-VD and 11 (55%) with Dara-RD. Moreover, 25% of patients suffered from a cardiovascular disease (hypertension, arrhythmia or valvulopathy). All patients had received premedication with chlorphenamine, paracetamol and corticosteroid; during first infusion, 14 patients (70%) had suffered of IRRs, grade 1, according to Common Terminology Criteria for Adverse Events (CTCAE). Common IRRs included conjunctivitis, rhinorrhoea, cough, wheezing or hypertension, reverted in a short time. Median number of daratumumab prior infusions for our cohort was 14. Premedication was carried out similarly to previous infusions. Short time infusion was realised administering daratumumab at rate of 200 ml/h for the first 30 minutes, increasing to 400 ml/h for the next 60 minutes. No patient experienced adverse events during infusion, neither 30 min after completion, in which patients were observed to assess for delayed IRRs. 90-min infusion were realised in safety even in patients who experienced grade I (40%) and II (10%) anaemia or in those with a history of immediate hypersensitivity reactions (20%) to drugs or aeroallergens. Ninety-minutes daratumumab infusion was therefore well tolerated, allowing a considerable saving of time for RRMM patients and encouraging their adherence to treatment.

P026

A REAL-WORLD EFFICACY AND SAFETY ANALYSIS OF CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE (KRd) COMBINATION IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

I. Caratozzolo, P. Tacchetti, R. Zambello, L. Pantani, E. Antonioli, K. Mancuso, G. Buda, S. Rocchi, L. Canepa, I. Rizzello, B. Gamberi, G. Barilà, N. Testoni, G. Marzocchi, E. Zamagni, M. Cavo

Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliera di Padova e Veneto, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria Univesitaria Careggi, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria Pisana, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliera ASMN, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliera ASMN, Azienda Ospedaliera di Padova e Veneto, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Italy

Carfilzomib-lenalidomide-dexamethasone (KRd) has been approved for the treatment of relapsed/refractory multiple myeloma (RRMM), based on results of the ASPIRE trial (Stewart, N Eng J Med, 2015). In this retrospective analysis, we aimed at further evaluating the efficacy and safety of KRd in a real-world setting. 197 RRMM patients (pts) who received KRd in routine care between January 2016 and March 2018 in 6 Italian haematologic institutions were identified and followed for a median of 12.5 months. At KRd initiation, median age was 63 years, 47% of the pts had ISS II or III, and 27% carried high risk cytogenetic abnormalities (HRCA) [del17p and/or t(4;14) and/or t(14;16)]. Median number of prior lines of therapy was 2 (1-8); nearly all pts (96%) received prior bortezomib (18% refractory) while 45% were exposed to lenalidomide (R) and 22% were refractory. KRd was administered according to the ASPIRE schema. For 53 (27%) pts R starting dose was ≤ 15 mg, according to standard recommendations for cytopenia or renal impairment. 98% of the pts received anti thrombotic prophylaxis. Median number of delivered cycles was 7 (1-27). At the data cut-off, 52% of the pts had discontinued treatment, 66% for progression, 20% for salvage transplant, 12% for adverse events (AEs) and 2% for other reasons. Main grade 3-4 AEs were neutropenia (21%), infections (11%)

and hypertension (6%). Overall, the response rate was 88%, including 22% complete response and 50% \geq very good partial response (VGPR). The median PFS was 19.8 months and 1-year OS rate was 82.6%. By subgroup analysis, extended PFS and OS were observed for pts who received ≤ 2 prior lines of therapy (HR=0.17, $p < 0.001$ and HR=0.35, $p = 0.001$, respectively), not refractory to prior R (HR=0.37, $p < 0.001$, and HR=0.47, $p = 0.024$), without HRCA (HR=0.33, $p = 0.005$ and HR=0.26, $p = 0.016$) and achieving \geq VGPR (HR=0.17, $p < 0.001$ and HR=0.18, $p < 0.001$). By multivariate analysis, a response \geq VGPR (HR 0.163, $p < 0.001$), a number of prior lines of therapy ≤ 2 (HR=0.410, $p = 0.034$) and the absence of HRCA (HR=0.425, $p = 0.036$) were independent predictors of prolonged PFS. OS was longer for pts achieving \geq VGPR (HR=0.072, $p = 0.009$), with del17p negativity (HR=0.098, $p = 0.01$) and ISS stage I (HR=0.17, $p = 0.046$). In conclusion, KRd demonstrated to be effective in RRMM patients treated in real-world setting, without new safety concerns. Better survival outcomes emerged for pts with ≤ 2 prior lines of therapy, achieving at least a VGPR, and without HRCA.

P027

MINIMAL RESIDUAL DISEASE (MRD) RATIO BEFORE AND AFTER AUTOLOGOUS STEM CELLS TRANSPLANTATION (ASCT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

E. Antonioli¹, C. Nozzoli², M. Staderini¹, R. Boncompagni², R. Caporale³, B. Peruzzi³, A. Bosi¹, R. Saccardi²

¹SODc Ematologia AOU Careggi; ²SOD Terapie cellulari e Med trasfusionale AOU Careggi; ³SOD Citofluorimetria AOU Careggi, Italy

In the last ten years, Multiparametric Flow Cytometry (MFC) has been standardized and routinely applied for the detection of MRD as a prognostic factor in multiple myeloma (MM) patients across different lines of therapy. We assessed the MRD carried out before and after ASCT in a series of consecutive MM patients in order to investigate whether the ratio of the two determinations might increase the prognostic potential. We collected bone marrow samples for MRD assessment at the end of induction therapy and 3 months after ASCT from 61 newly diagnosed MM patients treated between 2013 and 2017 with a bortezomib-based induction therapy and achieving at least a VGPR, according to the most recent International Myeloma Working Group (IMWG) criteria. MFC-determined MRD was evaluated according to EuroFlow recommendations. Post-induction therapy MRD was found predictive of post-ASCT MRD status. Indeed, patients transplanted in a MRD positive status had a significantly increased risk to maintain a MRD positivity status after transplantation (Odds Ratio - OR - 15,053, $p = 0,002$). Detection of post-ASCT MRD had a negative impact on median PFS (28 months vs not reached respectively, $p = 0,001$). In Cox-Regression analysis, a complete remission status (CR) with an undetectable MRD after the ASCT resulted to be the major protective factor from relapse (Hazard Ratio - HR - 0,012, $p = 0,005$), while patients with a detectable MRD before and after the ASCT had the worse PFS (22 months, HR 2,958; $p = 0,029$). Risk analysis showed 3 different PFS risk groups: "high" for the patients with MRD detectable before and after the ASCT, "intermediate" for patients with MRD positivity before the ASCT who achieve a negativity after, and "low" in the case of MRD undetectable before and after. MFC is a relatively recent method to assay MM MRD, and its role in MM therapeutic path is still under investigation. According to our data, a detectable MRD after the ASCT is a major relapse risk. Interestingly we found that it can be early predicted by the post-induction MRD status and its negativization after ASCT has a modest impact on this. Therefore, we support the concept of treatment escalation when a CR is not reached after the induction treatment, in order to undergo to the ASCT in the best possible response. However, double MRD determination before and after ASCT may increase the prediction potency of currently validated

P028**EARLY SALVAGE TREATMENT WITH SECOND-GENERATION NOVEL AGENTS AT BIOCHEMICAL RELAPSE PROLONGS OVERALL SURVIVAL: A REAL-WORLD SINGLE CENTER EXPERIENCE**

A. Romano, V. Del Fabro, U. Markovic, V. Leotta, E. Martino, M. Parisi, V. Calafiore, G. Sapienza, A. Orofino, M. Di Giorgio, S. Poidomani, A. Petronacci, F. Di Raimondo, C. Conticello

Division of Hematology, Azienda Policlinico-OVE, University of Catania, Italy

Background: Multiple Myeloma (MM) remains largely incurable and patients invariably develop relapsed or refractory (R/R) disease. Relapse can be characterized according to disease aggressiveness and the presence of clinical symptoms. Aggressive relapse can occur at biochemical level, due to rapid and relevant increase of monoclonal component or LDH, or at clinical level, defined by the presence of extramedullary disease, acute renal injury or progression to secondary plasma cell leukemia.

Aim: To identify the clinical outcome upon treatment with second generation novel agents (pomalidomide, carfilzomib) and monoclonal antibodies (elotuzumab, daratumumab), in early salvage treatment to RRMM.

Study design: According to regulatory of the Italian regulatory agency, patients (N=128) received one of the following regimens: KRd (N=42, group A), Poma-Dex (N=77, group B), Daratumumab (in monotherapy, N=12; associated to bortezomib and Dex, N=18, associated to Rd, N=3), or Elotuzumab associated to Rd (N=12, group C).

Results: From July 2014 to January 2019 we evaluated 165 treatments in 128 patients (58% males, median age 62 years, range 45-78), relapsed (59%) and RR (41%) patients, treated at biochemical (68%) or clinical relapse (32%). Median number of previous lines was 3 (1-13), including autologous stem cell transplantation (ASCT) in half of cases (N=64, 50%). FISH before to start the second-generation drugs was available in 53 cases: 11 carried on high-risk (del 17p, t4;14 or t14;16) and 42 standard-risk cytogenetics, without any additional marker compared to the matched sample at diagnosis. Overall survival (OS) showed a better outcome for patients treated at biochemical relapse (27.8 vs 15.2 months, p=0.0005), for relapsed patients compared to RR (34.1 vs 19.3 months, p=0.0004), for patients who received less than 3 lines of therapy (33.6 vs 19.1 months, p=0.002), those carrying standard-risk cytogenetics (20.4 vs 11.2 months, p-value not significant for low numbers in two groups) without any significant difference for previous ASCT or the type of second-generation treatment chosen. In multivariable analysis only type of relapse (biochemical versus clinical) and refractory status were independent predictors of overall survival (p<0.0001).

Conclusions: In our community setting data, heavily pretreated patients achieved a median OS >12 months, using second generation agents. Our data indicate that the tumor mass and mechanisms of resistance play a major role in response to the novel agents.

P029**IRON INDUCES BORTEZOMIB RESISTANCE IMPROVING MITOCHONDRIA FITNESS AND ENERGETIC METABOLISM IN MULTIPLE MYELOMA CELLS**

G. Camiolo¹, A. Barbato¹, C. Giallongo¹, D. Cambria¹, N. Parrinello¹, F. Puglisi¹, C. Conticello¹, A. Romano¹, V. Del Fabro¹, G.A. Palumbo², G. Li Volti³, D. Tibullo³, F. Di Raimondo¹

¹Section of Haematology, Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy. Division of Haematology, Azienda Policlinico-OVE, Catania; ²Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy. Division of Haematology, Azienda Policlinico-OVE, Catania; ³Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

Background: Multiple myeloma (MM) is a non-curable B cell malignancy in which iron metabolism plays an important role. Due to the high rate of proliferation and increased metabolism, myeloma cells have an increased need for iron which may represent the Achilles' heel of the disease. Clinical studies showed that MM patients present low or normal serum iron levels and elevated serum ferritin levels. Consistently with impaired iron mobilization and release, bone marrow biopsies are characterized by an increase in hemosiderin-laden macrophages with

normal or increased iron stores. In this work we investigated the role of iron metabolism in MM drug resistance.

Results: *In vitro* pre-treatment of MM cells (U266, OPM2, H929) with iron (400uM Ferric Ammonium Citrate, FAC for 24h) or hemin (20uM for 24h) reduced cell-sensitivity to Bortezomib (BTZ) compared to cell not treated with FAC (p<0.01), and this effect was abolished by co-administration of deferasirox, an iron chelator (p<0.001). Interestingly, BTZ resistant MM cell line (U266-R) showed an increased expression of genes involved in iron metabolism (i.e. FPN1, HMOX1, Nrf2, TFRC1 and DMT-1). Moreover, in U266 cells FAC or hemin treatment resulted in a significant increase of ROS levels and lipid hydroperoxides formation accompanied by a significant mitochondrial membrane depolarization which was recovered following 24h. Cell response to damage consisted also in a significant increase in mitochondrial mass (p<0.001), upregulation of genes associated to mitochondria biogenesis (i.e. PGC1a and TFAM), OXPHOS genes and energetic metabolism (ATP synthase, ND4, CytB). Consistently, glutathione synthetase (GSS) gene and GSH levels resulted also significantly increased in response to oxidative stress. Finally, regarding Iron Responsive Element-Iron Regulatory Protein system (IRE-IRP), we observed a downregulation of CD71 protein and upregulation of DMT-1, HMOX1, TFRC1 and FPN1 gene expression. Interestingly, iron or Hemin treatment also increased autophagy in myeloma cells as observed in H929-LC3-GFP-mCherry myeloma cell line.

Conclusion: Iron metabolism is involved in reduced BTZ sensitivity of MM cell via autophagic pathway and improving mitochondrial fitness and metabolism.

P030**CLINICAL FEATURES AND OUTCOMES OF NEWLY DIAGNOSED 1q+ MULTIPLE MYELOMA PATIENTS RECEIVING NEW-DRUG CONTAINING REGIMENS**

M. Offidani¹, S. Morè¹, L. Corvatta², V. Mori¹, L. Maracci¹, S. Trappolini¹, S. Gentili³, G. Discepoli¹, A. Olivieri¹

¹Clinica di Ematologia AOU Ospedali Riuniti di Ancona; ²Divisione Medicina Ospedale di Fabriano; ³Divisione Medicina Ospedale di Civitanova Marche, Italy

Amplification of chromosome 1q21 (1q+) represents one of the most common cytogenetic abnormalities in multiple myeloma (MM) but results, mainly retrospective, evaluating the impact of 1q+ on outcome of MM patients are fairly conflicting. Some retrospective studies (Biran et al, 2014; Shah et al, 2017; Schmidt et al, 2018) showed that patients with 1q+ had a poor outcome despite novel agent-based induction regimens and autologous stem cell transplant (ASCT). Others, (Cavo et al., 2016; Chen et al, 2019) found that bortezomib-based therapy are able to overcome poor prognostic value of this aberration. We collected data for 324 newly diagnosed MM patients who were seen at Clinica di Ematologia di Ancona between 2010 and 2018 with the aim to compare clinical characteristics and outcomes of patients with 1q+ with those of a standard risk group (SR) defined by the absence of del17p, t(4;14) or t(14;16) by FISH analysis. Amplification 1q was detected in 45 patients (14%) who were compared with 126 SR MM patients. No significant difference were found in terms of age, sex, performance status but patients with 1q+, compared with SR group, were more likely to have LDH level above the upper limit of normal (19% vs 7%; p=0.037) and to present with stage III ISS (37% vs 22%; p=0.05). Induction regimens were IMiDs-based and PI-based (bortezomib or carfilzomib) in 24% and 40% of patients with 1q+ vs 23% and 41% in SR patients whereas 36% of both groups received IMiDs plus PI combinations. Forty percent and 44% of 1q+ and SR patients underwent ASCT, respectively. Moreover, no difference was seen in the number of patients enrolled in clinical trials, being 62% in the first group and 59% in second one. Response ≥ VGPR was achieved in 73% and 67% in 1q+ and SR patients, respectively. After a median follow-up of 32 months, no differences were seen in terms of PFS and OS between the two groups of patients (median PFS 47.7 vs 44.7 months; median OS was 88.7 and 95.6 months in 1q+ and SR patients, respectively). Multivariate analysis demonstrated that LDH > N (p=0.035) and ISS stage III (p=0.023), but not 1q+ (p=0.795), were factors affecting PFS and OS. Our experience suggested that 1q+ alone is not an adverse prognostic factor for any outcomes but it has to be taken into account that most of our patients received PI-based induction therapy that was found to overcome negative impact of 1q+ and that we were not able to provide copies of 1q21.

Myeloma and Monoclonal Gammopathies 2

P031

CARFILZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: THE REAL-LIFE EXPERIENCE OF RETE EMATOLOGICA PUGLIESE (REP)

A. Mele¹, E. Prete¹, C. De Risi¹, G. Greco¹, S. Citiso¹, N. Cascavilla², A.P. Falcone², G. Sanpaolo², D. Pastore³, G. Mele³, A. Giannotta³, N. Di Renzo⁴, C. Vergine⁴, G. Rendiconto⁴, P. Mazza⁵, G. Palazzo⁵, S. Sabatelli⁵, G. Tarantini⁶, C. Germano⁶, R. Miccolis⁶, G. Specchia⁷, P. Curci⁷, R. Rizzi⁷, A. Guarini⁸, S. Capalbo⁹, A. Greco¹, R. De Francesco¹, S. Sibilla¹, M.R. Morciano¹, V. Pavone¹

¹Department of Hematology and Bone Marrow Transplant, Hospital Card. G. Panico, Tricase; ²Department of Hematology and Bone Marrow Transplant, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo; ³Department of Hematology, Hospital Perrino, Brindisi; ⁴Department of Hematology, Hospital Vito Fazzi, Lecce; ⁵Department of Hematology and Bone Marrow Transplant, Hospital G. Moscati, Taranto; ⁶Department of Hematology and Bone Marrow Transplant, Hospital G. Moscati, Taranto; ⁷Department of Hematology and Bone Marrow Transplant, Policlinico, University of Bari; ⁸Department of Hematology, Hospital IRCCS Oncologico, Bari; ⁹Department of Hematology, Hospital University Riuniti, Foggia, Italy

Background: Carfilzomib, lenalidomide and dexamethasone (KRd) has been approved for the treatment of Relapsed and Refractory Multiple Myeloma (RRMM). However, its effectiveness and safety profile in real clinical practice should be further assessed.

Aims: We retrospectively evaluated 120 RRMM patients treated with KRd, in 9 hematology departments of Rete Ematologica Pugliese (REP), between December 2015 and August 2018.

Methods: The patients' baseline characteristics are presented in Table 1. The median number of previous treatment lines was 1 (range 1-11). Half of the patients (52%) had a previous Autologous Stem Cell Transplant (ASCT). The median time from the diagnosis to start of KRd was 40 months (range 5-295). Thirty patients were treated with KRd early (≤ 18 months from diagnosis). Disease status at the start of treatment with KRd was refractory in 33 patients (29%) and 13 patients (12%) were refractory to lenalidomide.

Table 1. Patients' baseline characteristics and details of the previous therapies.

	N= 120
Median age, years (range)	66 (34-81)
Age ≥ 70 years, n (%)	41 (34)
III ISS disease staging, n (%)	50 (44)
Extramollard disease, n (%)	13 (11)
Cytogenetic profile, n (%)	
Unkown	77 (64)
Standard Risk	35 (29)
High Risk	8 (7)
Elevated LDH, n (%)	51 (46)
Median Time since initial therapy, months (range)	40 (5-295)
≤ 18 months	30 (25)
Median number of previous lines of therapy (range)	1 (1-11)
Number of previous lines of therapy, n (%)	
2 / ≥ 3	24 (20) / 33 (29)
Previous autologous transplant, n (%)	62 (52)
Previous allogeneic transplant, n (%)	6 (5)
Previous therapy, n (%)	
Bortezomib / Lenalidomide	113 (94) / 41 (34)
Bortezomib and Lenalidomide	39 (33)
Pomalidomide / Monoclonal Antibodies	8 (7) / 3 (2)
Refractory, n (%)	33 (29)
Bortezomib	18 (16)
Lenalidomide	11 (10)
Bortezomib and Lenalidomide	2 (2)
Refractory to last therapy, n (%)	24 (21)

Results: The overall response rate (ORR) was 84% (n93), with 23% (25) complete response (CR) and 50% (55) \geq Very Good Partial Response (VGPR). The median duration of response was 12,9 months (range 3,33-

27,7). ORR was higher in patients relapsed after a previous ASCT (56% vs 37% in those relapsed without prior ASCT; p 0,05). Patients treated in late relapse had a better ORR (44%) vs those in early relapse (19%; p 0,02). After a median follow-up of 13,4 months, median PFS was not reached (NR) and 2y-PFS was 61%, Table 1. PFS was longer in responding patients (\geq PR) to those with $<$ PR (median PFS NR vs 4,9 months; p 0,0001). Median PFS in patients relapsed after a prior ASCT was NR vs 20 months in those without prior ASCT (p 0,002). Patients achieving ASCT after KRd had a better PFS to those without ASCT (median NR vs 9 months, p 0,001). 28 patients (24%) performed 4 KRd cycles as bridge treatment to ASCT. The 64% of patients reached a VGPR and 67% received ASCT, with 9 upgraded from VGPR to CR after ASCT. The treatment discontinuation rate due to adverse events (AEs) was 13%. In 11% of patients a KRd dose reduction was necessary. The most frequent AE was neutropenia (43%) and anemia (41%). Infections occurred in 10% of patients. Cardiovascular events occurred in 8% of patients.

Conclusions: Our analysis confirmed that KRd is effective in RRMM patients. It is well tolerated and applicable to the majority of patients outside clinical trials. A longer PFS was shown in patients achieving a partial response (PR), relapsing after previous ASCT and in those with the possibility to perform ASCT after KRd treatment.

P032

UTILITA' DELLA 18FDG-PET/CT NEL MIELOMA: UNA ESPERIENZA "REAL-LIFE"

S. Galimberti¹, W. Bertolami¹, P.A. Erba², D. Caramella³, G. Buda¹, E. Orciuolo⁴, F. Caracciolo⁴, E. Benedetti⁴, R. Fazzi⁴, N. Cecconi⁴, G. Consani⁴, G. Cervetti⁴, F. Mazziotta^{1,5}, L. Iovino^{1,6}, F. Martini¹, R. Morganti⁷, M. Petri¹

¹Department of Clinical and Experimental Medicine, Section of Hematology, University of Pisa, Pisa, Italy; ²Department of Translational Research and New Technologies in Medicine and Surgery, Section of Nuclear Medicine, University of Pisa, Pisa, Italy; ³Department of Translational Research and New Technologies in Medicine and Surgery, Section of Radiology, University of Pisa, Pisa, Italy; ⁴UO Hematology, AOUP, Pisa, Italy; ⁵School of Doctorate GenOmeC, University of Siena, Siena, Italy; ⁶School of Doctorate in Clinical and Translational Sciences, University of Pisa, Pisa, Italy; ⁷Statistical support to clinical studies, AOUP Pisa, Italy

Background: Bone involvement is found in 50% of multiple myeloma (MM) patients at diagnosis, and in 90% during the course of disease. The conventional radiography gives 10-20% of false-negative results; sensitivity and specificity of CT are superior, and also MR is a valid imaging technique, with 50% of patients negative on X-ray showing focal lesions on MR. With sensitivity of 80-90% and specificity of 80-100%, FDG-PET/CT is a new tool: by combining it with MR of the spine and pelvis, 92% of patients with active disease are correctly diagnosed.

Aim: We retrospectively compared results from PET/CT with CT, MR and X-ray in 160 MM patients who underwent or not to autologous transplantation.

Results: At diagnosis, PET/CT offered an advantage of 14% and 19% in terms of sensitivity in respect of X-ray and CT, respectively, whereas it resulted fully comparable to MR. During follow-up, in 7 out of 427 occasions (0.9%) PET/CT failed to identify bone involvement; on the other hand, in 50 cases (11.7%) it was positive compared to the negative results obtained by the other imaging techniques. Overall, during follow-up sensitivity of the PET/CT was 53%, specificity 74%, positive predictive power 77%, and negative predictive power 48%. Then, we assessed if PET/CT could predict the quality of response or survival: it was not predictive, even if a trend to better OS and PFS was observed in cases with a negative PET/CT.

Conclusions: In terms of sensitivity and specificity, we confirmed the good performance of PET/CT: with a sensitivity and specificity about 70%, our results confirm those becoming from a published meta-analysis based on 32 clinical trials where the sensitivity of FDG-PET/CT ranged from 67% to 100%. PET/CT resulted more sensitive than X-ray either at diagnosis or during follow-up, more sensitive than CT at diagnosis but comparable during follow-up and comparable to MR, both before and after treatment. Thus our study, even if retrospective but involving a large number of patients, confirms in a "real-life" experience that PET/CT and MR are the best imaging techniques in terms of accurate assessment of bone involvement in MM and that FDG-PET/CT

could represent an optimal approach for those centres where the WBMR is still not available. Sure, it remains the problem of its international standardization, but this process has been already started.

P033

REAL-LIFE DATA ON LEN/DEX COMBINATION AT FIRST-LINE THERAPY OF MULTIPLE MYELOMA

V. Leotta, A. Romano, V. Del Fabro, M. Di Giorgio, F. Di Raimondo, C. Conticello

Division of Hematology, Azienda Policlinico-OVE, University of Catania, Italy

Background: Lenalidomide plus dexamethasone (Rd), has recently become a new standard of care for newly diagnosed multiple myeloma (NDMM) patients. The FIRST phase 3 trial showed that continuous therapy with Rd is superior in PFS and OS compared to fixed therapy or triplet including melphalan, thalidomide and prednisone (MPT), leading to approval of Rd for NDMM. However, little is known about safety and efficacy of Rd in frail and ultra-frail patients in the real life setting.

Experimental design and aims: From March 2017 up to now, 54 consecutive frail NDMM patients were treated with Rd regimen, according to the FIRST schedule. Lenalidomide dosage was reduced in patients with low platelets count and/or renal failure according to manufacturer's guidelines. We evaluated safety and efficacy of Rd combination and the prognostic significance of several parameters on PFS in 54 consecutive myeloma patients treated with Rd in first line, according to routine clinical practice of our Institution.

Results: Median age was 78 years (range 68-95), most patients were males (59%). 25 patients had an ECOG higher or equal to 2, 18 higher or equal to 3. Creatinine clearance was lower than 50 ml/min in 31 patients (57%). 46 patients (85%) had an evaluable FISH, 7 (12%) were classified as high risk for the presence of del(17p) or t(4;14) or t(14;16), and 16 (23%) carried acq1q. Five patients have an extramedullary disease. Median number of Rd cycles was 3.5 (1-19), with a mean duration of treatment of 5.6 months; 29 (54%) patients received more than 4 cycles, 30 (55%) are still on treatment. Hematological adverse events were reported in 19 patients, grade 4 anemia was reported only in one patient treated with blood transfusion. Grade 2 neutropenia and anemia were treated with granulocyte growth factors and/or erythropoietin, according to local policy. Grade 2 thrombocytopenia was managed by delaying next cycle. Non hematological toxicity was reported in 28 patients. Grade 3 fever of undetermined origin (FUO), pneumonia or thromboembolic events were more frequent events (N= 3, 3 and 3 patients respectively) and induced treatment interruption. Among 29 evaluable patients who received at least 4 cycles, the overall response rate was 39% (N=21), including 9 (17%) very good partial response (VGPR) and 12 (22%) partial response (PR), without any complete response. Overall, median PFS was 4 months; 5 (9%) patients had biochemical or clinical relapse during the treatment and 28 (52%) patients had clinical response to therapy (defined as no further need of transfusion, stable disease, regression of pain). In univariate analysis, clearance creatinine <30 ml/min and the ratio of absolute count of neutrophil/lymphocyte (NLR) >2 were strong negative predictors of overall survival (respectively, HR=2.8, 95% intervals of confidence, 0.8-9.4, p=0.02 and HR=2.4, 95% C.I. 0.9-6.3, p=0.04), while acq1q detected by FISH, LDH>200 U/L and ISS 3 stage were not associated to inferior outcome in our series. Only clearance creatinine <30 ml/min was a negative prognostic factor for OS and PFS in multivariate analysis.

Conclusion: These real-world data confirm the efficacy of Len/Dex combination in frail patients; more importantly, it is demonstrated for the first time outside of a clinical trial that high ECOG or age >75 years do not contraindicate treatment.

P034

AUTOLOGOUS STEM CELL TRANSPLANTATION IS SAFE IN SELECTED ELDERLY MULTIPLE MYELOMA PATIENTS

M. Staderini¹, E. Antonioli¹, C. Nozzoli², G. Buda³, R. Boncompagni², M. Petrini³, R. Saccardi², A. Bosi¹

¹SODc Ematologia AOU Careggi; ²SOD Terapie cellulari e medicina trasfusione AOU Careggi; ³UO Ematologia, Italy

Autologous stem cell transplantation (ASCT) is currently the "gold standard" first line treatment for multiple myeloma (MM) patients (pts) younger than 65. To evaluate the safety and the efficacy of this procedure in pts older than 65, we retrospectively analyzed a cohort of MM pts aged 65 or older, who were consecutively allocated to upfront ASCT at bone marrow transplantation Units of Florence e Pisa. From January 2009 to November 2017, we enrolled 105 pts. Although initially intended to, 22 pts did not receive ASCT eventually because considered unfit for the procedure later on or because of a suboptimal response to induction treatment. Our analysis thus focused on 83 pts actually transplanted. Their median age was 67 (range 65-74). Induction therapy was bortezomib-based in 75 (90%)pts, while 10% received thalidomide plus dexamethasone. Peripheral blood stem cells were collected in all 83 pts, mainly after high-dose cyclophosphamide plus G-CSF (89%). The conditioning regimen consisted of melphalan 100 mg/sqm, 140 mg/sqm and 200 mg/sqm in 40, 15 and 28 pts, respectively. A median number of 4.35×10^6 CD34+ cells/Kg was reinfused (range 2.09-10.44). Median neutrophils' and platelets' recovery were 11 (range 8-25) and 12 days (range 7-45), respectively. Forty percent of pts experienced treatment-related adverse events of any grade, the large majority of which were mild-graded; no transplant-related deaths were observed. After ASCT, VGPR and CR rates increased from 70% to 87% and from 19% to 39%, respectively. The independent predictive value of at least VGPR after induction for achievement of CR was confirmed in multivariate analysis (p 0.016); also including factors as FISH analysis, ISS, age over 69 years or higher melphalan dose. After a median follow-up of 46 months (range: 11-124 months), 52 pts (64%) relapsed. Median progression free survival (PFS) was 23 months (19-27 months), while median OS was not reached. The achievement of CR and VGPR before and after ASCT are the only significant prognostic factors for PFS. Multivariate analysis confirmed the association between high grade response to induction therapy and longer PFS (95% IC HR 2,53; p=0,014). No association was found between PFS and FISH analysis, ISS, age over 69 or higher melphalan dose. ASCT is an effective and safe first-line treatment approach also in fit elderly MM pts. A careful pts selection can reduce the toxicity of the procedure.

P035

MAINTENANCE THERAPY vs RE-TREATMENT AT BIOCHEMICAL RELAPSE vs OBSERVATION IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS: RESULTS OF A PHASE II, RANDOMIZED STUDY

R. Mina, A. Larocca, A. Belotti, C. Conticello, F. Monaco, M. Offidani, S. Ronconi, M. Marchetti, R. Ria, A. Conconi, N. Pescosta, M. Grasso, A. Capra, R. Zambello, M. Poggiu, N. Giuliani, S. Ballanti, C. Cellini, A.M. Liberati, T. Caravita, M.T. Petrucci, A. Palumbo, P. Musto, P. Sonneveld, M. Boccadoro

GLMEMA/ European Myeloma Network Italy; Takeda Pharmaceutical; Department of Hematology, Erasmus Medical Center, the Netherlands

Background: The pattern of multiple myeloma (MM) relapses includes biochemical relapses (increase of monoclonal paraprotein without organ damage) and clinical relapses (increase of monoclonal protein and organ dysfunction), with salvage treatment indicated at clinical relapse. We report the results of a randomized, phase II study assessing efficacy and safety of bortezomib as continuous treatment until progression or as a re-treatment at biochemical relapse, as compared to observation until clinical relapse in MM patients.

Methods: Patients with RRMM after 1-3 prior lines of therapy, the last one including bortezomib, without evidence of disease progression, were enrolled within 45 days of completion of the previous salvage cycle. In arm A, patients received maintenance treatment with bortezomib (1.3 mg/m², days 1,15) and dexamethasone (20 mg, days 1,2,15,16); in arm B, patients were treated at biochemical relapse with

six 4-week cycles of bortezomib (1.3 mg/m² on days 1,8,15,22) and weekly dexamethasone (40 mg); in arm C, patients received no treatment until clinical relapse. The primary endpoint was time to progression (TTP), calculated from the time of enrolment to both biochemical relapse (TTBR) and clinical relapse (TTCR).

Results: 60 patients were evaluated (arm A=16; arm B=23; arm C=21). Median number of prior therapies was 2 (1-3). At enrolment, there were no significant differences in terms of response to prior bortezomib-salvage treatment in the 3 arms. Median follow-up was 39 months. In patients receiving bortezomib maintenance, median TTBR and TTCR were 18 and 22 months, respectively; in patients receiving bortezomib-retreatment at biochemical relapse, median TTBR and TTCR were 8.4 and 20 months; in the observation arm, median TTBR and TTCR were 10.5 and 11 months (Figure 1). Median progression-free survival² was longer in patients receiving bortezomib maintenance (45 months) or re-treatment (31 months) than in patients in the observation arm (26 months). The rate of grade 3-4 adverse events was low (<20%), without significant differences in patients receiving bortezomib as maintenance or re-treatment.

Conclusions: in RRMM patients treated with a bortezomib-based regimen, bortezomib maintenance or re-treatment with bortezomib at biochemical relapse were safe and prolonged TTCR as compared to observation, without a negative impact on the efficacy of subsequent therapies.

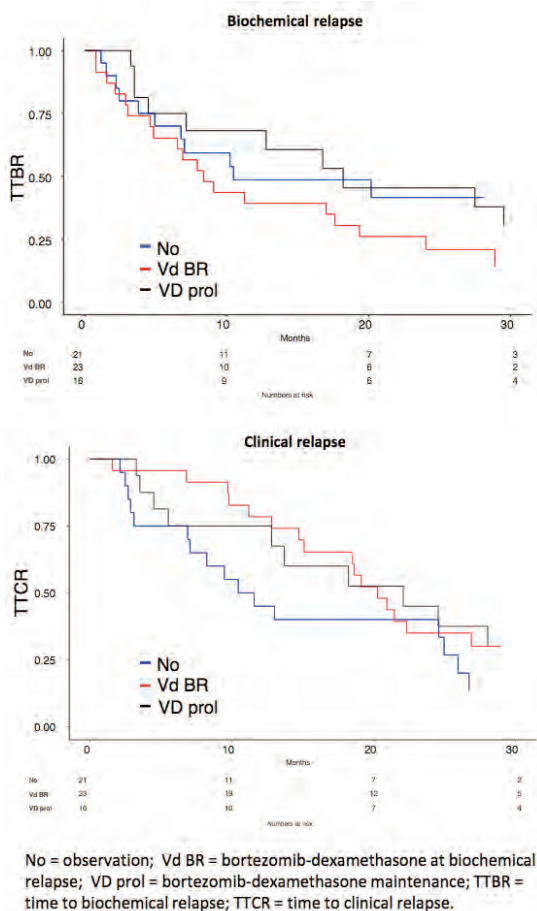


Figure 1.

P036

HIGH PROPORTION OF PATIENTS WITH MULTIPLE MYELOMA RECEIVED MANY DIFFERENT LINES AFTER FRONTLINE THERAPY: A SINGLE ITALIAN TERTIARY CENTRE EXPERIENCE

S. Morè¹, L. Corvatta², V. Mori¹, L. Maracci¹, G. Mancini¹, I. Scortechini¹, S. Gentili³, A. Olivieri¹, M. Offidani¹

¹Clinica di Ematologia AOU Ospedali Riuniti di Ancona; ²Divisione Medicina Ospedale di Fabriano, ³Divisione Medicina Ospedale di Civitanova Marche, Italy

Despite some studies from Europe and USA, data on real-world management of Multiple Myeloma (MM) patients in Italy are lacking. However, they are important to know because of national difference in approval status and reimbursement of drugs. This study analyzed treatment strategy at first and subsequent lines of therapy in a single tertiary centre using our database on MM. From 2005 to 2019, we managed 324 MM patients requiring treatments. Median age was 68 years (range 42-93), half of patients were male and 132 (43%) were older than 70 years. The most common monoclonal component was IgG (53%). Revised International Staging System (R-ISS) was high-risk in 13% and low-risk in 30% of 278 patients with available cytogenetics and LDH. Twenty-two percent of patients had three or more comorbidities and 18% of them had renal failure. Overall, 147 patients (45%) were enrolled in clinical trials in at least one line of therapy. First line therapy included a combination of immunomodulatory agents (IMiDs) and a proteasome inhibitor (PI) in 35% of 324 patients, PI-based regimens in 34%, IMiDs-based regimens in 29% and chemotherapy in only 2%. One hundred and forty two patients (44%) underwent ASCT, double in 36% of cases. Among 179 relapsed patients, 155 (86.5%) received a second line that was PI-based (33.5%), IMiDs-based (33%), monoclonal antibodies (MoAb)-based regimens (19%) and IMiDs + PI combinations (10%). Out of 86 patients with a second relapse, 67 (78%) received a third line treatment, particularly IMiDs-based (52%); 15% pomalidomide-based). A fourth line of treatment was administered to 39 (75%) of 52 patients who had a third relapse as well as a fifth line to 23 patients (62%) with a fourth relapse. The main reason to hold subsequent therapies was death that occurred quite in patients older than 65 years. With a median follow-up of 40 months, median PFS and OS were 45 and 98 months, respectively, overall. Median 2ndPFS, 3thPFS, 4thPFS and 5thPFS (defined as the time from previous treatment to relapse-progression disease) were 23, 13.2, 7 and 4.5 months, respectively. In conclusion, the proportion of patients receiving second and subsequent lines of therapy was higher than that reported in a recent European survey (Raab *et al.*, BJH 2016) leading to remarkable outcomes. However, it should be outlined that in our centre many patients were enrolled in clinical trials, thus new drugs have been offered also in advanced disease.

P037

PROFILING PERIPHERAL BLOOD LOX-1+ GRANULOCYTES IN THE TUMOR IMMUNE MICROENVIRONMENT OF MGUS AND MULTIPLE MYELOMA DISCLOSED IMMUNE SUPPRESSIVE PROPERTIES OF HIGH-DENSITY NEUTROPHILS

N.L. Parrinello^{1,2}, F. Puglisi¹, V. Simeon³, C. Bellofiore¹, C. Giallongo¹, D. Tibullo¹, G. Schininà, V. Leotta¹, V. Del Fabro¹, C. Conticello¹, P. Musto³, F. Di Raimondo¹, A. Romano¹

¹AOU Policlinico Vittorio Emanuele di Catania - Divisione di Ematologia; ²Dipartimento di Scienze Mediche - Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia" Catania; ³Department of Hemato-Oncology - IRCCS - Rionero in Vulture, Potenza, Italy

Background: In the tumor immune microenvironment (TIME) of Multiple Myeloma (MM), immune-suppressive granulocytes obtained after a gradient-density based centrifugation, are distinguished in high-density neutrophils (HDN) and granulocytic myeloid-derived suppressor cells (G-MDSCs). A recent study identified Lectin-type oxidized LDL receptor-1 (LOX-1) as a distinct surface marker for human PMN-MDSC, detectable without recurring to gradient-density based centrifugation.

Methods: We provided the detailed phenotypic and functional profile of HDN and G-MDSCs in the MM-TIME to investigate the existence LOX-1+ PMN-MDSC in MGUS and MM patients by multidimensional flow cytometry (MFC) and in vitro experiments.

Results: The percentage of cells with a CD14-CD15+CD33+HLADR-phenotype was higher in BM than in PB ($p=0.03$), but similar in the BM of MGUS and MM patients (about 50% of white blood cells), with differential reactivity against CD11b, Lox-1 and the immune-suppressive marker ARG-1. Both CD11b+ CD14-CD15+CD33+HLADR- and Lox-1+ PMN-MDSC were higher in MM than MGUS and healthy subjects ($p=0.01$); ARG-1 was higher in MM than MGUS ($p=0.01$) and expressed almost exclusively by Lox-1+PMN-MDSC. After a density-gradient-based centrifugation, Lox-1 was equally distributed between HDN and G-MDSC, disclosing that HDN obtained after density-based centrifugation include Lox-1+PMN-MDSC both in MGUS and in MM. According to similar studies in literature showing that LOX-1+CD15+PMN-MDSCs significantly reduced proliferation of T-cells, our functional studies confirmed that HDN and G-MDSC in MM had comparable immune-suppressive activity against allogeneic T-cells, reverted by inhibition of Arginase-1 (ARG1). Gene-expression profile disclosed the upregulation of IL-6/JAK/STAT3 signaling in MM-HDN and that STAT3 and STA5 gene expression was positively associated with ARG-1. STAT-3 and STAT-5 were inducible in vitro by exposure to MM sera, without affecting ARG1 levels.

Conclusions: We have determined the phenotypic and immunosuppressive potential of unique granulocytic subsets and unveiled that, in contrast to previous findings, HDN have immunosuppressive potential, associated to the upregulation of IL-6/JAK/STAT3 signaling.

P038

CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA

C. Cerchione¹, G. Martinelli¹, D. Nappi², A.E. Pareto², M. Di Perna², K. Ferrara², G. Musuraca¹, A. Lucchesi¹, A. Ferrari¹, G. Simonetti¹, F. Pane², L. Catalano²

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; ²Hematology, AOU Federico II, Italy

Carfilzomib is an epoxyketone proteasome inhibitor of second generation, proved to be effective and safe in relapsed and refractory Multiple Myeloma (rrMM), in combination with dexamethasone or lenalidomide and dexamethasone. In this retrospective observational trial, it has been evaluated efficacy and safety of carfilzomib, in combination with lenalidomide-dexamethasone (KRD) as salvage regimen in patients with rrMM, refractory to lenalidomide, whose prognosis is particularly severe. 41 patients (23 M/18 F), with rrMM, median age at diagnosis 63.7 years (r. 43-82), median age at start of treatment 67 years (r. 48-84) previously treated with several lines of treatments (median 3, r. 2-11), underwent to KRD regimen (ASPIRE trial schedule) for a median treatment cycles of 8 (r 2-18). ISS was equally distributed, and all patients had previously been treated with bortezomib and IMiDs, and were refractory to this agents. 61% (19/31) of them had undergone at least to a single ASCT. According to IMWG criteria, after a median follow-up of 9 months (r. 2-18), ORR was 68,2% (28/41: 9 CR, 12 VGPR, 7 PR) with 5 progressive diseases (PD) and 8 patients in stable disease (SD): this can be considered as an impressive result in this subset of rrMM patients, refractory to lenalidomide. In particular, for 11 patients, KRD was, after having achieved at least a PR, a bridge to second/third autologous SCT. Median time to response was 1.3 months (r.1-4), median OS from diagnosis was 62 months (r. 9-170), median OS from start of Carfilzomib was 11 months (r. 2-18). Carfilzomib was well tolerated, with grade 2 anemia in 39%(16/41) of patients, successfully managed by ESAs, without necessity of blood transfusions; 29% (12/41) grade 3-4 neutropenia (pegfilgrastim in primary prophylaxis was given, no hospitalization was required, no septic shocks were observed); 34% (14/41) grade 2, 21% (9/41) grade 3 and 12% (5/41) grade 4 thrombocytopenia, without hemorrhagic events and transfusion-dependency. Moreover, it was observed pneumonia in 39% (16/41) of patients, treated by common antibiotic drugs and always solved. A cardiac monitoring was performed for all patients: hypertension (grade 2-3) in 34% (14/41) of patients; fatigue in 39% (16/31) of patients. Carfilzomib-Lenalidomide-Dexamethasone has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, also lenalidomide, and it could be considered as a bridge to a second autologous or allogeneic SCT.

P039

BONE MARROW PLASMA CELLS INFILTRATION, MONOCLONAL COMPONENT LEVEL AND EVOLUTION ARE SIGNIFICANT PREDICTORS OF PROGRESSION IN SMOLDERING MYELOMA PATIENTS

M. Pontone¹, E. Antonioli¹, M. Staderini¹, A. Buzzichelli¹, M. Messeri¹, V. Nencini¹, C. Nozzoli², R. Caporale³, B. Peruzzi³, A. Bosi¹

¹SODc Ematologia, AOU Careggi; ²SOD terapie cellulari e medicina trasfusione, AOU Careggi; ³SOD Citometria AOU Careggi, Italy

Smoldering Multiple Myeloma (SMM) is an asymptomatic plasma-cell dyscrasia that comprises a spectrum of distinct entities with different risk of progression. Several studies have developed risks models to stratify SMM patients and reported possible predictors of progression. This single centre retrospective study analysed the impact of different characteristics at diagnosis on evolution to symptomatic disease. We reviewed our electronic database, 255 diagnosis of SMM were made at the Hematology Unit of AOU Careggi, Florence from 01/01/2003 to 31/12/2018. Inclusion criteria were having at least 10% clonal PC in the bone marrow and/or serum monoclonal component (MC) ≥ 3 gr/dl. Progression of SMM was defined as development of organ damage attributable to PC dyscrasia (CRAB or Myeloma Defining Events for patient with a diagnosis after 2014), initiation of therapy for MM or development of amyloidosis. Time to progression (TTP) was calculated from SMM diagnosis to progression or at the date of last follow-up. 55 patients were excluded from the analysis due to insufficient clinical data. Over an estimated median follow-up period of 54 months (range 2-191 months), progression occurred in 70 (35%) patients; the median TTP was 35 months (range 2-191 months). In the univariate analysis significant risk factors included: presence of urinary MC [HR: 0.4 (CI, 0.2-0.7); $p=0.009$], serum MC level ≥ 3 gr/dl [HR: 0.09 (CI, 0.04-0.18); $p<0.0001$], MC evolution [HR: 0.24 (CI, 0.1-0.4); $p<0.0001$], presence of immunoparesis [HR: 0.6 (CI, 0.37-0.98); $p=0.041$], FLC ratio <0.125 or >8 [HR: 0.28 (CI, 0.15-0.51); $p<0.0001$], bone marrow infiltration (BMPC%) $>25\%$, -cutoff defined by ROC curve analysis- [HR: 0.26 (CI, 0.15-0.47); $p<0.0001$]. Other factors, such as sex, age, prior MGUS, MC and light chain isotype and high-risk cytogenetics abnormalities were not significantly associated with progression. BM flow cytometry was performed in 84 patients, 40% had more than 95% of aberrant PC and a significantly higher relative risk of progression [RR: 2,3 (CI, 1.1-5.1); $p=0.01$] without a difference in terms of TTP (62 vs 63 months). BMPC% $> 25\%$ [HR: 2.9 (CI, 1.1-7.5); $p=0.024$]; MC ≥ 3 g/dL [HR: 9.1 (CI, 3.1-26.8); $p<0.0001$] and MC evolution [HR: 3.8 (CI, 1.5-9.4); $p<0.004$] independently predicted shorter TTP in multivariate analysis. Few laboratory data, at diagnosis and over a short follow-up, can be used to identify patients with high-risk SMM who need close monitoring.

P040

THE ROLE OF CHEMOTHERAPY IN THE ERA OF NEW DRUGS: A MONOCENTRIC EXPERIENCE ON ADVANCED PHASE MULTIPLE MYELOMA PATIENTS TREATED WITH CYCLOPHOSPHAMIDE

F. Massaro, E. Rivolti, F. Soci, M. Quaresima, L. Tognazzi, B. Gamberi, F. Merli

Hematology, AUSL-IRCCS Reggio Emilia, Italy

Multiple myeloma (MM) is invariably characterized by disease progression. The use of proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) or monoclonal antibodies (MAbs), especially when is not allowed a three-drug combination, is not frequently associated to durable responses, sometimes representing an unbalanced cost-effectiveness solution. Cyclophosphamide is an alkylating agent which has been widely used in myeloma patients, especially for peripheral blood mobilization of hematopoietic stem cells. Several reports describe its activity as single agent also at lower doses and in advanced phases of disease. We report our single center experience in 30 relapsed MM patients treated with intravenous cyclophosphamide (1.2 g/sqm on days 1 and 3 and dexamethasone 40 mg/day from day 1 to day 4, every 4 weeks) from September 2009 to March 2018. The median age was 67 years (range 52-87), with a median time from diagnosis to cyclophosphamide treatment of 50 months (range 11-140). Patients population had received a median number of 3 prior lines of treatment (range 2-8) and particularly 97% had received bortezomib, 90% lenalidomide and

63% at least one ASCT. The median best progression-free survival (PFS) achieved by patients prior to cyclophosphamide treatment was of 25 months (range 4-57). We recorded an overall response rate (ORR) of 60% and particularly 27% of partial response (PR), 30% of very good partial response (VGPR) and 3% of complete response (CR), with a median time to best response of 3 months (range 2-7). A stable disease (SD) was maintained in 33% of patients while disease progression (PD) was reported in 7% of cases. Median PFS and overall survival (OS) were 7 months (range 2-31) and 8 months (range 2-51) respectively. Interestingly, 4/30 patients (7%) experienced a better or equal PFS time if compared to best PFS achieved before cyclophosphamide treatment. Concerning safety profile, we reported 53% of grade 3-4 infections, mostly febrile neutropenia and pneumonia. Five patients (17%) had treatment interruption not related to disease progression: in 4 cases, it was related to hematological toxicity while one case was associated to death due to grade 5 infection. Despite the availability of novel drugs, the achievement of durable responses in advanced phases of MM still represents a challenging goal. Patients experiencing relapses after PIs and IMiDs could still be sensitive to alkylating agents. Cyclophosphamide could represent a feasible solution, related to a satisfactory disease control and a favourable cost-effective profile.

Myeloproliferative Neoplasms 1

P041

HISTOPATHOLOGICAL AND CLINICAL ANALYSIS IN MYELOPROLIFERATIVE NEOPLASMS, UNCLASSIFIABLE (MPN-U): A NEW DIAGNOSTIC CHALLENGE

A.G. Giannone, P. Tralongo, A. Mondello, P. Ortenzio, S. Bellavia, P. Lohneis, R. Büttner, A.M. Florena

Anatomia patologica, Università degli Studi di Palermo, Institut für Pathologie, Universität zu Köln

Myeloproliferative Neoplasm, unclassifiable (MPN-U) underpins a heterogeneous group of chronic myeloid neoplasms in which there is a clear evidence of myeloproliferative neoplasms (MPN) features without meeting all the necessary diagnostic criteria for a specific subset of MPN. Nowadays, in most cases it is possible to provide an early mutational screening, so that the subsequent bone marrow biopsies show ambiguous clinical and morphological features due to their precocious identification. The aim of our study was to adopt supplementary morphological and clinical criteria for a better evaluation of the evolution or identification of these cases. In our investigation we collected 25 cases of MPN-U from the Institutes of Pathology of the University of Palermo, Italy, and University of Cologne, Germany within a timespan from 2012 to 2018, examining scrupulously their molecular, clinical, and morphological patterns. Among the major abnormalities we found out a hypercellular bone marrow (72%) with quantitative increased megakaryopoiesis (76%), increased granulopoiesis (60%), normal erythropoiesis (48%), normal myeloid/erythroid ratio (48%), signs of dysmegakaryopoiesis, and an absent or mild reticulin/collagen fibrosis. Anemia and organomegaly were the most common clinical alterations; none of our cases showed recurrent infections. The mutational state confirmed the JAK2 V617F mutation in 60% of cases, while among the JAK2-negative cases a triple-negative state and an unusual NPM-1 mutation were observed respectively in 16% and 8%. Finally, we adopted a specific analytic model with the aim to identify different nosographic subsets with histological features reliable enough to address the diagnosis toward one of the most common Philadelphia-negative MPN. In conclusion, we could state that this proposal for a novel classification of MPN-U could be useful not only for a better histopathological categorization, but also to provide a possible first therapeutic approach for these entities.

References

- Gianelli U, Cattaneo D, Bossi A, Cortinovis I, Boiocchi L, Liu Y-C, et al. The myeloproliferative neoplasms, unclassifiable: clinical and pathological considerations. *Modern Pathology*. 2016;30(2):169-79.
- Gianelli U, Bossi A, Cortinovis I, Sabattini E, Tripodo C, Boveri E, et al. Reproducibility of the WHO histological criteria for the diagnosis of Philadelphia chromosome-negative myeloproliferative neoplasms. *Modern Pathology*. 2013;27(6):814-22.

P042

IMPACT OF VARIANT ALLELE FREQUENCY (VAF) OF JAK2V617F AND CALR MUTATIONS ON THROMBO-HEMORRHAGIC RISK IN ESSENTIAL THROMBOCYTHEMIA

I. Bertozzi, A.M. Lombardi, G. Bogoni, T. Vezzano, F. Fabris, M.L. Randi

Dipartimento di Medicina – DIMED, Università di Padova, Italy

In Essential Thrombocythemia (ET), the most common causes of morbidity and mortality are thrombotic and hemorrhagic complications. The presence of JAK2V617F, CALR and MPL mutations in ET can differently impact on thrombo-hemorrhagic risk. JAK2 mutation is a well-known risk factor for thrombosis and higher VAF of JAK2 correlates with increased risk of thromboses and hemorrhages in Myeloproliferative neoplasms (MPN). No data are available about impact of CALR VAF on thrombo hemorrhagic risk in ET. Here we compare the impact of JAK2 and CALR VAF on thrombo hemorrhagic complications in a large cohort of patients with ET. We selected 167 consecutive ET patients [48 M, 119 F, mean age 52.9±16.8 y, 129 JAK2 (77%) and 38 CALR (23%)] followed for median 9.1 y (0.1-39), in which VAF has been determined. Complete medical history and anti-thrombotic drugs use were recorded. 34 (26.3%) JAK2 and 1 (2.6%) CALR patients had

a thrombosis at diagnosis, while 27 (20.9%) JAK2 and 5 (13.1%) CALR developed thrombotic complication during follow-up. JAK2 patients with thrombosis had a significantly higher median VAF (22, 3-97) compared to those without thrombotic events (19, 2-89; $p=0.001$); no difference has been observed in median VAF among CALR patients with (46, 37-49) or without (39.5, 11-49) thrombotic complications ($p=ns$). Only 1 (0.8%) JAK2 and no CALR patients bleed at diagnosis. 11 (8.5%) JAK2 and 6 (15.8%) CALR patients suffered for hemorrhages during follow-up. JAK2 hemorrhagic patients carry significantly higher VAF compared with those without hemorrhagic complication ($p=0.016$). Median VAF of CALR patients with or without hemorrhage was not statistically different ($p=ns$). In multivariable analysis, VAF of CALR has no impact on thrombo-hemorrhagic risk while higher JAK2 VAF is confirmed as an independent risk factor both for thrombosis and hemorrhages during follow-up, in separated Cox regression models adjusted respectively for age plus presence of cardiovascular risk factors ($p=0.009$) and antithrombotic drugs use ($p=0.05$). In ET, mutational status influences thrombo hemorrhagic profile and JAK2 positive MPN patients with higher VAF carry high thrombo hemorrhagic risk. In this study, we confirm a direct correlation between higher JAK2 VAF values in ET and increased risk of bleeding and thrombosis during follow-up. On the contrary, we fail to demonstrate a role of CALR VAF on thrombo hemorrhagic risk of ET patients.

P043

OBSERVATIONAL EPIDEMIOLOGIC REGISTRY OF POLYCYTHEMIA VERA IN LATIUM FROM 2011 TO 2015, AN UP TO DATE

M. De Muro¹, A. Di Veroli², M. Montanaro², R. Latagliata³, C. Santoro³, M. Breccia³, M. Cedrone⁴, B. Anaclerico⁴, M. Trawinska⁵, E. Abruzzese⁵, A. Rauco⁶, S. Leonetti-Crescenzi⁴, N. Villivà⁷, G. Cimino⁸, A. Romano⁹, A. Spadea⁹, E. Rossi¹⁰, V. De Stefano¹⁰, L. Petriccione¹¹, A. D'Addosio¹², L. Maurillo¹³, G. Mazzucconi³, A. Andriani¹¹

¹Department of Hematology, Stem Cell Transplantation, Campus Bio-Medico University Hospital, Rome, Italy; ²Department of Hematology, Belcolle Hospital, Viterbo, Italy; ³Department of Cellular Biotechnology and Hematology, University of Rome "La Sapienza", Italy; ⁴Department of Hematology, S. Giovanni Addolorata Hospital, Rome, Italy; ⁵Department of Hematology S. Eugenio Hospital, Rome, Italy; ⁶ Department of Oncology San Camillo De Lellis Hospital, Rieti, Italy; ⁷Department of Hematology, ASL Roma1, Italy; ⁸Hematology Unit (2U) ICOT/S, Maria Goretti Hospital, AUSL Latina, Italy; ⁹Department of Hematology, Regina Elena National Cancer Institute, Rome, Italy; ¹⁰Department of Hematology, Università Cattolica del Sacro Cuore, Agostino Gemelli University Hospital, Rome, Italy; ¹¹Department of Hematology, Fabrizio Spaziani Hospital, Frosinone, Italy; ¹²Department of Hematology, Fatebenefratelli Villa S. Pietro Hospital, Rome, Italy; ¹³Department of Hematology, University "Tor Vergata", Rome, Italy

Background: Polycythemia Vera (PV) is a Ph negative Chronic Myeloproliferative Neoplasm (Ph-MPNs) which features are dominated by myeloproliferation (erythrocytosis, often leucocytosis and/or thrombocytosis) and a tendency to thrombosis and transformation to myelofibrosis or acute myeloid leukaemia. The pathophysiology of this condition has been defined as JAK/STAT pathway activation, almost always due to mutations in JAK2 exons 12 or 14 (JAK2 V617F). In the present report, we aimed to evaluate the incidence and the general features at presentation of PV patients in the Latium area (approximately six millions of residents) across a 5-year period of prospective observation.

Method: The present epidemiologic analysis was conducted in 17 hematological Centers (5 academic/12 community-based Hospitals) in Latium, collecting data at onset in all newly diagnosed PV patients aged > 18 years from January 2011 to December 2015. Diagnosis of PV was made according to 2008 WHO and revised 2016 WHO criteria.

Results: In the 5-year period of observation, 339 adults received a new diagnosis of PV. The overall incidence rate was 1.1/10⁵ regional residents in 2011, 1.2/10⁵ in 2012, 1.3/10⁵ in 2013, 1.0/10⁵ in 2014 and 0.9/10⁵ in 2015. Baseline patient features are summarized in the Table 1. We analyzed the incidence of the main thrombotic risk factors at diagnosis: diabetes, dyslipidemia, smoke attitude, essential hypertension and thrombophilia were observed in 7.9%, 11%, 21.5%, 25.7% and

1.4% of the patients, respectively. Among other more common comorbidities observed at diagnosis, arrhythmia and a previous or co-existing neoplasia were reported in 3.8% and 4.7% of the patients, respectively. At a minimum follow-up period of 36 months, all patients started antithrombotic prophylaxis with aspirin (79.8%), oral anticoagulants (4.2%) or other drugs (clopidogrel, ticlopidine) (16.0%). A cytoreductive treatment was started in 240 patients (70.9%) [204/240 patients with hydroxyurea (85.2%) and 36/240 (14.8%) with other drugs]: the remaining 99 patients (29.1%) were managed with phlebotomies only. 8 patients (2.3%) has developed a thrombotic event during follow up.

Conclusions: Preliminary data of this prospective epidemiologic study in a real-life unselected population of PV patients confirm the overall incidence of PV previously reported in others European cancer registries, with an annual incidence in our region relatively stable during the observational period.

Table 1. Baseline features in 339 PV observed in the Latium area from Jan 2011 to Dec 2015.

PV	
M/F, n°	193/146
Median Age, yrs	67.7 (24 – 87.5)
Median WBC count, x 10 ⁹ /l	9.70 (4.6 – 25.8)
Median PLTs count, x 10 ⁹ /l	464 (154 – 965)
JAK-2 V617F pos, %	93
Spleen enlargement > 5 cm, %	4.8
Liver enlargement >5 cm, %	1.3
Previous thrombosis, %	16.6
Symptoms:	
• Pruritus, %	7.1
• Astenia, %	8.6
• Headache, %	4.0
• Vascular, %	4.5
• No symptoms, %	75.8

P044

REAL WORLD DATA ON RUXOLITINIB (RUX) IN MYELOFIBROSIS (MF): 6 MONTHS (MOS) INTERIM ANALYSIS OF ITALIAN ROMEI STUDY(CINCA24AIT04).

M. Breccia¹, P. Guglielmelli², A. Malato³, F. Mendicino⁴, G.A. Palumbo⁵, C. Selleri⁶, D. Cilloni⁷, P. Mazza⁸, S. Siragusa⁹, E. Abruzzese¹⁰, R. Latagliata¹, M. Crugnola¹¹, D. Pastore¹², G. Micucci¹³, G. Benevolo¹⁴, M. Bonifacio¹⁵, G. Carli¹⁶, F. Pane¹⁷, V. Pavone¹⁸, M. Morelli¹⁹, F. Palandri²⁰, F. Passamonti²¹ in behalf of ROMEI Study Group

¹Department of Biotechnologies and cellular Hematology-Sapienza Università; ²CRIMM, Center for Research and Innovation of Myeloproliferative Neoplasms, AOU Careggi, Department of Experimental and Clinical Medicine, University of Florence; ³UOC di Ematologia con UTMO, Ospedali Riuniti Villa Sofia-Cervello; ⁴U.O.C. di Ematologia, Azienda Ospedaliera "Annunziata"; ⁵Dipartimento di Scienze Mediche Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia", Università degli Studi di Catania; ⁶UOC di Ematologia e Trapianti di Cellule Staminali Emopoietiche, AOU San Giovanni di Dio e Ruggi D'Aragona; ⁷University of Turin; ⁸C/O Ospedale Moscati Dept. of Hematology-Oncology; ⁹UOC di Ematologia, Azienda Ospedaliero-Universitaria Policlinico "P. Giaccone"; ¹⁰Hematology Unit, S.Eugenio Hospital, Tor Vergata University; ¹¹Hematology and BMT Unit, "Azienda Ospedaliero-Universitaria di Parma"; ¹²Hematology, A. Perrino Hospital; ¹³Clinic of Hematology, AOU-Ospedali Riuniti Umberto I; ¹⁴Dipartimento di Oncologia e Ematologia, A.O.U. Città della Salute e della Scienza di Torino; ¹⁵Department of Medicine, Section of Hematology, University of Verona; ¹⁶Hematology Department, Ospedale San Bortolo; ¹⁷Azienda Ospedaliera Universitaria Federico II di Napoli Università; ¹⁸Haematology, "G. Panico" Hospital; ¹⁹Novartis Oncology, Novartis Farma SpA; ²⁰Department of Hematology/Oncology, "Seràgnoli" Institute of Hematology, University of Bologna School of Medicine; ²¹Università degli Studi dell'Insubria, Italy

ROMEI is a prospective, observational study involving 42 Italian sites focused on quality of life, disease burden and safety of MF patients (pts)

treated with RUX. Symptoms and quality of life were evaluated at baseline and after 6 mos of therapy by the Myeloproliferative Neoplasm (MPN)-10 and -7, and the EQ-5D-5L general health questionnaires, respectively. Out of 219 screened pts, 215 have been enrolled. At data cutoff (01/2019), 214 pts entered the 6-mos interim analysis; 117 were male, median age 67 years (range 24-86), 170 were at Int-2 or High IPSS risk. Median time from MF diagnosis to RUX start was 5.3 mos, with 69% starting therapy within 2 years from diagnosis. Mean MPN-10 total score improved from 34.86 (range 0-88) at baseline to 21.55 (range 0-70) at 6 mos, with mean change equal to -13.31. Similarly, mean MF-7 total score improved from 26.91 (range 0-70) to 16.68 (range 0-40). Conversely, mean EQ-5D-5L index values were stable during the observation period. Almost all pts had some degree of reduction in spleen length as shown in Figure 1. The proportion of evaluable pts with reduction $\geq 50\%$ in palpable spleen length increased at each visit: from 0.37 at wk4 to 0.63 at wk24 and percentage of change in spleen length from baseline to 6 mos was -48.35%. Mean RUX baseline dosage was 29.80 mg; 134 pts had at least one change of dose during treatment. Thirty-one (15%) pts had at least one temporary interruption and 28 pts (13%) had a permanent discontinuation: the main reasons were allogeneic stem cell transplantation (21%), lost to follow-up (18%), death (14%) and adverse events (AEs, 14%). AEs were reported for 160/211 evaluated pts (76%); 114 pts had at least one drug-related AEs and, similar to what reported in literature, the most common drug related AEs were anemia (all grade (G) 50%, G3-4 9,5%) and thrombocytopenia (all G 35%, G3-4, 6%). Overall, 3/211 pts (1.4%) had Herpes Zoster infection (max G2), 4 (1.8%) had urinary tract infection (3 of G2 and one of G1) and 5 pts (2.3%) had pneumonia (1of G2, 2 of G3 and 2 of G5); no tuberculous infections were reported. Six pts died: 1 of disease progression, 1 of gastrointestinal carcinoma, 2 of pneumonia, 1 of septic shock and 1 of unknown reason. Overall, these 6 mos results confirmed the beneficial effects of RUX on symptoms and spleen responses, comparably to those observed in the expanded-access JUMP trial. The safety profile of RUX as reported in the ROMEI study is aligned with previous reports.

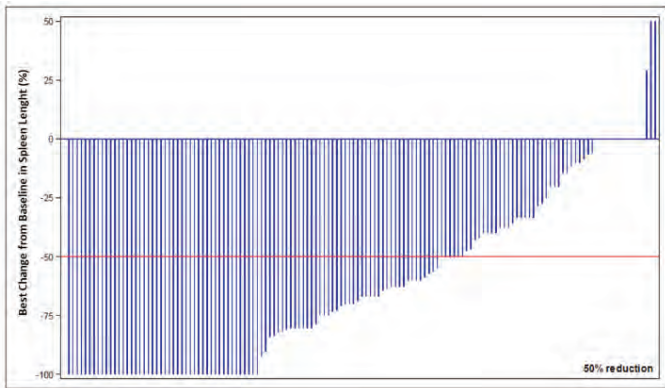


Figure 1.

P045

EPIDEMIOLOGIC UPDATE OF ESSENTIAL THROMBOCYTHEMIA IN THE PROSPECTIVE OBSERVATIONAL REGISTRY OF THE LATIUM REGIONAL NETWORK FROM 2011 TO 2015

A. Di Veroli¹, M. De Muro², M. Montanaro¹, R. Latagliata³, C. Santoro³, M. Breccia³, M. Cedrone⁴, B. Anaclerico⁴, M. Trawinska⁵, E. Abruzzese⁵, A.M. Rauco⁶, S. Leonetti-Crescenzi⁴, N. Villivà⁷, G. Cimino⁸, A. Romano⁹, A. Spadea⁹, E. Rossi¹⁰, V. De Stefano¹⁰, L. Petriccione¹¹, A. D'Addosio¹², R. Porrini¹³, F. Spirito¹⁴, L. Maurillo¹⁵, G. Mazzucconi³, A. Andriani¹¹

¹Department of Hematology, Belcolle Hospital, Viterbo, Italy; ²Department of Hematology, Stem Cell Transplantation, and Cellular Therapy, Campus Bio-Medico University Hospital, Rome, Italy; ³Department of Cellular Biotechnology and Hematology, University of Rome "La Sapienza", Italy; ⁴Department of Hematology, S. Giovanni Addolorata Hospital, Rome, Italy; ⁵Department of Hematology S. Eugenio Hospital, Rome, Italy; ⁶Department of Oncology San Camillo De Lellis Hospital, Rieti, Italy; ⁷Department of Hematology, ASL Roma1, Italy; ⁸Hematology Unit (2U) ICOT/S, Maria Goretti Hospital, AUSL

Latina, Italy; ⁹Department of Hematology, Regina Elena National Cancer Institute, Rome, Italy; ¹⁰Department of Hematology, Università Cattolica del Sacro Cuore, Agostino Gemelli University Hospital, Rome, Italy; ¹¹Department of Hematology, Fabrizio Spaziani Hospital, Frosinone, Italy; ¹²Department of Hematology, Fatebenefratelli Villa S. Pietro Hospital, Rome, Italy; ¹³Department of Hematology, Sant'Andrea Hospital, Rome, Italy; ¹⁴Department of Hematology, San Camillo Hospital, Rome, Italy; ¹⁵Department of Hematology, Tor Vergata University Hospital, Rome, Italy

Background: Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm (MPN) characterized at diagnosis by thrombocytosis (PLTs > 450x10⁹/l). In the follow-up, ET is associated with thrombo-hemorrhagic complications and transformation to myelofibrosis (MF) and/or acute myeloid leukemia (AML). To date, however, available epidemiological studies and prospective analysis of ET patients in a real-life context are rare in Italy. In the present report, we aimed to evaluate the incidence and the general features at presentation of ET patients in the Latium area, which accounts for approximately six millions of residents, across a 5-year period of prospective observation.

Method: The present epidemiologic analysis was conducted in 17 hematological Centers (5 academic and 12 community-based Hospitals) in Latium, collecting data at onset in all newly diagnosed ET patients aged > 18 years from January 2011 to December 2015. Diagnosis of ET was made according to 2008 WHO criteria.

Results: On the whole, in the 5-year period of observation, 627 adults received a new diagnosis of ET. The overall incidence rate was 2.2/105 regional residents in 2011, 2.6/105 in 2012, 2.3/105 in 2013, 1.9/105 in 2014 and 1.2/105 in 2015. Baseline patient features are summarized in the Table. We also analyzed the incidence of the main thrombotic risk factors at diagnosis: diabetes, dyslipidemia, smoke attitude, essential hypertension and thrombophilia were observed in 7%, 4%, 12%, 19.5% and 3.5% of the patients, respectively. Among other more common comorbidities observed at diagnosis, arrhythmia and a previous or co-existing neoplasia were reported in 4.9% and 2.8% of the patients, respectively. At a minimum follow-up period of 36 months, 552 patients (88.1%) started antithrombotic prophylaxis and 484 patients (77.3%) started cytoreductive treatment: the different approaches are reported in the Table 1. 12 patients (1.9%) has developed a thrombotic event during follow up.

Conclusions: Preliminary data of this prospective epidemiologic study in a real-life unselected population of ET patients confirm the overall incidence of ET previously reported in others European cancer registries, with an annual incidence in our region relatively stable during the observational period. A longer follow-up is needed to correlate baseline features to different endpoints during ET course.

Table 1. Baseline features in 627 ET observed in the Latium area from Jan 2011 to Dec 2015.

ET	
M/F, n°	240/387
Median Age, yrs (range)	65.1(29 – 91.5)
Median WBC count, x 10 ⁹ /l (range)	8.60(4.4 – 20.1)
Median PLTs count, x 10 ⁹ /l (range)	700(448 - 1839)
#JAK-2 ^{V617F} pos, %	66
#CALR pos, %	29
Spleen enlargement > 5 cm, %	2
Liver enlargement >5 cm, %	0.7
Previousthrombosis, %	17
Symptoms:	
• Vascular symptoms, %	4.6
• No symptoms, %	95.4
Cytoreductive Therapy:	
• HU alone, %	72.3
• Anagrelide, %	2.5
• INF alfa, %	2.5
• No cytoreductivetherapy, %	22.7
Antithrombotic prophylaxis:	
• Aspirin, %	71.6
• Oral Anticoagulants, %	4.0
• Other (Clopidogrel, ticlopidine, other), %	12.5
• No prophylapxys, %	11.9

P046

MULTICENTER SURVEY ON MANAGEMENT AND OUTCOME OF RARE AND UNPREDICTABLE ORPHAN DISEASES: ADULT ONSET HISTIOCYTOSIS.

D. Lazzarotto¹, A. Candoni¹, L. Melillo², M. Criscuolo³, M. Dargenio⁴, F. Forghieri⁵, P. Minetto⁶, F. Vianello⁷, D. Capelli⁸, M.E. Zannier¹, C. Fili¹, R. Fanin¹

¹Division of Hematology and Stem Cell Transplantation, Azienda Sanitaria Universitaria Integrata di Udine; ²Division of Hematology, IRCCS Casa Sollievo della Sofferenza di San Giovanni Rotondo; ³Division of Hematology, Policlinico Gemelli; ⁴Section of Hematology, Vito Fazzi Hospital; ⁵Section of Hematology, Department of Surgical and Medical Sciences, University of Modena and Reggio Emilia; ⁶Division of Hematology, Policlinico San Martino, University of Genova; ⁷Hematology and Immunology Unit, Department of Medicine, University of Padova School of Medicine; ⁸Division of Hematology, Azienda Ospedaliero-Universitaria Ospedali Riuniti di Ancona, Italy

Introduction: Histiocytoses are diseases that mainly affect children. Adult onset Histiocytoses (AH) are very rare and their management is mainly derived from small retrospective studies, expert opinions and the pediatric experience. The history of the disease is widely variable and often unpredictable in the single patient. This is a multicenter survey on presentation, management and outcome of AH in the last 16 years (2002-2018).

Patient and Results: We report the data regarding 22 patients (pts) affected by AH. Eighteen/22 (82%) were affected by Langerhans Cells Histiocytosis, 2 (9%) by Rosai-Dorfman Disease and 2 (9%) by Histiocytic Sarcoma. Median age at diagnosis was 42 (range 18-67). Seventeen/22 (77%) pts presented with growing tumor mass symptoms and 8/22 (36%) with systemic aspecific symptoms. BRAF mutation was tested in 8/22 pts (the most recent) but none of them was positive. Eight/22 (36%) pts presented with Single Site involvement (3 bone, 3 lymph nodes, 1 CNS, 1 lung). The three pts with bone involvement were treated with surgery alone, while the others with a combination of surgery and systemic therapy (mean of 1.4±0.7 therapy lines). The 5-year OS of these pts is 80%, but 63% had an active disease at last follow up. Fourteen/22 (64%) pts presented with Multiple Sites involvement (bone, lymph nodes, lung the main involved organs). All these pts received systemic therapy (mean of 2.7±2.3 therapy lines) combined with surgery in 3 pts and radiotherapy in 2 pts. The best response achieved was a CR in 4/14 pts (28,5%) and a PR in 6/14 (43%) pts; 4/14 (28,5%) were refractory to all treatments. The 5-year OS of these pts is 77% but 79% had an active disease at last follow up. Overall, 15/22 (68%) patients achieved at least a PR, but long-term PFS was achieved in only 25% of them. Also, 4/18 surviving pts developed irreversible organ damage (pulmonary emphysema, chronic pancreatitis, persistent diabetes insipidus, severe bone lesions).

Conclusions: AH are chronic diseases in which the achievement of a complete and durable response with the current treatments is unusual. Pts live together with their disease, its complications and the side effects of multiple therapies. Further studies are needed to discover other molecular alterations besides BRAF V600E (that is not always found in AH forms) that can be targeted by specific therapies, to improve the response rates and allow pts to live free from the disease and its related complications.

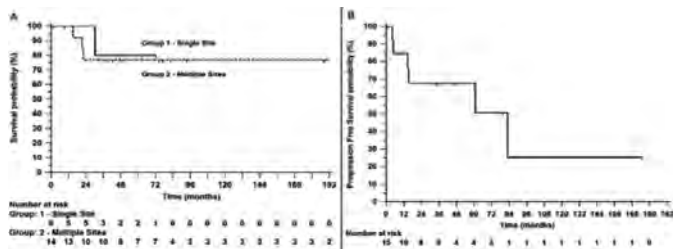


Figure 1.

P047

THE NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) IS A CHEAP SURROGATE OF EXPANSION OF MYELOID-DERIVED SUPPRESSOR CELLS IN MYELOFIBROSIS AND MAY PREDICT RESPONSE TO RUXOLITINIB

A. Romano¹, N.L. Parrinello^{2,3}, C. Giallongo³, F. Puglisi³, D. Cambria¹, G. Camiolo³, D. Tibullo⁴, E. Lambusta², F. Di Raimondo³, G.A. Palumbo^{2,3}

¹Department of Surgery and Medical Specialties, University of Catania; ²Division of Hematology - AOU Policlinico Vittorio Emanuele di Catania; ³Dipartimento di Scienze mediche chirurgiche e tecnologie avanzate - University of Catania; ⁴Biometec - Dipartimento di Scienze Biomediche e Biotecnologiche - University of Catania, Italy

Ruxolitinib (Ruxo) is the first-in-class Jak1/2 inhibitor recently approved for treatment for myelofibrosis (MF). Myeloid-derived suppressor cells (MDSC) are important regulators of immune responses in cancer, poorly investigated in MF. Recent reports have identified NLR, the ratio between absolute neutrophils count (ANC) and absolute lymphocyte count (ALC), as biomarker of myeloid-related immune suppression in cancer. To investigate NLR and MDSC in MF patients undergoing treatment with Ruxo, we evaluated in peripheral blood of 40 healthy volunteers and 52 consecutive MF patients NLR and MDSC by flow cytometry and functional essays. Findings were correlated to clinical outcome, including spleen response, time to progression (TTP) to acute myeloid leukemia (AML) and overall survival (OS)

Results: PMN-MDSC were higher in MF than controls ($p < 0.001$) and showed increased p-STAT3 and p-STAT5 ($p < 0.001$), markers of activation in myeloid suppressor cells. Accordingly, immune-suppressive cytokines IL-6, IL-8, TGF-beta and aminoacid-degrading enzymes Arg-1 and IDO-1 were significantly increased ($p < 0.001$). After 12-weeks treatment with ruxolitinib PMN-MDSC and p-STAT5 reduced to values measurable in healthy subjects ($p = 0.003$), but not p-STAT3. NLR was increased upon progression of disease and positively associated to PMN-MDSC percentage (r-square 0.52, $p = 0.003$) and to fibrosis grading as detected by immune-histochemistry. Patients with lower bone marrow fibrosis had lower median NLR (grade 0: 4.1, G1: 4.8, G2: 5.2, G3: 8.1, $p < 0.001$), while cases positive for JAK2V617F had increased NLR ($p = 0.002$). Receiving operator curve identified NLR=6 as cut-off to distinguish healthy and MF subjects with 97 % sensitivity and 89% specificity (AUC=0.92, $p < 0.001$), and it was applied for further evaluations. Patients with NLR>6 before starting Ruxo had a lower probability of a spleen reduction more than 50% at 12 weeks ($p = 0.001$) and 100% at 24 weeks ($p = 0.001$). However, after a median follow-up of 30.2 months, NLR at diagnosis was not predictor of neither TTP nor OS.

Conclusions: In MF, PMN-MDSC are increased and positively associated to NLR, mutational status and bone marrow fibrosis. NLR is a useful, simple and early predictor of response and spleen reduction in patients treated with Ruxo, despite it does not have any impact on TTP and OS.

P048

SYSTEMIC MASTOCYTOSIS WITH ASSOCIATED HEMATOLOGICAL NEOPLASM: SINGLE CENTER EXPERIENCE

M. Criscuolo¹, L. Fianchi¹, A.M.E. Maraglino², L. Pagano²

¹Dipartimento Scienze Radiologiche Radioterapiche ed Ematologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS; ²Dipartimento Scienze Radiologiche Radioterapiche ed Ematologiche, Fondazione Policlinico A. Gemelli IRCCS-Università Cattolica del Sacro Cuore, Italy

The WHO classification recognizes cutaneous (CM) and systemic mastocytosis (SM), further divided in indolent SM (ISM), aggressive SM (ASM) and SM associated to another clonal hematological disease (SM-AHN). Among 50 patients with mastocytosis, including 8 CM, 17 ISM and 13 ASM, we collected clinical and laboratory data of 12 patients diagnosed with SM-AHN in the last 13 years. The M/F ratio was 1, with a median age of 74 years (range 44-89). All patients performed bone marrow evaluation for cytopenia: 2 had acute myeloid leukemia (AML), 8 myelodysplastic syndrome (MDS), 1 multiple myeloma (MM) and 1 lymphoproliferative disease (LPD). The main reported symptoms were diarrhea (4, 33.3%), skin lesions with urticaria and/or pruritus (3, 25%), fever and fatigue (4, 33.3%). 1 patient (8.3%) presented bone os-

teolysis due to concomitant diagnosis of MM. Median basal tryptase levels was 15.8 (range 10.3-1310). C-KIT D816V mutation was detected in 4/12 tested patients only (33.3%). 2 elderly female patients (16.7%) were diagnosed with AML: they presented the highest basal tryptase levels and C-KIT D816V mutation. They were treated with intermediate dose citarabine plus dasatinib and died after 2.2 and 3.8 months respectively. 8 patients (66.6%) were diagnosed with MDS (7 lower and 1 higher risk): the M/F ratio was 5/3, with a median age of 74 (range 59-89). 3 patients (25%) had skin lesions and urticaria. Median basal tryptase level was 14.1 (range 10.3-200), C-KIT D816V was positive in 2 cases. 5 patients received best supportive care and 1 received azacytidine. In 2 cases a mastocytosis-oriented treatment was started: 1 was treated with dasatinib with no response, 1 achieved durable response with IFN. Median overall survival was 25.6 months (range 1-99.1). 2 patients (16.7%) were diagnosed with LPD: 1 is receiving chemotherapy for associated MM and 1 with low-grade LPD is in watchful follow-up. C-KIT D816V was negative in both cases. The median overall survival of the whole population was 5 months with a wide range (1-99). SM-AHD seems to be mainly associated to myeloid neoplasms. In most cases mastocytosis does not require specific treatment: the associated malignancy is treated considering mastocytosis as a concomitant manifestation. Anaphylaxis, skin lesions and gastrointestinal symptoms are reported in about 30% of cases. Positivity of C-KIT D816V and level of tryptase widely vary among patients. Therapy and outcome depend from the predominant disease

P049

SPLEEN VOLUME EVALUATION BY ULTRASOUND EVALUATION IS A VALID SUBSTITUTE OF MAGNETIC RESONANCE IN MYELOPROLIFERATIVE NEOPLASM. CAN WE COUNT ON IT?

N. Pugliese, R. Malafronte, C. Giordano, R. Della Pepa, F. Fanelli, G. Delle Cave, S. Vitiello, A. Leone, G. Piccolo, F. Pane, V. Martinelli, M. Picardi

Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi Di Napoli Federico II, Italy

Introduction: Splenomegaly is a common feature of chronic myeloproliferative neoplasms (MPN): in 30% of Polycythemia Vera (PV), in 5%-20% of Essential Thrombocythemia (ET) and in more than 50% in Primary Myelofibrosis (PMF) patients. Spleen physical examination lacks both accuracy and reliability thus IWGMRT and ELN experts recommend response assessments in MPN by MR. This study evaluated whether US and MR SV measurements are comparable in MPN patients.

Materials and Methods: MPNs patients undergoing ruxolitinib treatment were recruited in this prospective study and received both US and MR before ruxolitinib (baseline) and at 32 weeks of treatment. Spleen LD and volume US assessment was performed as described by Picardi et al. (Blood. 2002;99(11):4228-30), using EPIQ 5 Philips with a 1-5 MHz broadband curvilinear probe. MR calculated SV using a computer-assisted method based on Sorensen et al. (J Clin Oncol. 2001;19(2):551-7), as described in RESPONSE study. SV was obtained by outlining the circumference of the organ and determining the volume using the validated technique of last squares. Patients received a satisfaction questionnaire regarding both exams (Clin Radiol. 1995;50(3):137-43). US volume estimations were compared to those of MR at baseline and during follow-up using Sperman's rank correlation (ρ).

Results: MPN diagnosis were 14 PV, 9 pre-MF, 9 overt MF, 4 post-PV MF and 4 post-ET MF. All patients received scheduled treatment with ruxolitinib. At baseline, the median US SV was 600 ml (range 200-5000 ml) while median SV at MR was 553.1 ml (range 172-5140 ml). The median US LD was 14 cm (range 8-30 cm) whereas median MR-measured LD was 15 cm (7-31 cm). At week 32, the median SV at US was 415 ml (range 130-4400 ml) while median SV at MR was 380.2 ml (range 111-4790 ml). There was a good correlation between US and MR volume measurements with spearman ρ of 0.957 (CI 95%: 0.918-0.978; p value <0.0001), between US and MR splenic length (spearman ρ of 0.906, CI 95%: 0.824-0.951; p value <0.0001) and between US and MR SV at week 32 (ρ 0.943, p value <0.0001). The questionnaires showed a greater appreciation for US in terms of exam duration and anxiety.

Conclusions: MPNs diagnosis need baseline SV evaluation. Significant spleen reduction responses of ruxolitinib in MF and PV have been as-

sessed by MR but is not easily applicable in routine clinical practice due to cost and exam duration. SV assessment by US is cheap, rapid and a positively accepted substitute for diagnosis and SV response assessment in patients treated with ruxolitinib.

P050

BONE MARROW MESENCHYMAL STEM CELLS AS FACILITATOR OF RUXOLITINIB-MEDIATED INHIBITION OF JAK2 MUTATED CELLS

L. Marino¹, B. Charlier¹, V. Giudice¹, F. Dal Piaz¹, M. Pingeon¹, R. Vitolo¹, R. Rosamilio¹, B. Izzo², G. Villani¹, N. Montuori², B. Serio¹, V. Izzo¹, A. Filippelli¹, C. Selleri¹

¹Department of Medicine and Surgery, School of Medicine "Scuola Medica Salernitana" University of Salerno; ²Department of Clinical Medicine and Surgery, University of Naples "Federico II", Italy

Idiopathic Myelofibrosis (IMF) is a chronic hematologic disorder in which the genetic signature is a somatic mutation (V617F) in the Janus Kinase 2 (JAK2) gene. Ruxolitinib, a JAK1/2 inhibitor, has demonstrated great efficacy in improving clinical symptoms of IMF patients. We hypothesized that bone marrow mesenchymal stem cells (BM-MSCs) might modulate uptake and release of Ruxolitinib in the bone marrow (BM) niche regulating Hematopoietic Stem Cell (HSCs) proliferation. BM-MSCs primary cell lines (n=5) were obtained from newly diagnosed IMF patients. After third replating, BM-MSCs were characterized by flow cytometry as recommended by the International Society for Cellular Therapy (ISCT), and treated with Ruxolitinib (5,000 ng/mL) for 24 h. After washing and re-seeding, culture medium was collected at 24, 48, and 72 h, and Ruxolitinib was quantified by high performance liquid chromatography (RP-HPLC). Ruxolitinib was detectable in 3 out of 5 primary cell lines at 24 h (mean, 106 pg/mL) and 48 h (mean, 71 pg/mL), and in 4 out of 5 BM-MSCs cell lines at 72 h (mean, 53 pg/mL). The anti-proliferative effects on JAK2+ SET-2 cell line of Ruxolitinib and conditioned medium from treated BM-MSCs were assessed by colorimetric CCK-8 assay and by determination of inhibitory concentrations (IC)₅₀. All conditioned medium at a 96-dilution factor produced a 50% inhibition of SET-2 cell line proliferation. Finally, treated and untreated BM-MSCs were co-cultured with SET-2 cells at various seeding ratios (BM-MSCs/SET-2 1:20; 1:100; 1:1000). The proliferative capacity was assessed by CCK-8 assay and SET-2 cells were used as reference for data normalization. In co-cultures with untreated BM-MSCs at 1:1,000 and 1:100 ratios, SET-2 cell proliferation decreased of 5-15% after 7-day incubation, and of 50% at 1:20 ratio. In co-cultures with Ruxolitinib-treated BM-MSCs at 1:1,000 and 1:100 ratios, the SET-2 proliferation rate was reduced of 15-25%; while, at 1:20 ratio, the proliferation rate was even lower than 80%. Our preliminary data suggest that BM-MSCs might play an important role in modulating Ruxolitinib uptake and release in the BM microenvironment. Indeed, BM-MSCs might function as drug reservoir that can slowly release it in the BM niche influencing HSCs proliferation rate.

P051

REVIEW MPNS ACCORDING TO THE 2016 WHO CRITERIA: CLINICAL/LABORATORY PARAMETERS AND OUTCOME

N. Pugliese, S. Vitiello, A. Leone, G. Piccolo, V. Marafioti, A. Giordano, F. Fanelli, G. Delle Cave, C. Cimmino, I. Cappuccio, V. Martinelli, F. Pane

Università degli Studi di Napoli Federico II - Dipartimento di Medicina Clinica e Chirurgia, Italy

Introduction: The recently revised WHO classification of myeloid neoplasms recognizes Prefibrotic Myelofibrosis (pre-MF) as a distinct entity and redefines laboratory criteria for Polycythemia Vera (PV) diagnosis. The aim of this study was to examine the clinical relevance of distinguishing Essential Thrombocythemia (ET) from pre-MF and PV.

Materials and Methods: Our cohort included 312 patients with a diagnosis of MPNs according to 2008 WHO criteria performed from 1989 to 2015 and a bone marrow biopsy report at disease onset. All diagnosis subsequently underwent a re-review according to the 2016 revised WHO criteria. The rate of thrombotic events and evolution to MF and AML were calculated among MPNs subgroups.

Hodgkin Lymphoma 1

P052

EFFECTIVENESS OF CHEMOTHERAPY AFTER ANTI-PD-1 BLOCKADE FAILURE FOR RELAPSED AND REFRACTORY HODGKIN LYMPHOMA AND PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A RETROSPECTIVE ANALYSIS.

G. Lolli, B. Casadei, C. Pellegrini, V. Stefoni, M. Carella, A. Broccoli, L. Nanni, M. Marangon, A. Morigi, N. Fabbri, G. Gabrielli, L. Argnani, M. Cavo, P.L. Zinzani

Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

Hodgkin lymphoma (HL) shares with primary mediastinal large B-cell lymphomas (PMBCL) the ability to escape the immune system likely as a result of the amplification of chromosome band 9p24.1, leading to the upregulation of programmed death ligands and JAK/STAT pathway. In this setting, programmed death-1 (PD-1) blockade is a new therapeutic option with a good efficacy and a favorable side-effective profile. However, a substantial proportion of patients progresses or loses response to anti-PD1 therapy with a median progression free survival of 12 months for both diseases. Retrospective analyses in various tumor types and in HL showed a potential improvement in response rate to chemotherapy (CT) after exposure to PD-1 inhibitors, suggesting that anti-PD-1 therapy could re-sensitize the tumor cells to CT. We retrospectively investigated the effectiveness of salvage therapies for unsatisfactory response to anti-PD-1 therapy. Event free survival (EFS) was chosen as principal endpoint to assess the ability of anti-PD-1 therapy to re-sensitize tumor cells towards subsequent CT. Responses were assessed according to the Lugano criteria. We analyzed 22 patients (19 with classical HL and 3 with PMBCL), who were highly pretreated (median of 4 prior therapies, including 41% autologous stem cell transplant and 82% brentuximab vedotin) and 77.3% refractory to the last therapy before antiPD1. Patients underwent nivolumab (n=8) or pembrolizumab (n=14). Overall response rate (ORR) was 31.8%. Seventy-seven percent had unsatisfactory responses to anti-PD1 therapy (5 partial responses [PR], 14 progressive and 1 stable diseases), whereas only 2 patients achieved complete response (CR). Median EFS after antiPD1 was reached at 10 months, while median EFS with first CT following antiPD1 blockade was reached at 7 months (difference not statistically significant). Interestingly, for 9 (41%) patients EFS for first therapy after antiPD1 is longer than the one obtained with antiPD1 by a median time of 12,5 (range 1-29) months. Furthermore, ORR with CT was 45.5% (3 CR and 7 PR). The 3 CR patients underwent subsequently allogeneic transplantation and are still in response. No cumulative toxicity was registered. The efficacy of CT following anti-PD-1 is not yet well known, especially in lymphoma patients. To note, in our series, a subset of patients increased response rates and EFS to CT given after exposure to immune checkpoint inhibitors.

P053

TREATMENT OF HODGKIN LYMPHOMA (HL) PATIENTS WITH DEAUVILLE SCORE 5 (DS 5) AT INTERIM PET (IPET) AFTER 2 ABVD CYCLES IN THE REAL LIFE CLINICAL PRACTICE: EXCELLENT OVERALL SURVIVAL DESPITE THE NEED OF MULTIPLE LINES OF TREATMENT.

A. Guidetti, A. Rossi, C. Pavoni, F. Delaini, M. Picardi, C. Giordano, L. Trentin, R. Zanotti, L. Lissandrini, C. Schiavotto, A. Romano, A. Rambaldi, S. Viviani, P. Corradini

Divisione di Ematologia Fondazione IRCCS Istituto Nazionale dei Tumori, Dipartimento di Oncologia ed Emato-Oncologia Università degli Studi di Milano, Divisione di Ematologia ASST Papa Giovanni XXIII, Divisione di Ematologia AOU Federico II, Divisione di Ematologia Dipartimento di Medicina Università degli Studi di Padova, UO Ematologia Azienda Ospedaliera Universitaria integrata di Verona, Divisione di Ematologia Ospedale San Bortolo, Divisione di Ematologia AOU Policlinico-OVE Università di Catania, Italy

Introduction: Patients (pts) with advanced stage HL and DS 5 at iPET after 2 ABVD cycles represent an unmet clinical need. PET-adapted treatment strategies resulted in PFS ranging between 35% and 62% in published studies, however outcome of pts treated outside clinical trials

Results: MPNs diagnosis, according to WHO 2008 criteria, were distributed as follow: 67 PV (21.5%), 222 ET (71.1%), 13 Primary Myelofibrosis (PMF) (4.2%), 10 unclassifiable MPNs (u-MPNs) (3.2%). Review led to a diagnosis of WHO 2016-defined PV in 93 cases (29.8%), ET in 185 cases (57.1%), pre-MF in 27 cases (8.7%); overt-MF in 14 cases (4.4%). Among 26 patients revised as PV, in 20 (76.9%) cases ET was initial diagnosis, whereas in 6 cases (23.1%) initial diagnosis was u-MPN. Patients with PV had higher Hb and HCT values ($p<0.0001$) and lower platelet count ($p<0.0001$) compared with ET patients. Among 27 patients re-classified as pre-PMF initial diagnosis was ET in 22 cases (81.4%), overt-MF in 3 cases (11.1%) and u-MPN in 2 (7.4%). Megakaryocytes with abnormalities and/or forming dense clusters were more frequently observed in pre-MF (92.6%) compared with ET patients (20%) ($p<0.0001$). BM fibrosis grade 1 was observed in 74% of pre-MF patients compared with 28.7% of ET patients ($p<0.0001$). Splenomegaly (65.4 vs 35.7%, $p=0.004$) and higher LDH values ($p<0.0001$) were more frequently observed in patients with pre-MF than in those with ET. Evolution to MF was observed in 6 ET patients (4%) compared with 4 patients (14.8%) in the pre-MF group ($p=0.009$). No differences were found in terms of thrombotic event rate and evolution to AML in PV vs ET vs pre-MF and between PV and ET in terms of MF evolution rate.

Conclusions: BM biopsy examination remains an integral part of MPNs diagnosis. Laboratory parameters at presentation may provide additional information to suspect pre-MF or PV in a patient with a presumptive clinical diagnosis of ET. A correct distinction between pre-MF and ET seems to be important to identify patients at higher risk of MF evolution.

is unknown. Aims of study: To evaluate in a multicenter retrospective study clinical outcome of iPET DS 5 pts treated in the real life clinical practice.

Methods: Data of clinical characteristics, treatments and outcomes of iPET DS 5 pts have been collected from medical records of 7 Hematological Centers in Italy. Survivals, calculated from the date of iPET, were estimated by the Kaplan-Meier method.

Results: Twenty-three pts treated between 2008 and 2017 with 2 ABVD had an iPET DS 5 and were eligible for this analysis. Main characteristics were as follows: median age 34 years (range, 18 – 53), stage III/IV 61%, B symptoms 74%; bulky disease 61%, extranodal involvement 48%. After iPET all pts shifted to second line therapy (L2) consisting in escalated BEACOPP (n=7), eBEACOPP followed by standard BEACOPP (n=8), IGEV (n=7), or DHAOX (n=1). Eleven pts (48%) achieved CR, whereas 52% failed L2. Among pts treated with BEACOPP or IGEV, CR rate was 60% and 28%, respectively (p=ns). L2 response was consolidated by autologous transplant (autoSCT) in 2 pts and by radiation therapy in 3 pts. Three pts in CR relapsed after a median of 6 months and achieved a second CR after Brentuximab Vedotin (BV) or BV+Bendamustine (n=2) and after check points inhibitors (CPI) (n=1) followed by autoSCT (n=1) or autologous and allogeneic SCT (n=2). Among 12 pts refractory to L2, 10 (83%) achieved a CR after a median of one further therapy line (range, 1–3); 9 pts achieved a CR after a BV-based regimen followed in 7 pts by autoSCT. Overall 52% of pts required BV or CPI to achieve first or second CR. The 3-year PFS and OS for the whole population was 39% and 93%, respectively. No differences in OS were observed between pts in CR after L2 or after >2 lines of therapy as well as between transplanted at any time (n=12) and non transplanted pts.

Conclusions: Pts with iPET DS5 have a poor PFS when treated with conventional second-line salvage regimens; however, the majority of them have been rescued by further treatments containing BV or CPI, this resulting in excellent OS. In iPET DS5 pts amelioration of first salvage therapy by incorporation of BV or CPI in an earlier phase should be investigated in further studies.

P054

CK2ALPHA REGULATES PD-L1 EXPRESSION AND PIVOTAL PATHWAYS IN CLASSICAL HODGKIN LYMPHOMA, AND IS TARGETABLE BY SILMITASERTIB

A. Visentin, F. Frezzato, F. Severin, M. Pizzi, S. Imbergamo, E. Scomazzon, S. Pravato, S. Manni, L. Quoti Tubi, N. Facco, C. Carlo-Stella, G. Semenzato, F. Piazza, L. Trentin

Hematology and Clinical Immunology Unit, Department of Medicine, University of Padua, Italy; Venetian Institute of Molecular Medicine, Padua, Italy. General Pathology & Cytopathology Unit, Department of Medicine, University of Padua, Italy; Department of Biochemical Sciences, Humanitas University, Milan, Italy

Most patients with Hodgkin lymphoma (HL) are cured with first line therapy. However, only a few relapsed patients treated with novel agents such as brentuximab vedotin (BV, antiCD30 antibody conjugated to the microtubule inhibitor monomethyl auristatin E [MMAE]) or PD-1 inhibitors achieve durable remissions. The identification of pivotal proteins and the development of new targeted therapies are needed in HL. CK2 is a pleiotropic kinase consisting of 2 a (catalytic) and 2 b subunits that sustains cancer signaling cascades through the activation of NF- κ B, PI3K, and STAT pathways. Despite CK2 has been extensively studied in hematological malignancies, it has never been investigated in HL. Considering that NF- κ B, STAT and PI3K pathways are key players in HL, also regulating the expression of PD-L1, CK2 likely plays a critical role in HL. Experiments were performed employing 4 HL cell lines (L-428, L-540, KM-H2, and HDML-2) cultured in RPMI for 24h, 48h and 72h. CK2a, CK2b, p65-Serine (S)529, p65, PARP, AKT-S473, AKT, STAT3-S727, STAT3, PD-L1 and actin expression levels were evaluated by western blot analysis (WB). Apoptosis was assessed by Annexin V/Propidium iodide (AV/PI) assay and PARP cleavage by WB. HL cell lines were treated with silmitasertib, a CK2 inhibitor, and/or MMAE. By WB and immunofluorescence, we found that all the 4 HL cell lines expressed higher levels of CK2a (p=0.004), but lower levels of CK2b (p=0.004), as compared with normal B lymphocytes. These data were confirmed by immunofluorescence and immunohistochemistry on primary lymph-nodes derived from patients with HL (n=15), showing that CK2a but not CK2b was highly expressed in Reed-Sternberg

cells. In order to evaluate the activity of CK2, we performed WB analysis of the phosphorylation status of CK2 substrates. At basal conditions AKT, p65 and STAT3 were phosphorylated in HL at their CK2-related serine residue (S473, S529, and S727, respectively). Treatment of HL cell lines with silmitasertib lead to cell cycle arrest, reduction of AKT-S473, RelA-S529 and STAT2-S727, time and dose-dependent apoptosis (p<0.01), down-regulation of PD-L1 but not of CD30, and enhanced apoptosis by MMAE. We demonstrated that CK2 could play a central role in classical HL. Silmitasertib, by inhibiting CK2, switches off some pivotal pathways and trigger apoptosis in HL cell lines. Moreover, the down-regulation of PD-L1, but not of CD30, suggests possible combinations with checkpoint inhibitors or MMAE/Brentuximab.

P055

PERSISTENT MEDIASTINAL FDG PET-CT POSITIVITY AFTER FRONTLINE THERAPY FOR HODGKIN LYMPHOMA: BIOPSY OR OBSERVATION?

M. Novo^{1,2}, G.S. Nowakowski¹, T.M. Habermann¹, T.E. Witzig¹, I.N. Micaleff¹, P.B. Johnston¹, D. Inwards¹, B. Botto², K.M. Ristow¹, J.R. Young³, U. Vitolo², S.M. Ansell¹

¹Division of Hematology, Mayo Clinic, Rochester, Minnesota, Division of Hematology; ²Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino; ³Division of Nuclear Medicine, Mayo Clinic, Rochester, Minnesota

Residual mediastinal FDG PET-CT uptake after frontline therapy for classical Hodgkin lymphoma (cHL) may constitute persistent disease or inflammatory changes. Thus, we analyzed the practice patterns at two institutions to determine how often a biopsy of PET-positive mediastinal sites after treatment was informative, influenced patient management and outcome. We retrospectively reviewed all cHL treated at Mayo Clinic Division of Hematology in Rochester, MN and abstracted from the Mayo Clinic Lymphoma Database from the Division of Hematology, and the Hematology Division of Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino in Turin, IT. Cases with a mediastinal mass at diagnosis (largest diameter \geq 5 cm) and isolated FDG-uptake at the site after standard front-line therapy were included into the study. Clinical approach adopted and outcomes were analyzed. For patients with FDG PET-CT images available, an independent radiological review was performed. Among 1270 cHL evaluated 42 were eligible for the study. Ten of 20 cases biopsied (group 1) showed persistent cHL and underwent salvage therapy (ST); 4/10 biopsy-negative cases were observed and 6/10 received consolidative radiotherapy (RT). Ten of the 22 not-biopsied cases (group 2) were observed, 12 received RT or ST. Biopsies performed in post-observation PET-positive cases resulted 100% positive. Deauville score was available for 39/42 patients, through radiological review or original report when images not available: 4/42 cases were considered DS 3 (10%), 13/42 were DS 4 (31%) and 16/42 were DS 5 (38%). Three of 42 (7%) patients previously felt to be PET-positive but without DS reported were classified as DS 2, 3/42 cases were judged to be thymic rebound (DS X) (7%). Stratifying cases by DS, DS5 showed higher risk of progression (62%). With a median follow up of 37.8 months (10.6-171.9 months) no survival differences between groups 1 and 2 was observed (P=0.813). Within the limitations of this small retrospective cohort, we conclude that 'wait-and-see' strategy with a repeat FDG-PET scan re-assessment after an interval of 4 or more weeks is a cost-effective approach for DS3-4 cases. For DS5 upfront ST must be considered.

P056**FEASIBILITY AND EFFICACY OF POST-TRANSPLANT CONSOLIDATION IMMUNOTHERAPY WITH NIVOLUMAB SUPPORTED BY THE REINFUSION OF UNSELECTED AUTOLOGOUS LYMPHOCYTES IN PATIENTS AFFECTED BY RELAPSED/REFRACTORY HODGKIN LYMPHOMA**

F. Guolo, P. Minetto, F. Ballerini, M. Clavio, L. Manconi, F.A. Canale, M. Frello, R. Marcolin, E. Coviello, A. Kunkl, E. Tedone, D. Fenoglio, M. Miglino, A. Bo, M.C. De Luigi, M.R. Ruzzenenti, P. Carlier, A. Serio, S. Luchetti, E. Marcenaro, P. Strada, J.L. Ravetti, M. Gobbi, R.M. Lemoli

Università degli studi di Genova, Ospedale Policlinico San Martino, Italy

Background: Salvage chemotherapy of refractory/relapsing Hodgkin Lymphoma (HL) patients is usually followed by autologous stem cell transplantation (ASCT). Nivolumab has shown promising results in HL patients relapsed after ASCT but the expected CR rate is only about 20%. Thus we speculated that early administration of nivolumab, as post-ASCT consolidation, may improve its efficacy. It has been reported that durable response to nivolumab requires patient immunocompetence, often compromised in pretreated patients. In this view, in very high risk HL patients there is a strong rationale for early post ASCT infusion of autologous lymphocyte together with nivolumab.

Aims of The Study: Herein we report preliminary results of a prospective trial investigating the feasibility, and the efficacy of post-ASCT Nivolumab with the support of unselected autologous lymphocyte reinfusions (ALI).

Methods: Patients under the age of 60 with high risk HL, identified by PET2 or PET6 positivity following ABVD, were scheduled for a pre-emptive lymphocyte apheresis. Patients failing to achieve at least PR with salvage chemotherapy proceeded to ASCT with FEAM conditioning and were given 4 incremental doses of ALI (starting from 1×10^4 T cells/kg to a maximum of 1×10^7 /kg) followed after 48 hours by a flat dose of 240 mg Nivolumab.

Preliminary Results: Five R/R HL patients have completed the treatment and 2 are currently under treatment. PET scan before ASCT showed progressing disease in all patients, with multiple-extra nodal involvement in 4 of them. All patients underwent ASCT with FEAM conditioning and achieved complete engraftment after a median of 10 days (8-12). A quicker immune-recovery in term of CD3+ count was observed at all timepoints ($p < 0.05$) in all patients, compared to HL patients receiving the same conditioning without ALI. After ALI, cytotoxic (CD56+, CD16+, CD57+) NK cells showed the most significant increase (Figure 1, $p < 0.05$). No grade 3 or 4 adverse events were recorded so far. All treated patients are alive and disease-free after a median follow-up of 17 months.

Preliminary Conclusions: Post-ASCT ALI proved to be feasible and effective and induced a faster immune recovery. Moreover, nivolumab and ALI were associated with an impressive clinical response. This combination strategy might therefore improve the low CR rate of anti-PD1 blockade therapy alone, providing a more effective option for refractory patients, who are not usually considered candidate for ASCT.

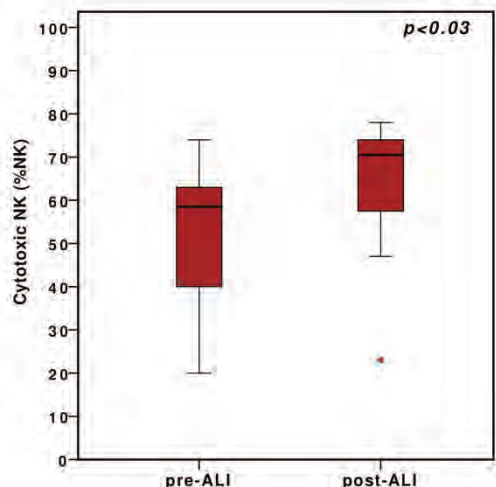


Figure 1. Cytotoxic NK cell/ul before and after ALI.

P057**SALVAGE TREATMENT OF RELAPSED/REFRACTORY (R/R) HODGKIN'S LYMPHOMA (HL): A SINGLE CENTER EXPERIENCE WITH DRUG-CONJUGATED ANTI-CD30 ANTIBODY BREN-TUXIMAB VEDOTIN (BV)**

B. Botto, G. Torsello, M. Novo, E. Santambrogio, F. Vassallo, A. Evangelista, A. Chiappella, C. Boccomini, L. Orsucci, U. Vitolo
SC Ematologia, A.O.U. Città della Salute e della Scienza, Italy

BV is a drug-conjugate anti-CD30 antibody highly effective in the treatment of R/R HL. Aim of our single center retrospective study is to evaluate clinical results and toxicity of BV in 30 patients with R/R HL treated in Turin between January 2010 and October 2017. Ten patients were treated with BV associated with Bendamustine (BBV) as first salvage treatment before autologous stem cell transplantation (ASCT). Twenty-two patients received BV as single agent during a second line salvage treatment. Two patients received both BBV and BV single agent. International Working Group (IWG) criteria were used to evaluate response to therapy with FDG-PET. Primary end points were complete (CR), partial (PR) remission, overall (ORR) response rates, 2-year progression free survival (PFS) and overall survival (OS). Survival data were obtained with Kaplan-Meier method and log rank tests were used to make a comparison with other conventional treatments. ORR before ASCT was 80% in patients treated with BBV: 8 patients (80%) were subsequently transplanted and final ORR was 70% (6 CR+1 PR). Three patients were refractory and 1 relapsed at the end of treatment. 2-years PFS was 60% for BBV (IC 25-83) vs 41% (IC 26-55) for 36 treated with other salvage chemotherapy (log rank $p=0.22$). 2 years OS was 100% for BBV vs 76% (60-86) for other chemotherapy. An ORR of 68% (10 CR+5 PR) was achieved in the 22 patients treated with BV as a single agent during a second line salvage treatment. The median number of treatments was 5 (range 4-16). Eight patients (36%) underwent ASCT with an ORR of 88%. Two patients (9%) received an allogeneic transplant with ORR 100%. Twelve patients (55%) were not transplanted with an ORR of 33%. 2-years PFS and OS were 50% (IC 27-70) and 80% (IC 53-92). Failures were 11/22 (50%), 8 refractory and 3 relapses. Total deaths were 4, 2 PD, 1 pneumonia and 1 acute GVHD. Adverse events were generally mild (grade 1-2 CTCAE): 11 events (37%) with 1 peripheral neuropathy grade 3 (3%), 1 grade 3 hematological toxicity (3%) and 2 infusion reaction (6%). In our study, with the limits of a retrospective one with low number of patients, BBV was confirmed as a valid alternative to conventional schemes in first salvage setting before ASCT. BV as second salvage in patients not suitable for ASCT or relapsed after ASCT, improved CR rate before transplant without a significant difference in terms of PFS and OS. Treatment was generally well tolerated without significant toxicity.

P058**AMINOACID DEPRIVATION DUE TO INCREASED ARG-1+ AND IDO-1+ HIGH-DENSITY NEUTROPHILS IN MICROENVIRONMENT TRIGGERS A METABOLIC ADAPTIVE RESPONSE IN HODGKIN LYMPHOMA**

F. Puglisi, C. Bellofiore, G. Schinina, N.L. Parrinello, C. Giallongo, G. Camiolo, D. Cambria, A. Bulla, A. Chiarenza, G. Motta, D. Tibullo, G.A. Palumbo, F. Di Raimondo, A. Romano

AOU Policlinico Vittorio Emanuele di Catania, Divisione di Ematologia, Italy

Our previous work showed that the arginine degrading enzyme arginase-1 (ARG-1) and the rate-limiting enzyme catabolizing tryptophan (trp) to kynurenine (kyn) indoleamine 2,3 dioxygenase (IDO-1) are increased in Hodgkin Lymphoma (HL) microenvironment. In order to evaluate their role in HL biology, we checked the effects of arg and trp deprivation on three human HL cell lines (L428, L1236 and KMHL), previously characterized for differential expression of CD30 and PDL1, by combining different assays (RT-PCR, immunofluorescence, flow cytometry and western blot). We found that both arg and trp deprivation induced cell cycle arrest in G0-G1 phase in all tested HL cell lines, without affecting viability, associated to increased IRF4 and activation of the transcription factor STAT3. Long-term deprivation of both arg and trp altered the cellular dependence on mitochondrial ATP generation via oxidative phosphorylation and increased glutamine anaplerosis, while intra-cellular amount of c-myc was reduced. Metabolic re-shape was associated to decreased mitochondrial membrane potential, as shown by reduced DiOC2(3) staining intensity by flow cytometry.

However, arg and trp deprivation mediated mitochondrial remodeling in different ways. Indeed, arg deprivation induced resting state associated to reduced expression of Fis1, OPA-1, DRP1, MNF2 and ATP5, compatible with fragmented mitochondria and low energy demand. Trp deprivation promoted fission to maintain respiratory activity, as shown by increased expression of Fis1 and DRP1, associated to reduced TFAM and OPA-1. High-density neutrophils and CD68+macrophages were the main IDO+ cell populations respectively in peripheral blood and in the lymph-node, as evaluated by flow cytometry and immunohistochemistry. Normal HDN cultured for 24 hours with conditioned media obtained from L428 HL-cell line activated both STAT-3 and STAT-5, associated to increased ARG-1 expression; conversely the exposure to 500nM pan-JAK inhibitor ruxolitinib, reduced the amount of activated STAT-3, STAT-5 and ARG-1. Taken together our findings suggest that soluble factors produced by HL cells can induce ARG-1 expression in HDN. Trp and arg shortage due to increased IDO and ARG1 in HDN and macrophages in HL microenvironment induce a mitochondrial remodeling and may contribute to metabolic adaptation of HL. Research is ongoing in our lab to investigate if this adaptive mechanism can be reverted by the pan-JAK inhibitor ruxolitinib.

Allogeneic and Autologous Transplantation 1

P059

RUXOLITINIB IN CHRONIC GVHD BEYOND THIRD LINE TREATMENT: HIGH OVERALL RESPONSE RATE IN 18 PATIENTS

M.T. Lupo-Stanghellini¹, C. Corti¹, C. Messina¹, S. Girlanda¹, F. Serpenti¹, C. Canzonieri¹, F. Pavesi¹, S. Markt¹, M.G. Carrabba¹, A. Assanelli¹, M. Bernardi¹, J. Peccatori¹, F. Ciceri^{1,2}

¹Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute; ²University Vita-Salute San Raffaele.

Background: Chronic Graft versus Host Disease (cGvHD) still represent a major issue after transplant with less than 20% of patients (pts) able to achieve meaningful response without need for additional treatment 1 year after diagnosis. Jak-inhibitors – such as ruxolitinib - are under investigation for the potential benefit in treatment of acute and chronic GvHD. Unfortunately, pts with more than 2 lines of treatment are not eligible to clinical trials actually ongoing.

Patients and Methods: Our study seeks to analyze the efficacy and safety of ruxolitinib in highly pre-treated cGvHD pts in our Centre between January 2015 and March 2019. Ruxolitinib was given off-label after provision of an informed signed consent and in the absence of alternative therapeutic options in 18 pts. Indication to treatment fulfilled criteria of trial NCT03112603, except for number of previous systemic treatment or time of prednisone exposure.

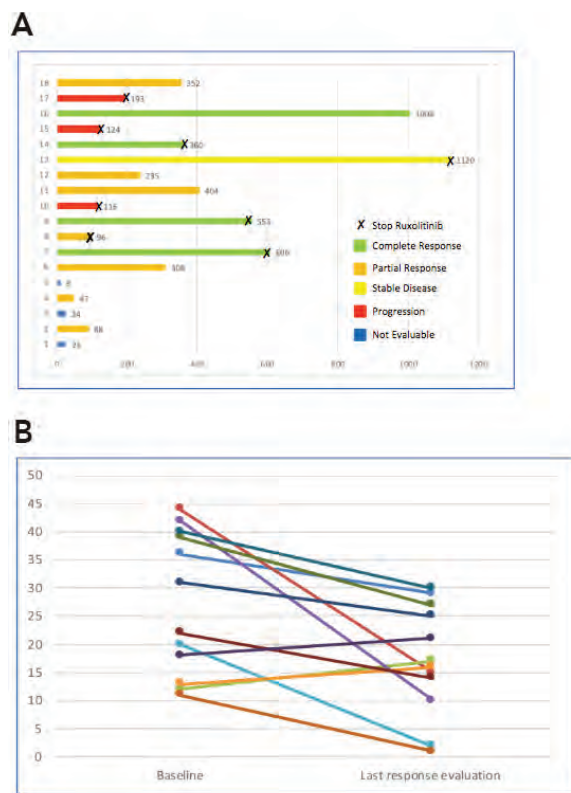


Figure 1. A) Treatment and response to ruxolitinib. B) Lee Symptoms Scale variation: baseline versus last response evaluation under ruxolitinib treatment.

Results: Median age was 47y (r 19-68). Donor was a match related in 7 cases, a match unrelated in 5 and a mismatch related one in 6. Median time from transplant to cGvHD diagnosis was 217 days; 12 pts were diagnosed with classic and 6 with overlap cGvHD; according to NIH 2014 4 were moderate and 14 severe (2 heart GvHD). All the pts received prednisone (0.5-1 mg/Kg) as 1st line treatment. The median time from transplant to ruxolitinib was 976 days, the median time from cGvHD diagnosis and ruxolitinib was 654 days. Ruxolitinib was initiated at a starting dose of 5mg twice daily, 7/18 pts increased the dose

to 10mg twice daily in absence of strong CYP3A4 inhibitors. The median time of ruxolitinib therapy was 214 days. Pts with at least 1 month of treatment were considered for response evaluation: 11 pts achieve a meaningful response (4 complete remission, 7 partial response). One patient maintained stable disease while 3 pts progressed during treatment (Figure 1 A). All pts completed the Lee symptoms scale: the results were consensual with the health-care provider evaluation (Figure 1 B) We did not observe toxicity due to drug-drug interactions and we did not register haematological toxicities G₂/=2. One patient required hospitalization due to CMV pneumonia and 2 pts developed sepsis (one after major surgery and one in the context of a community acquired pneumonia).

Conclusions: Ruxolitinib is well tolerated and effective also in very highly pre-treated and advanced cGVHD pts, confirming the potential leading role in the treatment of cGVHD.

P060

FREQUENCY AND IMPACT OF EARLY MIXED CHIMERISM AFTER MYELOABLATIVE CONDITIONING REGIMEN WITH FLUDARABINE AND BUSULFAN (FB4) AND ATG FOR ALLOGENEIC TRANSPLANTATION IN MYELOID MALIGNANCIES

A. Grassi, C. Pavoni, C. Micò, M.C. Finazzi, F. Lussana, D. Taurino, A. Rambaldi

ASST Papa Giovanni XXIII, Bergamo, Italy

The combination of fludarabine with myeloablative doses of busulfan (FB4) represents a standard of care conditioning regimen before allogeneic transplantation (alloHSCT) in patients with myeloid malignancies. FB4 has potent antileukemic activity and is associated with low transplant-related mortality and acute GvHD. However, early after transplantation a proportion of patients may not convert to a full donor chimerism, particularly if anti-T lymphocyte globulin (ATG) is used. We retrospective analyzed 104 patients who underwent an alloHSCT after FB4 at our hospital, from November 2007 to August 2018. The median age was 51 y and the main diagnosis was AML (76%). The disease status at transplantation was: CR1 in 59%, CR2 in 5% and active disease in 36% of patients. The stem cell source was represented by PBSC and anti-T lymphocyte globulin (ATG) was used both in more than 95% of cases. The donor was a HLA identical sibling (26%) or an unrelated donor (74%). Hematopoietic chimerism was molecularly evaluated by Variable Number of Tandem Repeats on bone marrow (BM) mononuclear cells and peripheral blood (PB) T lymphocytes, purified by immunomagnetic positive selection. After 30, 60 and 90 days the proportion of patients with a full BM chimerism was 95%, 97% and 98%, respectively while the PB T cell full chimerism was 49%, 79% and 98,5%. Before day 100, 10 patients required DLI to treat a pending or overt hematologic relapse and 12 patients to convert the lymphoid chimerism from mixed to complete (successfully in 7 cases). After day 100, 13 additional patients required DLI to treat disease relapse or progression and 8 patients to improve the chimeric status or the immune reconstitution. At 5 years, the Overall Survival is 64%, with a relapse and non-relapse mortality of 21 % and 11%, respectively. By multivariable analysis, AML diagnosis and a mixed BM chimerism before day 100 were associated with Relapse and OS. A mixed PB T-lymphoid chimerism before day 100 does not adversely impact on non-relapse mortality, cumulative incidence of relapse, leukemia-free and OS. In conclusions after FB4 and ATG, a progressive increase of PB lymphoid donor chimerism develops gradually in most of cases without the need of DLI. Early mixed lymphoid chimerism does not compromise the main long-term clinical outcomes and may at least partially explain the low aGVHD incidence.

P061

ANTICORPI ANTI-HLA DONATORE SPECIFICI (DSA) NEL TRAPIANTO DI CELLULE STAMINALI EMATOPOIETICHE E STRATEGIA DI DESENSIBILIZZAZIONE. STUDIO PROSPETTICO UNICENTRICO

U. La Rocca¹, A.P. Iori¹, M.P. Perrone², P. Cinti², M. Gozzer², B.M. Shafiqi², S. Pepe¹, W. Barberi¹, L. Quattrocchi¹, C. Cavallari², P. Gesuiti², R. Lattanzi², R. Ricci¹, L. Laurenti², R. Foà¹, G. Girelli²

¹Hematology, Policlinico Umberto I, Department of Translational and Precision Medicine, Sapienza University; ²Immunohematology and Transfusion Medicine, Policlinico Umberto I, Sapienza University

Background: In the setting of hematopoietic stem cell transplantation (HSCT), the detection of antibodies (Ab) directed against donor specific HLA antigens (DSA) represents a contraindication to proceed with the same donor. In many cases, there is insufficient time to search for alternative donors and it is necessary to plan an immunosuppressive strategy to decrease the DSA levels, thus reducing the risk of graft failure. To date, there is no consensus on desensitization standards to manage DSAs in HSCT. The aim of this study was to evaluate the efficacy of our desensitization strategy.

Methods: Anti-HLA Ab research was carried out using the Luminex bead assay (Lifecode Screen and LSA I/II-Immucor). The results were expressed as mean fluorescence intensity (MFI); MFI >1000 was considered positive. In case of a mismatched related donor, a flow cytometric crossmatch test (FCXM) was performed. If the patient had DSAs and only one available donor, the following desensitization strategy was employed: Rituximab on day -15, single-volume plasmapheresis procedures (PP), usually on day -9 and -8, intravenous immunoglobulins on day -7, infusion of HLA selected platelets for DSA absorption in case of persistent antibodies directed against class I HLA antigens. The aim of this schedule was to avoid interferences with chemotherapy and anti-T-cell globulins infused during the conditioning regimen.

Results: Between August 2014 and April 2019, we prospectively screened for DSA 159 consecutive patients candidates to an allogeneic HSCT. Thirty-eight patients (23.9%) showed anti-HLA Ab and 9 of them (5.7%) had DSAs: 6 were treated with the desensitization strategy, applied according to the MFI score and the FCXM result, and they all obtained an engraftment; in 2 cases, an alternative donor was selected and in 1 case the research for an alternative donor is still underway. DSA detection was performed every 7 days after HSCT for the first month and 60, 180 and 365 days following HSCT. Neither a DSA rebound nor other complications were observed during the follow-up.

Conclusions: Our prospective analysis underlines the necessity to routinely evaluate the presence of DSAs before an allogeneic mismatched HSCT. Our desensitization schedule, based on the combination of PP, rituximab, IVIG and platelet absorption, proved successful in reducing DSAs. Transplant and transfusion specialists should join to define a consensus for a standard desensitization strategy.

P062

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION USING THE TCR/CD19-BASED DEPLETION OF G-CSF-MOBILIZED PERIPHERAL BLOOD PROGENITOR CELLS IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES

I. Manfra, S. Bonomini, C. Schifano, R. Segreto, G. Sammarelli, G. Todaro, M. Sassi, I. Bertaggia, B. Cambò, B. Dalla Palma, A. Spolzino, E. Follini, L. Craviotto, L. Pochintesta, D. Vallisa, L. Prezioso

Azienda Ospedaliero Universitaria di Parma, Azienda Ospedaliera di Piacenza, Italy

The innovative strategy based on the selective depletion of T and CD19 positive lymphocytes from G-CSF-mobilized peripheral blood precursor cells has shown very promising results in the setting of the pediatric transplantation. This approach has been adopted in our Unit, focusing on adult population. Since January 2013, 62 patients with median age of 51 years (range 20-73) lacking a matched donor have been treated. Patient were affected by hematological malignancies: 41 leukemia, 14 lymphoma, 3 Myelofibrosis, 4 multiple myeloma). Conditioning chemotherapy consisted in ATG (6 mg/kg), Treosulfan (36 gr/sqm), Fludarabine (150 mg/sqm) and Thiotepea (10 mg/Kg). No additional immune suppression was given after transplantation as GvHD

prophylaxis. Grafts contained a median of $11 \times 10^6/\text{kg}$ (range 5-19) CD34^+ cells, $5.3 \times 10^6 \text{ CD3}^+\text{T cells}/\text{kg}$ (0.1-36), $8.4 \times 10^4/\text{kg}$ (0.4-62) T cells , $5.4 \times 10^6 +\text{T cells}/\text{kg}$ (1-37), $8 \times 10^4 \text{ B cells}/\text{kg}$ (1.5-32) and $22 \times 10^6 \text{ CD56}^+\text{NK cells}/\text{kg}$ (5-91). Median T and B cell depletion was 4.5 log and 3.2 log, respectively. A full donor sustained engraftment was achieved in 59/62 patients (95%) patients. Neutrophils and platelets recovered at a median of 12 (range 9-18) and 11 days (range 6-30), respectively. Thirty-five patients (56%) survive at a median time of 27 months (range 0,6-70): 17 patients (41%) among acute leukemia and 17 (80%) among the remaining group of diseases. Overall, 14/62 patients (22%) died without relapsing: 10 due to infection, 1 TMA, 1 acute distress syndrome and 2 acute GVHD. Among GVHD, one of the 2 patients had received the highest dose of $+ \text{T cells}$ ($3.7 \times 10^5/\text{kg}$) and the second, affected by 6GPDH deficiency, experienced a late onset hepatic GVHD. Ten patients had developed skin limited aGVHD (7 grade I-II and 3 grade III-IV) and two patient chronic GvHD. Seventeen patients relapsed (10/26 in advanced stage at time of transplant, 7/36 in remission). A rapid increase in peripheral blood T-cell and B-cell populations was observed. Four patients experienced CMV reactivation (\geq two episodes) and in three cases a CMV disease occurred. Notably, in three patients a significant expansion of pathogen-specific CD8^+ T cells was documented and it contributed to clear viral load spontaneously. A T cell depletion approach, can offer the benefit of a GvL effect in absence of both acute and chronic GvHD and it could be offered as a viable option in the early stages of the disease to high risk hematological malignancies without a matched donor.

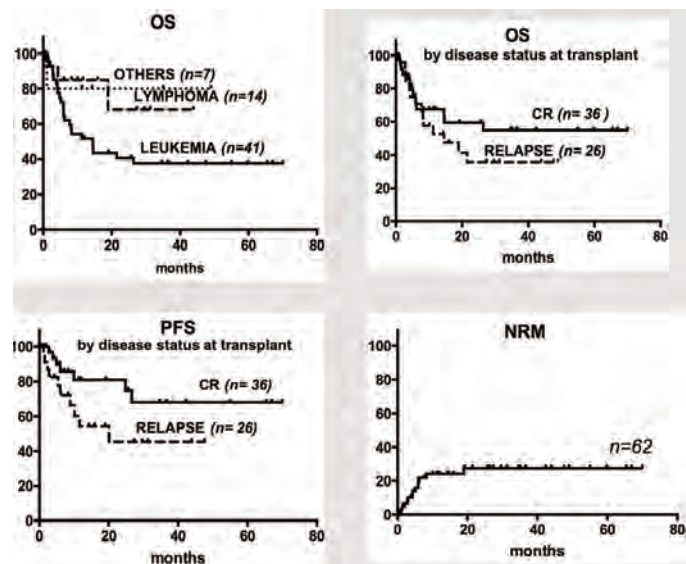


Figure 1.

P063
PERIPHERAL BLOOD HEMOPOIETIC STEM CELL MOBILIZATION REGIMENS IN POEMS SYNDROME: A RETROSPECTIVE STUDY AT TWO HAEMATOLOGICAL ITALIAN CENTRES

F. Autore¹, N. Piccirillo¹, A. Nozza², I. Innocenti¹, R. Putzulu¹, P. Chiussolo¹, F. Sorà¹, G. Zini¹, A. Bacigalupo¹, L. Castagna², S. Sica¹, S. Bramanti², L. Laurenti¹

¹Fondazione Policlinico Universitario A. Gemelli IRCCS; ²Humanitas Cancer Center, Italy

Introduction: POEMS syndrome is a rare paraneoplastic condition associated to an underlying plasmacellular dyscrasia. The use of alkylating agents and autologous peripheral blood stem cell transplantation (aPBSCT) seem to be the best strategy. In this undefined scenario, we retrospectively collected over the years patients with POEMS syndrome mobilized with different regimens and compared the outcome of these regimens. We are now reporting the results of this study, aimed at identifying, if possible, the best CD34^+ cell mobilization strategy.

Patients and Methods: We collected clinical and laboratory data of patients with POEMS syndrome undergoing HSC mobilization for aPB-

SCT from 2003 to 2018. We compared two HSC mobilization regimens: CY 4 g/m² followed by G-CSF 5 g/kg (regimen A), or G-CSF 10 g/kg/day alone for 5 consecutive days (regimen B).

Results: Our data set consisted of 25 patients, of whom 11 were mobilized using regimen A and 14 patients were mobilized with regimen B. All patients were successfully mobilized and underwent a collection procedure. Three patients, because of low CD34^+ cells after the administration of G-CSF alone, were submitted to plerixafor infusion. All patient underwent aPBSCT after HD-Mel conditioning regimen receiving an infusion of $4.7 \text{ CD34}^+\text{cell}/\text{kg}$ body weight and achieved a successful engraftment. At present all the patients are alive and in remission. Data analysis according to mobilization schedule was reported in Table 1. Analysing mobilization efficacy, chemo-mobilized patients achieved a higher pre-apheresis CD34^+ cell count (57 vs 33 cells/ μL , $p < 0.05$). This result allowed a significantly shorter procedure (2.3 TBV vs 3 TBV, $p < 0.001$). Patients receiving G-CSF alone, had a significantly higher WBC than chemo-mobilized patients ($40 \times 10^9/\text{L}$ vs $8.1 \times 10^9/\text{L}$, $p < 0.05$). The incidence of poor mobilization was low (3 out of 25 patients, 12%) and not statistically different between the two mobilization schedules. No statistically significant differences were seen between the two groups in terms of engraftment.

Discussion: These results suggest that both approaches for the mobilization of peripheral blood progenitors (CY plus G-CSF vs G-CSF alone) were able to harvest a sufficient CD34^+ cell dose and to allow a rapid and permanent engraftment and, despite the small number of patients ($n=25$), this remains one of the largest published series.

Table 1. Peripheral blood stem cell collection characteristics according to mobilization schedule.

	Tot 25 pts	Cyclophosphamide plus G-CSF 11 pts	G-CSF 14 pts	P
Age	54 (39-67)	55 (39-62)	54 (41-67)	0.9
Pre-collection peripheral CD34^+ μL	40 (12-308)	57 (12-308)	33 (14-75)	0.035
Pre-collection peripheral WBC $\times 10^9/\text{L}$	29.4 (2.5-87)	8.1 (2.5-31.9)	40 (20.2-87)	0.0002
Processed TBV	2.85 (0.9-3.5)	2.3 (0.9-3)	3 (2.4-3.5)	0.0013
CD34^+ collected cell dose $\times 10^6/\text{kg}$	3.85 (0.8-15)	3.85 (1.6-15)	3.85 (0.8-7.5)	0.41
2 nd procedure	11	4 pts	7 pts	0.68
Poor mobilizers	3	1 pt	2 pts	0.69
CD34^+ cell dose infused $\times 10^6/\text{kg}$	4.7 (1.5-8.4)	4.4 (2.986-7.5)	5 (1.5-8.4)	0.57
Time to engraftment PMN $0.5 \times 10^9/\text{L}$	14.5 (10-48)	14 (10-31)	19 (12-48)	0.12
Time to engraftment PLTs $25 \times 10^9/\text{L}$	14 (10-67)	13 (10-35)	17 (11-67)	0.14

P064
IMMUNE RECONSTITUTION OF CD4^+ AND NK CELLS AT SIX MONTHS AFTER ALLOGENIC TRANSPLANTATION STRONGLY CORRELATES WITH SURVIVAL

D. Taurino¹, M. Micò¹, C. Pavoni¹, A. Algarotti¹, F. Delaini¹, A. Grassi¹, G. Borleri¹, A. Rambaldi²

¹Hematology and Bone Marrow Transplantation Unit, ASST Papa Giovanni XXIII, Bergamo, Italy; ²University of Milan, Milan, Italy and ASST Papa Giovanni XXIII, Bergamo, Italy

Background: An adequate immune reconstitution (IR) is crucial to reduce the transplant toxicity, the relapse rate and mortality after allogeneic stem cell transplantation (allo-HSCT). The aim of this single center retrospective study was to investigate the correlation between the lymphocyte subpopulations recovery with the main transplant outcomes, including overall survival (OS), disease free survival (DFS) and

non-relapse mortality (NRM).

Patient and Methods: We analyzed the IR of 177 adult patients who underwent allo-HSCT between January 2013 and March 2017. Median age at transplant was 52 years. Thirty-nine donors were HLA-identical siblings (22%), 11 haploidentical (6%), 109 matched unrelated (62%), 11 MUD (6%) and 7 cord blood (4%). Bone marrow was used in 30 patients (17%), cord blood in 7 (4%) and peripheral blood in 140 (79%). The conditioning regimen was myeloablative in 99 (56%) transplant, reduced intensity in 75 (42%) and immunosuppressive in 3 (2%). GVHD prophylaxis was based on calcineurin inhibitors with Methotrexate or Mofetil Mycophenolate. ATG was used in 81% of patients. The peripheral blood lymphocyte subsets were analyzed by flow cytometry at 1, 2, 3, 6, 12 and 24 months after HSCT. Post-transplant engraftment was molecularly determined by VNTR analysis.

P065

SECOND TRANSPLANTATION FOR PRIMARY GRAFT FAILURE, FOLLOWING HAPLOIDENTICAL GRAFTS WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE

S. Giammarco¹, A.M. Raiola³, P. Chiusolo², F. Sorà², S. Sica², C. Di Grazia³, F. Gualandi³, R. Valardo³, L. Laurenti², E. Angelucci³, A. Bacigalupo²

¹Istituto di Ematologia, Fondazione Policlinico Universitario Gemelli IRCCS; ²Università Cattolica del Sacro Cuore, Roma; ³IRCCS AOU San Martino IST, Genova, Italy

Rejection, following haploidentical transplants (HAPLO) with post-transplant high dose cyclophosphamide (PT-CY) has been described and has been associated with donor specific antibodies (DSA). The aim of the study is to assess the incidence of rejection in patients with hematologic malignancies, prepared with a myeloablative conditioning regimen and the outcome of a second transplant. We studied 482 patients with hematologic malignancies, receiving an unmanipulated HAPLO marrow graft. Median age was 52 (17-74) and 38% had advanced disease. All patients received GvHD prophylaxis with Cyclosporin, mycophenolate and PTCY. The conditioning regimen used, was one of the following three: # Fludarabine (FLU) 40 mg/m²x3 + Total body irradiation (TBI) 9.9-12 Gy (n=80); # Thiotepa (THIO) 5 mg/kgx2, busulfan (BU) 3.2 mg/kg/dayx3, FLU 50 mg/m²x3 (TBF) (n=209); # THIO 5 mg/kgx2, BU 3.2 mg/kg/dayx2, FLU 50 mg/m²x3 (n=193). Rejection was seen in 1/80 patients after TBI (1.2%), 5/209 after TBF with 3 days of Busulfan (2.4%) and in 11/193 in patients receiving TBF with 2 days of Busulfan (5.7%) (p=0.02). We could not find a correlation of rejection with donor age, patients age, diagnosis, phase of the disease, ABO mismatch, or bone marrow cell dose infused. Second transplantation: 17 patients failed to recover neutrophils by day +28, and were found to have 100% recipient chimerism. Of these 17 patients, one was infused with CD34 selected cells from the same donor, without a conditioning regimen, and died without recovery, after having failed a third transplant. 16 patients received a second graft (at a median interval of 43 days, range 33-66) with the Baltimore protocol and PB from the same donor (n=13) or another family member (n=3). GvHD prophylaxis was again CyA and PTCY. 13/16 had trilineage recovery (81%) and became 100% donor: a neutrophil count of 0.5x10⁹/L was reached on day 18 (16-21) and a platelet count of 20x10⁹/L on day 35 (18-80). Acute GvHD grade I occurred in 46% and grade II in 8%. Moderate-severe chronic GvHD developed in 23%. One year actuarial survival of the 16 patients who received a second graft is 76%.

Conclusions: (a) the risk of rejection is dependent on the intensity of the conditioning regimen: it is very low after TBI (1.2%) and with full dose TBF (2.4%); (b) full engraftment can be achieved with a second transplant, in over 80% of patients rejecting. Mortality due to rejection, in this series of 482 patients, was 4 patients (82%)

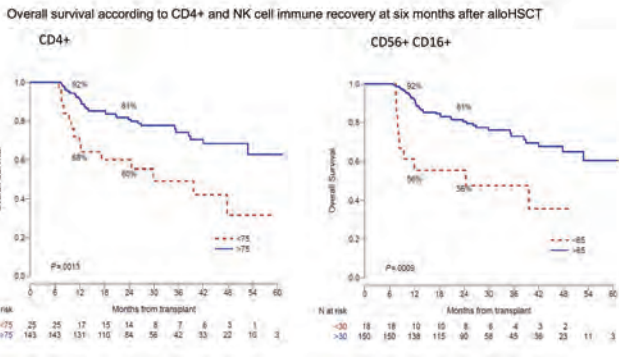


Figure 1.

Table 1.

Peripheral blood lymphoid subpopulations (median absolute values/ μ L, with range) and proportion of BM and PB T lymphocyte chimerism, at different time points after alloHSCT

Days after alloHSCT	+30	+60	+90	+180	+360	+720
Lymphocytes	483 (290.5-841.5)	787 (418-1120)	873 (506.8-1288)	1103 (739.8-1690)	1326 (898.5-2078)	1778.5 (1237-2629)
CD19+	2 (0-6)	14 (2.5-82.5)	27 (3-70.8)	56 (14-132.8)	148 (46.8-243.5)	268 (160.8-478.2)
CD3+	213 (78.5-531)	444 (199.5-863.8)	596 (296-974)	744 (397.5-1236)	890.5 (592.5-1533)	1152.5 (831-1813)
CD3+ CD4+	84.5 (23.2-145.8)	95 (48-147.5)	118.5 (87.8-180.5)	185.5 (108.2-285.8)	238.5 (186.2-350.5)	342 (254.5-458.2)
CD3+ CD8+	100 (33-278)	280 (87.5-563)	433.5 (189.8-874.8)	469.5 (207-837.5)	574 (345-995.2)	737 (459.8-1118)
CD56+ CD16+	202 (107.5-319.5)	166 (83-291.5)	160 (110-270.5)	172 (106.8-278.2)	177 (106-288)	237.5 (115-335.8)
BM donor chimerism	97%	90%	95%	90%	95%	97%
Peripheral blood T cell donor chimerism	87%	75%	79%	80%	96%	99%

Results: As detailed in Table 1, the proportion of full donor chimerism analyzed in the peripheral blood T lymphocytes improved progressively after transplantation and the same pattern was observed when the chimeric status was measured in unselected bone marrow cells. To favor the achievement of a full donor chimerism, DLI were performed in 26 patients starting at the median of 96 days after transplant. With a median follow-up observation of 25 months, the 1 year OS and NRM was 88% and 5%, respectively. At 6 months after allo-HSCT, the achievement of values higher than 75, 175 and 65/ L for CD4+, CD8+ and NK cells, respectively, was significantly associated to a better OS (Figure 1), DFS (p=0.05), and to a lower NRM (P<0.001 for CD4+ and CD8+, P= 0.0034 for NK). A better lymphoid reconstitution was observed after the use of either a sibling or a haplo donor than a MUD or cord blood donors. The use of ATG was significantly associated with a delayed CD4+ recovery but with a faster NK cells reconstitution.

Conclusions: At six months after alloHSCT, recovery of CD4+ and NK cells predicts survival. Monitoring of immune recovery may help to guide pre and post-transplant treatment strategies.

Table 1.

Clinical characteristics of 482 patients undergoing a HAPLO transplant

Conditioning	FLU-TBI	TBF (BU3)	TBF (BU2)	p
	9.9-12 Gy	3.2 mg/die per 3	3.2 mg/die per 2	
Number	80	209	193	
Recipient's age	30 (15-58)	49 (17-72)	61 (18-74)	
Year of Transplant	2012 (11-17)	2015 (11-17)	2015 (11-17)	
Advanced disease (>CR2)	24%	30%	44%	
Donor: HLA HAPLO family	100%	100%	100%	
Stem cell source BM	100%	100%	100%	
Rejection: n.pts (%)	1 (1.2%)	5 (2.4%)	11 (5.7%)	0.02

Allogeneic and Autologous Transplantation 2

P066

DETERMINATION OF B CELL SUBSETS IN PATIENTS RECEIVING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Bellesi, E. Metafuni, F. Marchionni, P.L. Puggioni, S. Giammarco, L. Teofili, N. Piccirillo, A. Bacigalupo, S. Sica, V. De Stefano, P. Chiusolo

Istituto di Ematologia Fondazione Policlinico A. Gemelli IRCCS Università Cattolica del Sacro Cuore, Roma, Italy

Few data exist about the role of B cell subsets in the contest of allogeneic hematopoietic stem cell transplantation (HSCT). Dysfunction of B cells may play a role in the pathogenesis of Graft versus Host disease (GvHD). Some authors described that B cells with regulatory properties were less frequent and functionally impaired in peripheral blood (PB) of patients (pts) with chronic GvHD (cGvHD). B cells were evaluated by AQUIOS Flow Cytometer by means of AQUIOS Designer Software, a tool for the creation of user-defined applications. Panel-1 CD19-FITC/CD10-PE/CD38-ECD/CD24-PC5/CD27-PC7 and Panel-2 CD19-FITC/CD5-PE/CD38-ECD/CD24-PC5/CD20-PC7 were specifically designed by Beckman Coulter for our center. Transitional B (TransB) cells were defined as CD24high/CD38high/CD27-, Mature B cells as CD24low/CD38low/CD27- and Memory B cells as CD24+/CD38-/CD27+. We enrolled 75 pts, submitted to HSCT from November 2017 to October 2018 in our center. Median age was of 56 years (range 16-69). Stem cells source was PB in 40 pts and bone marrow in 35 pts. We analyzed B cell subsets at baseline for pts and donors, in grafts products and at day +90. Mann Whitney U Test was applied to compare B cell subsets percentages and absolute count with the occurrence of GvHD. Any association was found between B cell subsets in donors and pts at baseline and acute GvHD (aGvHD) occurrence. Analyzing B cells in graft products, a lower median percentage of TransB cells was detected in pts with aGvHD as compared to pts without aGvHD (5.5% vs 9.1%, $p=0.002$). In addition, pts with aGvHD showed a higher median percentage of Memory B cells in graft product as compared to pts without aGvHD (34.8% vs 23.3%, $p=0.03$). No difference was seen for Mature B cell median percentage according to aGvHD occurrence. Multivariate analysis confirmed that TransB cell percentage in graft product could be considered as predictive variable for aGvHD development (HR 0.88, 95%CI 0.80-0.97, $p=0.008$). Among 26 pts with a follow-up of at least one year after HSCT, we found a lower level of TransB cells at day +90 after HSCT in pts who developed chronic GvHD as compared to pts without cGvHD, both in percentage ($p=0.05$; 0.01 vs 47.3%) than in absolute level ($p=0.02$; 0.0 vs 27.6/ μ l). These data need to be integrated with functional assay to confirm a regulatory activity of TransB cells. Moreover, the association between B cell subsets and GvHD need to be confirmed in a larger cohort of pts.

P067

GMP PRODUCTION AND CRYOPRESERVATION OF PURIFIED DONOR T REGS CELLS FOR INFUSION IN PATIENTS W/ CHRONIC STEROID REFRACTORY GVHD

A. Orlando¹, G. Chirumbolo¹, S. Rizzi¹, T. Montemurro², S. Budelli², M. Sessa¹, G. Tolomelli³, M. Cavo¹, F. Bonifazi¹, M. Arpinati¹

¹Institute of Hematology "L. & A. Seragnoli", Sant'Orsola Hospital; ²Cell Factory, Unit of Cellular Therapy and Cryobiology, Fondazione IRCCS Ca' Granda Ospedale, Maggiore Policlinico; ³Hematology, Infermi Hospital, Italy

We are evaluating the role of multiple infusions of purified cryopreserved donor T regulatory cells (Tregs) in patients with refractory chronic Graft Versus Host Disease (cGVHD). The effects of cryopreservation on Tregs population have not been well defined. Tregs were purified in GMP conditions from the original stem cell donors using immunomagnetic separation for depletion of CD8 and CD19 positive cells and enrichment of CD25high positive cells. Six purified Tregs products were prepared with a solution composed by sodium chloride, human albumin, DMSO and ACD-A, then divided in three fractions at escalating doses, finally cryopreserved. The infusion program includes three escalating total doses of cryopreserved Tregs for three patient cohorts (1st cohort: 0.5×10^6 cells/Kg; 2nd cohort: 1×10^6 cells/Kg; 3rd cohort: 2×10^6 cells/Kg). The cells were stored in liquid nitrogen until the infu-

sion and thawed in 37°C water bath. The patients of the first cohort have completed the treatment, while the patients of the second cohort have started the infusion program. We have analysed donor Tregs purity, viability, phenotype and inhibitory function before and after thawing. We have analysed the number of Tregs in the peripheral blood (PB) of treated patients before and after (24 h later) each infusion. The purity and recovery of Tregs were stable after thawing. However we observed a mild, but significant decrease of the viability of donor cryopreserved Tregs (shown as % of 7-AADcells): thawed Tregs mean $77.1 \pm 13\%$ vs fresh Tregs mean $90.5 \pm 9\%$ ($p \leq 0.05$). We observed that the expression of FoxP3 decreases in thawed Treg cells. CD15s expression and CD62L expression appear also to be reduced in thawed donor Tregs (mean CD15s $3.7 \pm 2\%$, mean CD62L $25.7 \pm 23\%$) vs fresh donor Tregs (mean CD15s $15.1 \pm 5\%$, mean CD62L $78.3 \pm 10\%$) ($p \leq 0.05$). However, the inhibitory capacity of thawed Tregs seems to be preserved compared to fresh Tregs. Finally PB Treg numbers increased after 24 hours in 7/10 infusions, although Treg numbers did not significantly change during the 3 month observation period. Use of donor cryopreserved GMP-isolated Tregs for immunotherapy of GVHD appears feasible. Treg purity is preserved following freezing and thawing. Expression of Treg specific markers such as FoxP3, CD15s and CD62L is affected but does not appear to significantly affect Treg function. Infusion of Treg cells may correlate with a short term increase in the number of circulating Treg cells.

P068

APPLICATION OF THE MYELOFIBROSIS TRANSPLANT SCORE (MTSS) IN ALLOGENEIC STEM CELL TRANSPLANTATION FOR PRIMARY AND SECONDARY MYELOFIBROSIS

P. Chiusolo^{1,2}, F. Sorà^{1,2}, E. Metafuni¹, S. Giammarco¹, A. Di Giovanni¹, E. Rossi^{1,2}, T. Za¹, L. Laurenti^{1,2}, I. Innocenti¹, F. Autore¹, V. De Stefano^{1,2}, A. Bacigalupo^{1,2}, S. Sica^{1,2}

¹Istituto di Ematologia Fondazione Policlinico Universitario Agostino Gemelli IRCCS Roma, ²Università Cattolica del sacro Cuore di Roma, Italy

Background: Recently Gagelman et al published a score for the evaluation of patients affected by primary and secondary myelofibrosis submitted to allogeneic stem cell transplantation (MTSS). The score consisted of the following parameters: Karnofsky performance status <90%, age >57 years, stem cell transplant with an HLA mismatched unrelated donor, the absence of CALR/MPL as driver mutations, the presence of ASXL1 as subclonal mutation, WBC at transplant $>25 \times 10^9/L$ and PLT $>150 \times 10^9/L$.

Aim: We evaluated the application of the score to a cohort of 35 consecutive patients submitted to alloSCT in our Institution.

Patients and methods: Patients' characteristics were as follow: MF 16/19, Median age 56 (range 35-72), 10pts were submitted to transplant from HLAid sibling, 1 from cord blood, 4 from unrelated donor and 20 from haploidentical donor. Conditioning regimen consisted of TBF in the majority of the pts (88%) and stem cell source was: PB in 14, cord blood in 1 and BM in 20 of them.

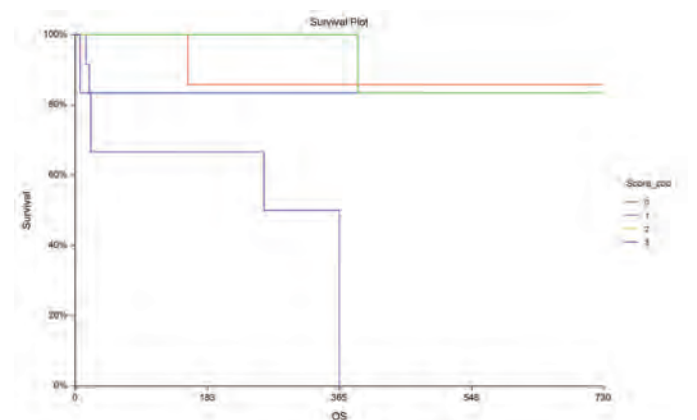


Figure 1.

Results: According to score 7 pts were included in the low score level

(LR), 12 pts in the intermediate (IR), 10pts in the high score (HR) and 6 in the very high level (VHR). The median time to PMN recovery was 23 days (range 13-94) and to PLT engraftment 38 (12-177). Eleven pts died (31%). Main causes of death were: relapse (4pts), sepsis (2 pts), multiorgan failure (2pts), GvHD (1pt), cerebral haemorrhage (1pt), pneumonia (1pt). The cumulative incidence of grade II-IV aGvHD and moderate severe cGvHD were respectively 28% and 14%. The median OS was 11 months after transplant (range 1-78). The 2ys OS and DFS according to each group were respectively 85% (LR), 83% (IR), 42% (HR) and 0% (VHR) ($p=0.009$) and 100% (LR), 100% (IR), 66% (HR) and 0% (VHR) ($p=0.0009$). The 2ys NRM was 14% (LR), 16.7% (IR), 16.7 and 50% (VHR) ($p=0.16$).

Conclusions: In our cohort of pts we confirmed the role of pre-transplant prognostication and decision making of MTSS. MTSS in fact confirmed its role in predicting OS and DFS. In particular the role was confirmed despite the kind of SCT, in fact in our casistic we had 57% of pts (20/35) submitted to haploidentical transplant, as compared to less than 2% (4/361) in Gagelman paper.

P069

HYPOFRACTIONATED TOTAL LYMPHOID IRRADIATION BY HELICAL TOMOTHERAPY AND HIGH DOSE CHEMOTHERAPY AS CONDITIONING FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA

F. Guolo, S. Vagge, P. Minetto, A. Dominiotto, F. Ballerini, L. Manconi, F.A. Canale, M. Clavio, R. Marcolin, C. Ghiggi, A. Ibatci, A.G. Congiu, M. Miglino, R. Corvò, M. Gobbi, E. Angelucci, R.M. Lemoli

Università degli studi di Genova, Ospedale Policlinico San Martino, Italy

Salvage chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard of care for patients with relapsed/refractory (R/R) lymphomas. Adding hyperfractionated total lymphoid irradiation (TLI) to conditioning regimen has shown promising results in advanced Hodgkin's Lymphoma, however, at the price of significant toxicity. Helical tomotherapy (tomo-TLI) is a novel technique that delivers highly conformal radiation dose reducing healthy tissues exposition. The aim of our study was to assess feasibility of tomo-TLI followed by high dose chemotherapy as conditioning strategy for ASCT in R/R lymphoma. From February 2011 to December 2018, 20 patients with R/R HL (n=7), diffuse Large B Cell NHL (n=11) and T-cell NHL (n=2) were treated.

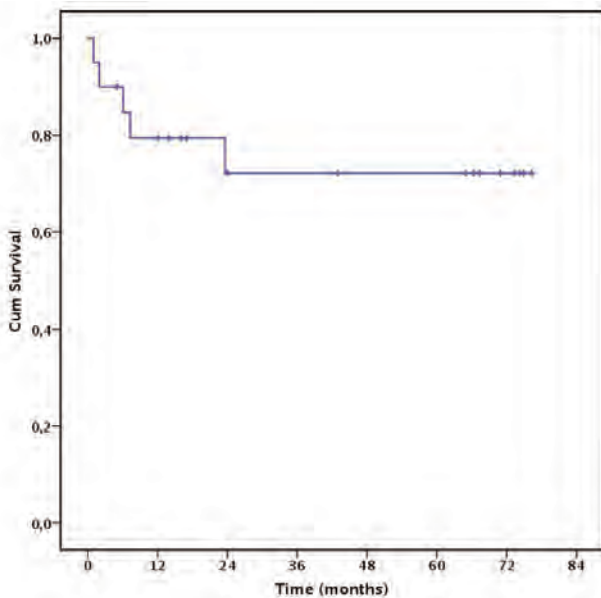


Figure 1. Overall Survival in all patients.

Median age was 42 years (range 20-68) and median previous lines of therapy was 3 (range 2-4). 4 patients had already received prior ASCT. Tomo-TLI total dose of 1200 cGy was delivered in a daily fraction of 400 cGy for 3 consecutive days, with a boost over the region of residual

disease. Conditioning chemotherapy consisted of high-dose Bendamustine (400 mg/sqm) and Melphalan 140 (mg/sqm) for patients aged >40 years (n=10) and conventional FEAM (Fotemustine, Ethoposide, Cytarabine and Melphalan) for younger patients. Salvage chemotherapy induced CR in 9 patients (5 HL, 4 NHL), PR in 5 (2 HL, 3 NHL), less than PR in 6. Eight patients (40%) experienced fever of unknown origin and 8 patients (40%) developed grade 3/4 mucositis. None experienced other grade 3/4 extra-hematological toxicity. The median number of CD34+ cells infused was $5,5 \times 10^6$ /kg (range 2,1 -11,6). All patients showed complete engraftment, median time to ANC and platelet recovery was 11 (range 9-21) and 12.5 days (range 9-21) respectively. Median follow-up was 64.9 months (CI 95% 22.5-107.3 months). All patients in PR or less before transplant achieved CR. No cases of treatment-related death were recorded. The 3-year overall PFS and OS were 55.5% and 72.8% (Figure 1) respectively. Post-ASCT relapse occurred in 9 patients (HL=2 and NHL=7) at a median time of 8 months. Our preliminary results show that tomo-TLI is safe in advanced lymphomas, reducing the damage to healthy tissues. With the limit deriving from the small size of this series, we observe that all patients achieved CR after the procedure, even if heavily pretreated, and that relapse rate was relatively low. Overall these results encourage the implementation of tomo-TLI in the standard conditioning for R/R lymphomas.

P070

MULTIPARAMETRIC PREDICTIVE SCORE FOR GRAFT VERSUS HOST DISEASE (GVHD) IN PATIENTS SUBMITTED TO ALLOGENEIC STEM CELLS TRANSPLANTATION (ALLO-SCT)

A. Turra¹, N. Polverelli¹, F. Corvini¹, E. Morello¹, M. Malagola¹, F. Arena¹, A. Bertulli¹, L. Gandolfi¹, T. Zollner¹, E. Accorsi¹, F. Cattina¹, M. Farina¹, B. Rambaldi¹, S. Bernardi², C. Zanaglio², C. Foroni², F. Re², M. Andreoli³, D. Russo¹

¹Unit of Blood Diseases and Stem Cell Transplantation, ASST Spedali Civili of Brescia, Chair of Hematology, University of Brescia, Italy; ²CREA Laboratory (Centro di Ricerca Emato-Oncologica AIL), ASST Spedali Civili of Brescia, Italy; ³Dietetics and Clinical Nutrition Unit, ASST Spedali Civili of Brescia, Italy

Background: GVHD is the major cause of morbidity and mortality for patients submitted to allo-SCT. Patient, donor and transplant characteristics, such as HLA-match, donor type, source of stem cells, female donor and age were described as predictors for acute (a) and chronic (c) GVHD. We analysed these variables together with parameters of metabolic and endocrine functions to identify possible predictors of GVHD for developing a risk score.

Methods: Clinical, transplant characteristics and laboratory data were analysed in 330 patients (Table 1) before SCT, at day +7, +14, +21, +28 and +3 months. Clinical data were: disease status, patient and donor age, sex, blood group and CMV serology. Laboratory variables were liver and renal function, protein and lipid metabolism, endocrine system tests and autoimmune parameters.

Table 1.

Patients characteristics	Patients N° (%)
Age at transplantation: median (range)	50 (17-70)
Sex:	
Male	203 (62%)
Female	127 (38%)
Diagnosis:	
Acute Leukemia	185 (56%)
Myelodysplastic Syndrome	19 (5%)
Chronic Myeloid Leukemia	9 (3%)
Lymphoma	53 (16%)
Chronic Lymphatic Leukemia	12 (4%)
Multiple Myeloma	33 (10%)
Myelofibrosis	10 (3%)
Other	9 (3%)
Status disease at transplantation:	
Up Front/1°CR	144 (44%)
2° CR	49 (15%)
Advanced	137 (41%)

Results: Total Body Irradiation (TBI) and Cyclophosphamide (HR 1,8; $p=0,011$) conditioning regimen, HLA mismatch <8/8 (HR 1,68; $p=0,024$)

and urea < 50 mg/dl at +7 day (HR 2,82; p=0,035) were evidenced as predictors for aGVHD. Score values for each factor are 1, 1 and 1,5 respectively and the score values ranged from 0 to 3,5 (Figure 1). The cumulative incidence of aGVHD was 21-37-69% at day +30 and 37-57-75% (score 0-3,5) at day +100 in low, intermediate and high risk respectively (p=0,0001). Previous aGVHD (HR 2,13; p=0.00024) and no ATG prophylaxis (HR 1,86; p=0,0018) resulted predictors for cGVHD, with score values of 1 and 1 and a score range from 0 to 2 (Figure 1). The cumulative incidence of cGVHD was 12-24-47% at month +12 and 15-30-50% (score 0-2) at month +24 in low, intermediate and high risk respectively (p=0,00001).

Discussion: Our study confirms TBI-based conditioning, HLA mismatch and no ATG use as risk factors for GVHD and revealed urea at day +7 as a novel independent prognostic factor for aGVHD that we used for developing an aGVHD score (Figure 1). Its levels depend on protein intake, endogenous catabolism and urinary excretion and may be a poor nutritional status index. The biological relevance of our results require a prospective validation and deeper studies of the complex network between metabolic, endocrine and immune-system functions.

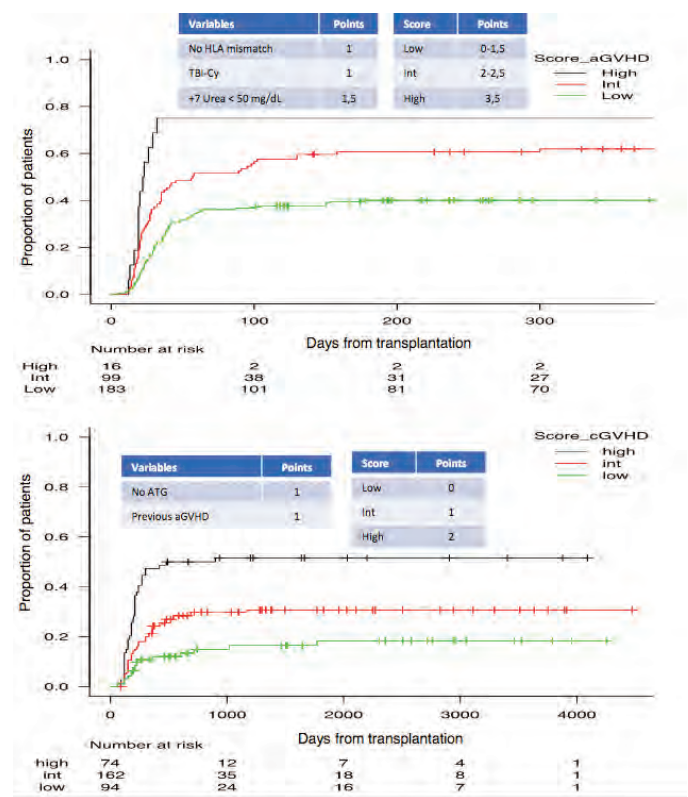


Figure 1.

P071

KINETICS OF CIRCULATING ENDOTHELIAL CELLS (CECs) DURING AUTOLOGOUS STEM CELLS TRANSPLANTATION (AUSCT) IN PATIENTS WITH MULTIPLE MYELOMA

O. Annibali¹, C. Gregorj¹, V. Tomarchio¹, M. Di Cerbo², L. Antonelli¹, D. Armiento¹, A. Scardocci¹, M. Tafuri¹, E. Circhetta¹, C. Sarlo¹, B. Vincenzi³, M.C. Tirindelli², G. Avvisati¹

Unit of ¹Hematology and Stem Cell Transplantation, ²Transfusion Medicine and Cellular Therapy and ³Oncology, University Campus Bio-medico, Rome, Italy

Introduction: CECs are considered a marker of neoangiogenesis in hematologic malignancies, in particular in MM. Recently, we proved, in a national multicenter study, the possibility to reach a high standardization level in CECs count and analysis by the use of a polychromatic flow cytometry Lyotube (BD).

Aim: The aim of our study was to assess the kinetics of CECs in patients with MM undergoing AuSCT.

Methods: MM (#26) patients responding to standard treatments were enrolled in the study. Samples of 9 ml were collected in three EDTA

tubes and for each sample, 20 106 leukocytes were processed as already described (Lanuti P. et al, Cytometry, 2016, 89.3: 259 and Lanuti P. et al, Scientific reports, 2018, 8.1: 5823). Cells, after erythrocyte-lysis step, were added to the lyophilized cocktail (Lyotube BD) of reagents plus 1 M Syto16 (Thermo Fisher Scientific, Eisai, Medipost - US). Samples were incubated in the dark for 30 minutes at 4 °C, and re-suspended in 1.5 mL of FACSFlow (BD Biosciences). By flow cytometry (FACSCanto II- BD), 1-2 106 events/sample with lymph-monocyte morphology were acquired. A threshold combination was used on Forward Scatter (FSC) and Fluorescein Isothiocyanate (FITC-Syto16) channels to get rid of very small and non-nucleated events. Compensations were calculated using CS&T bright beads (BD Biosciences). CECs were identified, as 7-ADDneg/Syto16pos/CD45neg/CD34pos/CD146pos. Venous blood samples for CECs were collected at different time points: before chemotherapy (T0), before HSC, on the day of HSC infusion (T1), 24 h after ASCT (T2), on the day of neutrophil engraftment (T3), at 100 days after transplantation (T4).

Results: Among the 26 MM patients, 11 (42%) were females and 15 (58%) males, median age was 59 years (range 38-70). All patients received high dose melphalan chemotherapy followed by AuSCT. The median CECs number at T0 was 78.0/ml. Considering the kinetics of CECs we observed a constant increase of CECs from T0 to T3 followed by decrease at T4 (Table 1). Using the median value of CECs at T3 we were able to divide our population in 2 groups > or ≤ 619 /ml. By this cut off, 9/13 patients with infective complications had a CECs value > 619/ml (p=.005).

Conclusions: This study suggest that CECs value may be a function of endothelial damage caused by conditioning regimen as demonstrated by their increase during the engraftment period. The further increase of CECs during infective complications support this pathogenetic mechanism as the main cause of CECs increase. These preliminary results require a larger population to be confirmed.

Table 1. Median values and range of CECs at different timepoints.

T ₀	T ₁	T ₂	T ₃	T ₄
78 CECs/ml (4-322)	241CECs/ml (44-1338)	239 CECs/ml (34-1288)	619 CECs/ml (46-16110)	114 CECs/ml (29-564)
Statistical Analysis by Mann-Whitney U-test				
T ₁ vs T ₀ (P=.000); T ₂ vs T ₀ (P=.000); T ₃ vs T ₀ (P=.000), T ₄ vs T ₀ (P=.032)				
T ₂ vs T ₁ (P= NS); T ₃ vs T ₁ (P=.046); T ₄ vs T ₁ (P=.001)				

P072

T /CD-19 DEPLETED HAPLOIDENTICAL TRANSPLANTATION IN RELAPSE AND REFRACTORY HODGKIN LYMPHOMA

I. Bertaggia, I. Manfra, S. Bonomini, C. Schifano, R. Segreto, G. Sammarelli, G. Todaro, M. Sassi, B. Cambò, B. Dalla Palma, A. Spolzino, F. Re, F. Russo, C. Plenteda, E. Follini, L. Craviotto, L. Pochintesta, D. Vallisa, L. Prezioso

Ematologia e CTMO Azienda Ospedaliero Universitaria di Parma, Ematologia e CTMO Azienda Ospedaliero di Piacenza, Italy

Hodgkin lymphoma (HL) is considered a curable disease, but in 10-15% of cases it is refractory or relapses after chemotherapy and autologous transplantation (autoSCT). In this setting of patients, allogeneic stem cell transplantation (alloSCT) can be considered the only potentially curative treatment because of Graft-Vs-Tumor effect exploited by donor lymphocytes. Nevertheless, both transplant-related toxicities, mainly consisting in GVHD and infections, and relapse rates in advanced stage disease, still remain a major concern. Recently, the introduction of new transplantation strategies, such as the post-transplant administration of cyclophosphamide in combination with reduced intensity conditioning regimens for unmanipulated haploidentical SCT and the improvement of graft manipulation in T-cell depleted haplo-SCT have contributed to reduce transplant related mortality and improve overall survival (OS).

Since 2013, 9 patients at median age 27 years (range 20-39) have been treated in our Unit with T/CD19 depletion approach, that removes from the graft only the -T lymphocytes, which are involved in GVHD induction, and preserve in the graft anti-tumoral and anti-infective function provided by NK cells, Dendritic cells, and -T lymphocytes. Conditioning regimen consisted of ATG 6 mg/kg, Treosulfan 36gr/sqm, Fludarabine 150mg/sqm and Thiotepa 10 mg/Kg. No additional immune suppression was given as GvHD prophylaxis. At time of diagnosis, six patients had advanced stage. All patients have been heavily pretreated, with a median of 5 prior lines of therapy. Seven patients had previously received an autoSCT and one an alloSCT from a matched unrelated donor. At the time of transplantation three patients were in second remission, while 6 patients had active disease. Relapse didn't occur in any patient. The OS was 77.8% with a median follow-up of 19 months (range 2-35). One patient died because of pulmonary infection after 4 months and one patient due to thrombotic microangiopathy after 19 months. Four patients had been treated with Nivolumab prior to Haplo-SCT, with a time range to transplantation of 1-10 months. Nevertheless, only one patient developed Skin-limited low grade GVHD, which was successfully controlled with steroids. Immune reconstitution was fast in terms of number and function of T lymphocytes. Despite the limited number of patients and the short follow up, this strategy seems to be promising and safe in heavily pre-treated patients with HL.

Acute Myeloid Leukemia 1

P073

ABSTRACT WITHDRAWN

P074

AN IDO1-RELATED 3-GENE SIGNATURE PREDICTS OVERALL SURVIVAL IN INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA

S. Ragaini¹, S. Wagner², G. Marconi¹, S. Parisi¹, C. Sartor¹, J. Nanni¹, G. Cristiano¹, A. Talami¹, M. Olivi¹, D. Ocadlikova³, M. Ciciarello¹, G. Corradi¹, E. Ottaviani³, C. Papayannidis¹, S. Paolini¹, J. Vadakekolathu², M. Cavo¹, S. Rutella², A. Curti³

¹Department of Experimental Diagnostic and Specialty Medicine Institute of Hematology "L. and A. Seràgnoli" University of Bologna; ²John van Geest Cancer Centre School of Science and Technology Nottingham Trent University Nottingham United Kingdom; ³Azienda Ospedaliero-Universitaria S. Orsola-Malpighi Department of Hematology and Oncology Institute of Hematology L. e A. Seràgnoli Bologna Italy

Introduction: ELN intermediate-risk AML poses considerable challenges to clinicians both in terms of accurate prognostication and optimal treatment. Indoleamine 2,3-dioxygenase 1 (IDO1) plays a central role as a mediator of immune tolerance in AML through the increase of Treg cells. IDO1 activity is negatively regulated by the BIN1 proto-oncogene. Herein, we analyzed the correlation between BIN1 and IDO1 expression in AML, also focusing on IDO1-interacting genes, with the aim to identify a predictive gene signature for OS.

Methods: Biological and clinical data of 732 patients with de novo AML were retrieved from public TCGA and HOVON datasets. Since details on chemotherapy regimens were not available in the HOVON dataset, we decided to exclude patients ≥ 65 years from survival analyses. IDO1-interacting genes were selected through a co-expression analysis performed on TCGA RNA-sequencing data accessed through cBioPortal. For each patient, we computed a gene expression score. Patients were split in three different groups using quartiles as a cut-off.

Results: In the HOVON dataset, IDO1 and BIN1 mRNA expression were negatively correlated ($r = -0.40$, $p < 0.0001$). Our analysis of TCGA data identified PLXNC1 as an IDO1-interacting gene and a predictor of patient survival (median split of mRNA expression, $p < 0.001$, survival analysis performed on the BloodSpot online portal). The correlation between PLXNC1 and IDO1 was validated in the HOVON dataset ($r = 0.24$, $p < 0.0001$). PLXNC1 expression was combined with IDO1 and BIN1 expression to obtain a gene expression score. The 3-gene score predicted survival in ELN intermediate-risk patients who did not receive allogeneic HSCT both in the HOVON dataset ($p < 0.0001$) and the TCGA dataset ($p < 0.05$). In particular, the highest expression of the three genes predicted the shortest OS.

Conclusions: Our study shows a negative correlation between IDO1 and BIN1 in AML, suggesting IDO1 inhibition by BIN1, and identifies for the first time PLXNC1, a receptor for semaphorines, as an IDO1-interacting gene potentially implicated in immune response regulation. This finding corroborates the role of IDO1 and its interacting genes in the promotion of a tolerogenic microenvironment in AML. Lastly, our gene expression score predicted OS in intermediate-risk AML patients not undergoing HSCT, a finding which has clinical implications for accurate patient stratification and for clinical decision making, i.e., bridging these patients to transplant.

P075

MONITORING RISK OF RELAPSE IN ACUTE MYELOID LEUKEMIA BY MULTIPARAMETRIC FLOW CITOMETRY (MFC): A CLINICAL ANALYSIS IN A PARTICULAR SUBSET OF PATIENTS

D. Gramegna, E. Borlenghi, M. Chiarini, V. Giustini, C. Cattaneo, E. Cerqui, A. Passi, C. Pagani, G. Rossi

ASST- Spedali Civili U.O Ematologia, Brescia, Italy

Postremission treatment in acute myeloid leukemia (AML) is mandatory, considering the high frequency of relapse after hematological complete remission (CR). Measurable residual disease (MRD) adds independent prognostic information to CR for risk stratification and treatment planning. Pending validation of NGS methods, a molecular approach by qPCR is currently the gold standard, but its applicability is limited to patients (pts) with available molecular markers. In pts with normal karyotype (NK) and lacking molecular abnormalities, MRD determination would be particularly useful, considering their still debated indication to allogeneic transplant. The aim of this study was to analyze the results of MRD analysis using multiparameter flow cytometry (MFC) in this subset of pts outside of clinical trials. At diagnosis a panel of 22 monoclonal antibodies was employed to detect leukemia-associated immunophenotypes (LAIPs). We evaluated MRD at 2 time points: after induction (TP1) and after consolidation (TP2) with positive thresholds at $>0.1\%$ and $>0.035\%$, respectively. Relapse-free survival (RFS) and overall survival (OS) were analyzed. Among 198 consecutive de novo non-M3 AML pts, aged 18 to 69y (median 53), treated at our Institution from Jan 2010 to Dec 2018, according to NILG protocol (EUDRACT: NCT00400673), 111 (56%) had NK and 26 of them (23.4%) lacked any molecular marker. CR after induction was obtained in 23/26 pts (88%) and in 14 (61%) LAIP was available. At TP1, 7 of 10 (70%) MFC-MRD-pos pts relapsed vs 1 of 4 MFC-MRD-neg ($p=0.24$). Evaluation at TP2 had higher accuracy: MFC-MRD was detected in 8 of 14 pts (57.1%), 7 of whom (87.5%) relapsed after a median of 6 months (2-41mo). Among 6 MFC-MRD-neg pts, only 1 relapsed after 27 mo (16%) ($p=0.025$). Clinical characteristics of MFC-MRD pos or neg pts were similar. At TP2 the median RFS was 9 mo in MFC-MRD-pos vs 46 mo in MFC-MRD-neg pts ($P=0.0064$); median OS was 15.7 mo in MFC-MRD-pos vs not reached in MFC-MRD-neg pts ($p=0.026$) (fig.1). Of 17 relapsed pts only 7 (41%) obtained 2nd CR and could receive allo-SCT in remission. In conclusion this real-life study highlights the usefulness of MFC-MRD in the subset of AML pts with NK lacking alternative molecular markers. In spite of the small number of pts analyzed, the high statistical significance reached as a relapse-predicting tool, while deserving confirmation on larger cohorts, may represent an indication to perform allo-SCT before overt relapse in TP2 MFC-MRD-pos pts.

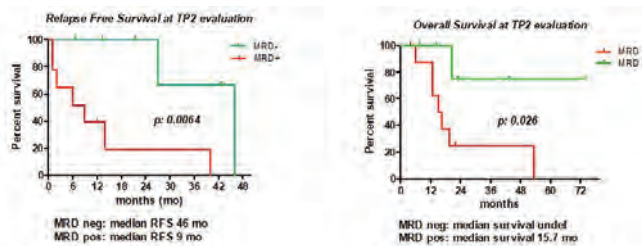


Figure 1.

P076

EXTRAMEDULLARY LOCALIZATIONS IN ACUTE MYELOID LEUKEMIA. AN EIGHT-YEAR MONOCENTRIC EXPERIENCE

L. Fianchi, M. Quattrone, M. Criscuolo, S. Bellesi, F. Sorà, P. Chiusolo, M. Bonanni, G. Dragonetti, S. Sica, A. Bacigalupo, L. Pagano

Dipartimento di diagnostica per immagini, radioterapia oncologica ed ematologia-Area Ematologica, Fondazione Policlinico Universitario A. Gemelli, IRCCS/Università Cattolica del Sacro Cuore, Italy

Background: The incidence, risk factors and prognostic significance of extramedullary involvement (EMI) in adult patients (pts) with acute myeloid leukemia (AML) have not been established yet.

Aims: This study analyzes clinical and biological characteristics, impact on prognosis and cumulative incidence of EMI in a monocentric retrospective study.

Methods: All adult pts with a diagnosis of AML observed in our institution between January 2010 and December 2017 were analyzed registering all cases of EMI; only pts who experienced EMI at the onset of AML and receiving induction therapy were evaluated for disease free survival (DFS) and overall survival (OS)

Results: Overall 346 AML were analyzed. The incidence of EMI was 11% (38 pts). The involved sites were: skin (66%), CNS (23%), pleura (7%), lymph nodes (5%), peritoneum (2%), spleen (2%), pancreas (2%), breasts (2%) and bones (2%). Most pts (91%) had only one site of EMI, while 9% had multiple sites affected. Twenty-eight (74%) pts showed signs of EMI at presentation of AML, while in the remaining 10 cases EMI occurred at relapse (26%). Cytogenetic, molecular, clinical and laboratory parameters were analyzed in order to identify risk factors. EMI had a significantly higher frequency in pts with monocytic and myelomonocytic leukemia subtypes ($p<0.0001$), MLL rearrangements ($p=0.001$), trisomy 8 ($p=0.02$) and a specific cytofluorimetry pattern (CD117-, $p=0.03$; CD56-/CD117-, $p=0.04$; CD56+/CD117-, $p=0.04$). 28 EMI pts experienced EMI at the onset of AML; 27/28 pts were treated with: conventional chemotherapy (21), hypomethylating agent (5) or low dose citarabine (1); 8 pts (28.5%) received consolidation with allogeneic stem cell transplantation (allo-HSCT). Complete remission (CR) rate after induction therapy was 22% with a median DFS of 7.4 months (range 2-79). Median OS of all 27 EMI pts was 11.6 months (range 2-79); this resulted significantly longer for 8 EMI pts who undergone allo-HSCT than OS of the remaining 19 EMI pts (16.7 vs 8.2 months respectively $p=0.02$). No differences emerged in OS according to site of EMI. Univariate and multivariate analyses showed that undergoing allo-HSCT was the main positive prognostic factors for survival in our population ($p<0.0001$).

Conclusions: Our data confirm poor prognosis for EMI pts with a median OS lower than 1 years. The allo-HSCT, applicable however only in some cases, seems to have a crucial role in the therapeutic approach of these pts, being associated with a better prognosis.

P077

ROLE OF FRAILTY SCORES IN VALIDATING FITNESS OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS: A SINGLE CENTRE STUDY

S. Aydin¹, M. Franzino¹, C. Frairia¹, E. Audisio¹, S. D'Ardia¹, G. Iovino¹, I. Urbino¹, A. Busca², C. Dellacasa², A. Evangelista³, G. Ciccone³, U. Vitolo¹

¹Hematology, AOU Città della Salute e della Scienza di Torino, Italy; ²Dept. of Oncology and Hematology, Stem Cell Transplant Center, AOU Città della Salute e della Scienza di Torino, Italy; ³Unit of Cancer Epidemiology, CPO Piemonte, AOU Città della Salute e della Scienza di Torino, University of Turin, Italy

Background: Intensive chemotherapy in elderly acute myeloid leukemia (AML) patients (pts) improves survival. But defining "pts fitness" requires objective criteria. Aim of the present study was to investigate three validation scores in assessing pts fitness at diagnosis in parallel to physician evaluation.

Methods: A total of 130 elderly (>60 years) newly diagnosed AML pts were evaluated by an haematologist and subsequently by an interdisciplinary board leading to therapy decision. In parallel and independently three scores were performed: i) a regional geriatric G8 screening tool, consisting of seven items from the Mini Nutritional Assessment

questionnaire, ii) the Sorror Index used for hematopoietic stem cell transplantation (HSCT) evaluation and iii) the AML score of the German AML Cooperative Group, predicting probability of complete remission (CR) and early death (ED) after intensive induction chemotherapy. Therapy response was defined by ELN criteria. Overall survival (OS) from diagnosis was compared between groups using the Cox model.

Results: Median age was 71,8 years (range 60-86). A total of 75 (57,7%) pts were evaluated "fit" by the medical board and treated intensively, 41 (31,5%) underwent semi-intensive therapy and 14 (10,7%) best supportive care. Fifty pts (38,5%) achieved a CR (10 successively allogeneic HSCT). Sixty-four (49,2%) were non responders and 16 (12,3%) died during the first cycle. Overall, the median OS was 11,2 months (95% CI 7,9-17,8). According to primary physician care evaluation a "fit" patient had a median OS of 17,6 months (95% CI 9-28,9) compared to "unfit" evaluated pts with 3,6 months (95% CI 1,8-8,8), $p < 0,001$ (HR unfit vs fit of: 3,04, 95% CI: 1,80-5,15). The local G8 screening tool distinguished "fit" pts with a median OS of 18,7 from "unfit" pts with a median OS of 7,9 months (HR unfit vs fit: 2,1, 95% CI: 1,23-3,58), $p = 0,007$. The Sorror Index (HR 2,14 with a 95% CI of 1,27-3,59) as well as the AML score ED (HR 1,94 with 95% CI of 1,16-3,24) separated also significantly "fit" from "unfit" pts, $p = 0,004$ and $p = 0,011$, respectively. There was no correlation between the scores as analyzed by the Spearman Correlation coefficient.

In conclusion, the frailty scores G8, Sorror Index and the AML Score discriminate significantly pts in terms of OS. They may represent an additional tool in frailty validation of elderly AML. In order to confirm this discrimination ability, a multi-centre study is planned.

P078

COMPARATIVE CLINICAL EFFECTIVENESS OF AZACITIDINE VERSUS DECITABINE FOR THE FRONT LINE TREATMENT OF ELDERLY ACUTE MYELOID LEUKEMIA (AML) PATIENTS

L. Maurillo, A. Candoni, A. Spagnoli, C. Papayannidis, E. Borlenghi, D. Lazzarotto, L. Fianchi, M. Sciumè, M.E. Zannier, F. Buccisano, M. Riva, M. Breccia, R. Fanin, E. Todisco, M. Lunghi, E. De Bellis, N. Fracchiolla, P. Musto, A. Venditti, G. Rossi

Dipartimento Oncoematologia Fondazione Policlinico Tor Vergata, Ematologia Azienda Sanitaria Universitaria Integrata, Università La Sapienza, Istituto di Ematologia BL and A. Seràgnoli, Asst Spedalicivili, Ematologia Azienda Sanitaria Universitaria Integrata, Ematologia Università Cattolica Sacro Cuore, Asst Spedalicivili, Ematologia Azienda Sanitaria Universitaria Integrata, Dipartimento Biomedicina e Prevenzione Università Tor Vergata, Ematologia Ospedale Niguarda, Ematologia Università La Sapienza, Azienda Sanitaria Universitaria Integrata, Divisione Oncoematologia IEO, SCU Ematologia Università del Piemonte Orientale, Dipartimento Onco-Ematologia Fondazione Policlinico Tor Vergata, U.O. Oncoematologia Fondazione IRCCS Ca' Granda Ospedale Maggiore, IRCCS CROB, Dipartimento Biomedicina e Prevenzione Università Tor Vergata, Asst Spedalicivili

The present observational retrospective multicentric study aimed to compare the efficacy of azacitidine (AZA) and decitabine (DEC) in elderly patients with previously untreated AML, diagnosed according to WHO criteria. In the two groups, we evaluated complete remission (CR), overall survival (OS) and disease free survival (DFS). The AZA and DEC cohort included 139 and 186 patients, respectively. To minimize the effects of treatment selection and observed confounding bias, adjustments were made using the propensity-score matching method for the following baseline characteristics: age, white blood cell count (WBCc) at treatment onset, cytogenetic risk group, AML type (de novo or secondary) and ECOG performance status (PS). The propensity score yielded two matched cohorts of 136 patients. In the AZA and DEC cohort, median age was 75 years in both groups, (range 61-88 and 61-85, respectively), median WBCc at treatment onset $2.5 \times 10^9/L$ (range 0.27-83) and $2.9 \times 10^9/L$ (range 0.34-75), median bone marrow (BM) blast count 36% (range 20-95%) and 35% (range 20-100%), respectively; 59 (43%) and 63 (46%) patients had a secondary AML, respectively. Karyotype was evaluable in 115 and 120 patients and, according to the refined Medical Research Council criteria, 80 (59%) and 87 (64%) had intermediate-risk abnormalities, 35 (26%) and 33 (24%) an adverse risk karyotype, respectively. The median number of AZA and DEC cycles delivered was 6 (range 1-60) and 4 (range 1-26). In AZA e DEC cohort, overall response rate was 39% and 35%, respectively; CR was 24%

and 29% and partial remission (PR) 15% and 6%, respectively. OS was not significantly different between AZA and DEC cohort: in fact, 2-year OS rates and median OS for AZA and DEC were 24% vs 26% and 12.6 vs 13.1 months ($p = ns$), respectively (Figure 1). Among patients with intermediate- and adverse-risk cytogenetic, frequency of WBCc $> 10 \times 10^9/L$ and $< 10 \times 10^9/L$, de novo and secondary AML, BM blast count $<$ and $\geq 30\%$, median OS of AZA and DEC cohort were 17.3 vs 15.8, 7.3 vs 7.1, 5.4 vs 7, 13.8 vs 13, 12.8 vs 11.9, 12 vs 16 months, 13.2 vs 15.8 and 10.9 vs 12 months, respectively ($p = ns$). DFS of patients who achieved CR/PR with AZA or DEC was 8 vs 11 months, respectively ($p = ns$). Our analysis indicates similar outcomes with AZA compared to DEC. Controlled, randomized clinical trials should be performed to confirm such a conclusion.

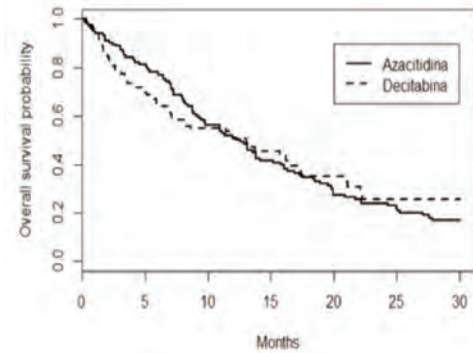


Figure 1.

P079

UP-REGULATION OF IMMUNE TOLERANCE GENES IN LEUKEMIC MESENCHYMAL STROMAL CELLS IS INDUCED BY ACUTE MYELOID LEUKEMIA CELLS THROUGH AN IFN-GAMMA-DEPENDENT INFLAMMATORY SIGNALING

M. Ciciarello, G. Corradi, S. Sangaletti, B. Bassani, G. Simonetti, G. Marconi, M. Colombo, G. Martinelli, M. Cavo, A. Curti

Azienda Ospedaliero-Universitaria S. Orsola-Malpighi Department of Experimental, Diagnostic and Specialty Medicine University of Bologna Institute of Hematology L. e A. Seràgnoli, Fondazione IRCCS, Istituto Nazionale dei Tumori; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST)

Mesenchymal stromal cells (MSCs) are a key component of the bone marrow (BM) niche by regulating hematopoietic stem cell (HSC) fate and having a unique immune-modulatory capacity mostly mediated by the Indoleamine 2,3-dioxygenase (IDO)-1 enzyme activity. Thus, in the leukemic milieu, MSCs not only can favor leukemic cell survival but also they can generate an immune-tolerant environment. Although recent findings have outlined a putative MSC role in hematological malignancies, MSC-dependent mechanisms potentially supporting leukaemia remain unclear. We hypothesize that leukemic cells can induce in BM-MSCs functional changes able to convert the BM microenvironment from hostile to permissive for leukaemia. We isolated acute myeloid leukaemia (AML) cells and generated AML-MSCs from the BM of AML patients. Next, we investigated gene expression in AML-MSCs and AML cells before and after co-culture experiments. In a Gene expression profile screening, we found that almost 40% of AML samples show an IFN- γ expression higher than the mean level of IFN- γ expression in healthy donors (HDs). In AML-MSC/AML cell co-culture experiments, we confirmed that AML cells produced IFN-gamma. Then, we analyzed MSC expression of IFN-gamma stimulated genes (ISGs) (e.g. IDO-1, Programmed death-ligand (PDL)-1, Nitric Oxide synthase (NOS)-2), which are known to regulate immunity and tolerance. In particular, we found that IFN-gamma positive, but not IFN-gamma negative AML cells, induced ISG expression in AML-MSCs, in a way similar to recombinant IFN-gamma. Moreover, an IFN- γ neutralizing antibody abrogated ISG upregulation. Finally, we found that AML-MSCs, after co-culture with IFN- γ -positive AML cells, were able to induce regulatory T cell in an IDO1-dependent manner. Our data suggest that inflammatory signals produced by AML cells are able to modify MSC functions, thus favouring an immune-tolerant and leukaemia supporting milieu. Overall, our results would likely contribute to unravel

MSC-dependent mechanisms promoting leukaemia and will help to provide novel applications for drugs already under experimentation (e.g. IDO-inhibitors, Checkpoint inhibitors) to translate into more effective therapies in AML patients.

P080

VALIDATION OF SIE, SIES, GITMO OPERATIONAL CRITERIA FOR THE DEFINITION OF FITNESS IN ACUTE MYELOID LEUKEMIA: A RETROSPECTIVE, MONOCENTRIC, OBSERVATIONAL STUDY

R. Palmieri¹, G. Paterno¹, E. De Bellis¹, E. Buzzatti¹, V. Rossi¹, A. Di Veroli², F. Esposito¹, C. Gurnari¹, P. Casciani¹, M.I. Del Principe¹, L. Maurillo¹, M.T. Voso¹, W. Arcese³, A. Venditti¹, F. Buccisano¹

¹Ematologia Dipartimento di Biomedicina e Prevenzione Università di Roma "Tor Vergata"; ²UOC Ematologia Ospedale Bel Colle; ³Ematologia Dipartimento di Biomedicina e Prevenzione Università di Roma "Tor Vergata", Rome Transplant Network, Italy

Background: Acute Myeloid Leukemia (AML) is considered a disease of the elderly, with a median age of 65 years (yrs) at onset. In the absence of shared scales of fitness, experts from the SIE (Italian Society of Hematology), SIES (Italian Society of Experimental Hematology) and GITMO (Italian Group for Bone Marrow Transplantation) summarized a list of criteria, identifying 3 modalities of treatment: 1) intensive chemotherapy (IC), to achieve complete remission (CR) for fit pts (FP); 2) non-intensive chemotherapy (NIC), to prolong survival, for unfit pts (UP); 3) supportive therapy (ST), to improve quality of life for frail pts (FrP) (Ferrara *et al.*, Leukemia 2013).

Aim: To retrospectively validate SIE, SIES, GITMO criteria in a series of AML pts diagnosed at our institution without age limit. Moreover to investigate the impact of fitness on Overall Survival (OS) and of treatment choice (tc) on the 3 categories.

Methods: From 2016 to 2018, 97 consecutive pts with a diagnosis of de novo AML were selected in the context of a harmonized diagnostic and care pathway program. SIE, SIES, GITMO criteria were retrospectively applied and agreement with the real-life treatment allocation was assessed.

Results: Overall, pts median age was 70 yrs (range 30-92), mostly above 65y (65/97, 67%). Forty-3/97 (44%) pts received IC, 26/97 (27%) NIC, 28/97 (29%) ST. High concordance was observed between tc and the one derived from the expert's criteria (90/97, 93%), especially for FrP pts (27/27, 100%), with minor discordance among the FP (42/47, 89%) and the UP (21/23, 91%) groups. The experts' criteria discriminated 3 categories (Figure 1) with a significantly different OS: 13.0 mos. (range 0.9-39.3) for FP vs 6.3 mos. (range 1.7-35.6) for UP and 0.9 mos. (range 0.9-2.0) for FrP, respectively ($p < 0.001$). When analyzing the correspondence between fitness and tc, the poor outcome of FrP (OS 1.0 mos, range 0.1-7.4) was confirmed, while for pts allocated to IC and NIC, OS slightly differed (10.6 mos., range 0.9-10.6, vs 7.9 mos., range 1.7-7.9, respectively) (overall $p < 0.001$). Higher incidence of toxicity of IC among older patients and NIC efficacy improvement may explain this similarity.

Conclusions: The SIE, SIES, GITMO criteria, regardless of age, reliably predict outcome and represent a valuable tool to modulate treatment intensity. Prospective validation of this process is locally underway.

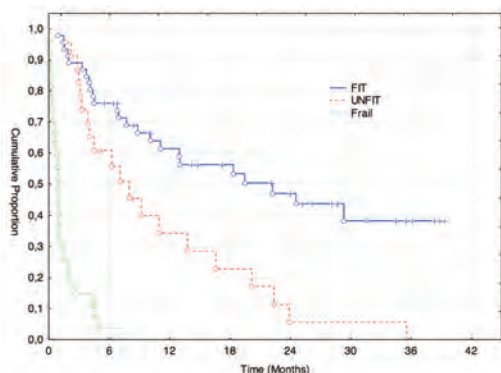


Figure 1.

P081

ABSTRACT WITHDRAWN

P082

IDENTIFICATION OF MUTATIONS AND MRD MONITORING BY NEXT GENERATION SEQUENCING IN ACUTE MYELOID LEUKEMIA: PRELIMINARY RESULTS FROM A PROSPECTIVE OBSERVATIONAL STUDY

C. Steidl, G.G. Sharma, C. Mastini, M. Fumagalli, P. Perfetti, L. Borin, C. Valentini, R. Piazza, C. Gambacorti-Passerini

University of Milano-Bicocca, Ospedale San Gerardo di Monza, Italy

Acute myeloid leukemia (AML) identifies a heterogeneous group of clonal disorders of the blood, both clinically and genetically. Numerous mutations have been described in AML, although only a few of them are currently employed in clinical practice to define prognosis and risk-oriented treatment. Moreover, Minimal Residual Disease (MRD) has been recognized as a potent, highly independent prognostic factor, especially in intermediate-risk AML. Next generation sequencing (NGS) allows for better understanding of the complex genetic background in AML and may direct individualized therapeutic strategies. In this study, we aim to identify mutations that are not routinely investigated in AML patients using a new NGS capture-based panel encompassing 101 genes and to evaluate how their oncogenic potential correlates with PFS. Secondary endpoints are: percentage of patients with actionable mutations detected by NGS and Overall Survival. Twenty-four patients aged 18 to 72 years with newly diagnosed AML were enrolled between January 2018 and April 2019. So far, 19 patients have undergone NGS-analysis. Twenty-eight mutations were detected in 15 patients (78.94%), with 5 patients (26.31%) harboring multiple mutations. We identified 6 actionable mutations in 6 patients (31.57%). In 4 patients (21.05%) with no detectable mutations using routine screening techniques, we were able to identify 6 genetic abnormalities. Eight fusion genes were also discovered in 7 patients (36.84%) using the TruSight panel coupled with our FusionAnalyzer bioinformatic tool. Furthermore, employing our custom-designed panel, we found 3 mutations (10.71%) in the BCOR, U2AF2 and EP300 genes, that are not covered by other NGS-based diagnostic solutions available on the market. Each mutation was weighed using OncoScore (<http://www.galseq.com/oncoscore.html>), a text-mining tool that ranks genes according to their oncogenic potential. The total OncoScore of our 19 patients ranged between 50.63 and 293.31 (median 106.66). In 5 patients (26.31%) with no available MRD markers at diagnosis, we were able to perform NGS-based MRD monitoring at different time points after the induction cycle using targeted deep sequencing. In all these patients, we observed mutational clearance after induction cycle. Overall, our study shows that NGS is a powerful and reliable tool in AML and should be employed both in routine diagnostic work up and in follow up analysis. Updated results will be presented at the meeting.

P083

A BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM-LIKE PHENOTYPE IDENTIFIES A SUBGROUP OF NPM1-MUTATED AML PATIENTS WITH WORSE PROGNOSIS WHILE HAS NOT PREDICTIVE VALUE IN NPM1-WT AML

P. Minetto, F. Guolo, M. Clavio, E. Tedone, R. Marcolin, M. Passanante, F. Ballerini, M. Miglino, A. Cagnetta, M. Cea, M. Gobbi, R.M. Lemoli

Università degli Studi di Genova, Ospedale Policlinico San Martino, Italy

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) are a rare and aggressive group of diseases included by WHO 2016 classification among the myeloid neoplasms, characterized by a recurrent phenotype (CD45^{low}/CD34⁺/CD56⁺/CD4⁺/CD123⁺), in the absence of lineage differentiation. We recently reported in acute myeloid leukemia (AML) patients with mutated NPM1 (NPM-AML) that a "BPDCN-like" phenotype, defined by the combination of at least two among CD56⁺/CD4⁺/CD123⁺, correlated with a dismal prognosis. The aim of the present study was to evaluate the incidence and the prognostic impact of BPDCN-like phenotype in a wider cohort of cytogenetically normal AML patients, irrespectively of NPM1-mutational status. We analyzed 83 younger (age <60 yrs), consecutive, AML patients with

normal karyotype, who have been intensively treated with the same fludarabine-containing induction and with immunophenotypic analysis available at diagnosis. Fifteen patients (18%) showed a BPDCN-like phenotype. The presence of BPDCN-like phenotype was not significantly correlated with NPM1, FLT3-ITD or biallelic CEBPA mutation. Median follow-up was 63 months, 3-year Overall Survival (OS) was 52%. In the whole cohort, NPM1 mutation or biallelic CEBPA mutation correlated with a better outcome ($p < 0.03$), whereas survival was not significantly influenced by the presence of FLT3-ITD mutation or BPDCN-like phenotype. However, as we previously reported, among the 30 patients with NPM1-mut AML, the presence of BPDCN-like phenotype conferred a dismal prognosis, if compared to BPDCN-negative patients (3-year OS 25% vs 77%, respectively, $p < 0.001$), irrespectively of mutational status for FLT3-ITD or other clinical features. Even if CR rate was not affected, all NPM1-mut patient with BPDCN-like phenotype failed to achieve minimal residual disease (MRD) clearance ($p < 0.05$). On the contrary, in the 53 NPM1-wt patients, presence of BPDCN-like phenotype did not correlate with a worse outcome or MRD clearance probability, if compared to BPDCN-negative patients (3-year OS 71% vs 35%, respectively, $p = 0.156$). Our extended analysis confirm that a peculiar BPDCN-like immunophenotype among NPM1-mut AML patients is associated with poor outcome. Interestingly, this observation was strictly restricted to NPM1-mutated AML, suggesting that BPDCN-like phenotype may identify a distinct subgroup among NPM-AML. Further gene-expression profiling studies are ongoing in order to explain our findings.

AML. We discovered different intermediates of lipid metabolism, as decreased 1-(1-enyl-palmitoyl)-2-palmitoyl-GPC ($p = 0,0049$), or involved in Krebs's Cycle, as reduced fumarate ($p = 0,0393$) in relapsed AML. Conversely, we observed a higher concentration of UDP-N acetylglucosamine/galactosamine ($p = 0,0044$), which is part of carbohydrate metabolism. By comparing CD34+ cells from relapsed or newly-diagnosed AML with cord blood, we defined a set of metabolic features which may suggest metabolic adaptation of the post-chemotherapy clone. It is worth noting the decrease in uridine concentration ($p = 0,03$), a metabolite of pyrimidine metabolism. Pyrimidine pathway is of great interest thanks to the development of new drugs as dihydroorotate dehydrogenase inhibitor, that decreases the levels of leukemia-initiating cells. Relapsed cells show reduced orotidine levels compared with cells from newly-diagnosed patients. KEGG and SMPDB annotation revealed enrichment for changes in compounds involved in Ketone Body Metabolism, Citric Acid Cycle and Mitochondrial Electron Transport Chain. Our data are in line with a metabolic reprogramming of AML cells at relapse, including a shift from pyruvate oxidation to fatty acid -oxidation, with altered mitochondrial oxidative phosphorylation in leukemia progenitor cells. The results suggest metabolic vulnerabilities of relapsed patients which could be novel therapy targets.

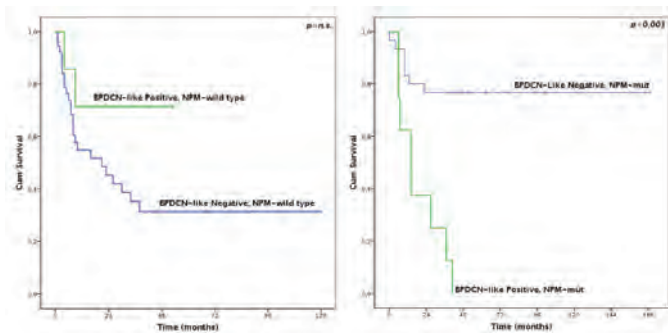


Figure 1. OS survival according to BPDCN-like signature in NPM1 wild type (A) and NPM1 mutated (B) patients.

P084

METABOLIC ANALYSIS OF CD34+ BONE MARROW STEM PROGENITORS IN RELAPSED AML PATIENTS

M. Ghetti, A. Padella, M. Pazzaglia, R. De Tommaso, M. Fontana, S. Bruno, M.T. Bochicchio, A. Ferrari, R. Napolitano, E. Franchini, J. Nanni, G. Marconi, C. Papayannidis, D. Remondini, G. Martinelli, G. Simonetti

Istituto Scientifico Romagnolo per lo Studio e Cura dei Tumori (IRST) IRCCS, Department of Experimental, Diagnostic and Specialty Medicine, "Seràgnoli" Institute of Hematology, University of Bologna, Department of Physics and Astronomy, University of Bologna, Italy

Leukemia stem cells are likely responsible for disease relapse. Several genomic studies of newly-diagnosed and relapsed AML have revealed different patterns of clonal evolution, but little is known about metabolic dysregulations among these two groups. Metabolomics can identify endogenous cell metabolite biomarkers for monitoring of disease progression. We aimed to identify relapse-specific metabolic alterations of CD34+ AML stem-progenitor cells. We analyzed metabolites using mass spectrometry (Metabolon) of CD34+ bone-marrow stem progenitor cells of 22 newly-diagnosed and 13 relapsed AML patients. We also compared our samples with CD34+ cells from umbilical cord blood (21 donors). Statistically-significant differences in metabolite concentrations between the groups were evaluated by Welch's t-test. KEGG and SMPDB annotation helped us in identifying the most relevant differences in terms of metabolic pathways. We identified differences in the concentration of 16 metabolites between newly-diagnosed and relapsed

Acute Lymphatic Leukemia

P085

ADULT TRIPLE NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA FUSION DETECTION: THE CHALLENGE FOR ALTERNATIVE THERAPIES

A. Ferrari¹, S. Vitali², C. Baldazzi³, G. Ferrari³, V. Robustelli³, A. Ghelli¹, E. Fonzi¹, M. Tebaldi¹, S. Salvi¹, C. Papayannidis³, S. Paolini³, A. Padella³, M.T. Bochicchio¹, G. Pasquini², G. Simonetti¹, G. Musuraca¹, D. Cangini¹, B.M. Giannini¹, C. Cerchione¹, A. Santoro⁴, J.M. Hernández-Rivas⁵, D. Calistri¹, N. Testoni³, D. Remondini², G. Martinelli⁶

¹Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS; ²Dipartimento di Fisica e Astronomia INFN Università di Bologna; ³Dipartimento di Medicina Specialistica Diagnostica e Sperimentale (DIMES). Ist. Di Ematologia; ⁴Hematology department A.O. Ospedali Riuniti Villa Sofia-Cervello; ⁵Fundación de Investigación del Cáncer de la Universidad de Salamanca; ⁶Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Italy

Background: The adult “triple negative” [Ph-/-; neg for t(9;22); t(1;19); t(4;11)]; 61% of adult B-ALL (Roberts KG, J Clin Oncol. 2017)] B-ALL group is characterized by a high genetic heterogeneity, lack of innovative target therapies and poor survival. Many chimeric genes have been recently identified (Gu Z, Nat Genet. 2019 Jan 14), that led to a refined classification of B-ALL and to, in some cases, tailored therapies; but identified them (also Ph-like) is challenging, cause to genetic complexity, low frequency and to expensive RNA-NGS approaches with hard bioinformatics pipelines.

Aims: To adopt RNAseq strategy on all Ph-/- pts to identify novel/described Ph-/- B-ALL subtypes/fusions in order to assess know or unknown fused targetable biomarkers. **Patients and methods:** we performed integrative genomic analysis including 1385 RNAseq gene Panel (illumina) and/or transcriptome sequencing (RNA-seq) of 60 Ph-/- cases from adult cooperative group studies. Fusion analyses were performed using 3 tools.

Results: We identify fusion genes, not conventionally detected, in 33 patients (pts) with a fusions rate of 55% (33/60), denoting that Ph-/- are not deeply characterized. In 10 cases, we found 2 fusions/pt and in 5 of them we capture also the reciprocal fusions. Many of these fusions were previously described in Ph- B-ALL (i.e. ZNF384-TCF3/EP300/TAF15, MEF2D-BCL9, KMT2A/MLL1, ABL1-2/RCSD1 and PAX5/ETV6) or in other diseases (i.e. NONO/TFE3; in Renal cell carcinoma). We studied 6 cases of paired diagnosis and relapse: we found 3 different patterns of evolution/involution. Notably a novel fusion was found also at the second relapse after the second relapse. Ph-/- fusion detection help to resub-classify our patients in the new B-ALL subgroups (23/33; 69.7%). We found: a) Rare MLL fusions 5% (3/60); b) MEF2Dr, PAX5alt and probable RUNX1-ETV6 like: 1,6% (1/60); c) ZNF384r: 11,6% (7/60); d) DUX4/ERG and BCL2/MYC: 3,3% (2/60); e) probable Ph-like fusions: 10% (6/60); DUX4.

Conclusions: We identified a high rate of “secondary” fusions in adult Ph-/- B-ALL patients (55%) that, considering the ample range of new described fusions, are difficult to well characterized with conventional diagnostic

Methods: A wide multigene NGS fusion approach is needed to detect fusions useful for a better classification and in some cases to find targetable fusions (i.e. ABL1-2/RCSD1), to give poor outcome pts an alternative therapy.

Supported by: ALL, FP7 NGS-PTL project, Harmony.

P086

NELARABINE AS SALVAGE THERAPY AND BRIDGE TO ALLOGENEIC STEM CELLS TRANSPLANTATION IN 118 ADULT PATIENTS WITH RELAPSED/REFRACTORY T-ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOMA. A CAMPUS-ALL, PHASE 4, STUDY

A. Candoni¹, D. Lazzarotto¹, A. Curti², F. Ferrara³, F. Lussana⁴, C. Papayannidis⁵, M.I. Del Principe⁵, M. Bonifacio⁶, F. Mosna⁷, M. Delia⁸, P. Minetto⁹, M. Gottardi¹⁰, N. Fracchiolla¹¹, V. Mancini¹², F. Forghieri¹³, P. Zappasoldi¹⁴, M. Cerrano¹⁵, A. Vitale¹⁶, E. Audisio¹⁷, S. Trappolini¹⁸, C. Romani¹⁹, M. Defina²⁰, S. Imbergamo²¹, N. Ciccone²², L. Santoro²³, B. Cambò²⁴, S. Iaccarino²⁵, M. Dargenio²⁶, L. Aprile²⁷, L. Elia¹⁶, R. La Starza²⁸, G. Pizzolo⁶, R. Foà¹⁶

¹Division of Hematology and SCT, Azienda Sanitaria Universitaria Integrata di Udine; ²Dipartimento di Oncologia e Ematologia, Policlinico S. Orsola-Malpighi, Bologna; ³U.O.C. Ematologia, AORN Antonio Cardarelli, Napoli; ⁴U.O.C. Ematologia, Ospedale di Bergamo ASST Papa Giovanni XXIII, Bergamo; ⁵U.O.C. Ematologia, AOU Policlinico Tor Vergata, Roma; ⁶Clinica Ematologica, Università di Verona; ⁷Ematologia e Centro Trapianto di Midollo Osseo, Ospedale Regionale San Maurizio, Bolzano; ⁸U.O. Ematologia con Trapianto, Azienda Ospedaliero-Universitaria Consorziale, Policlinico di Bari; ⁹Clinica Ematologica, Ospedale San Martino, Genova; ¹⁰U.O.C. Ematologia, Ospedale di Treviso; ¹¹U.O. Ematologia, IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano; ¹²Dipartimento di Ematologia e Oncologia, Ospedale Niguarda, Milano; ¹³S.C. Ematologia, Azienda Ospedaliero Universitaria di Modena; ¹⁴Clinica Ematologica, IRCCS Policlinico San Matteo, Pavia; ¹⁵SC Ematologia 1, A.O.U. Città della Salute e della Scienza di Torino (Presidio Molinette), Torino; ¹⁶Sezione di Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, “Sapienza” Università di Roma; ¹⁷S.C. Ematologia 2, AO Città della Salute e della Scienza, Torino; ¹⁸SOD Clinica Ematologica, Azienda Ospedaliero Universitaria Ospedali Riuniti, Ancona; ¹⁹U.O. Ematologia, ATS ASSL di Cagliari; ²⁰UOC Ematologia, Azienda Ospedaliero Universitaria Senese, Siena; U.O. di Ematologia e Immunologia Clinica, Università degli Studi di Padova; ²²S.C. Ematologia, Azienda Ospedaliero Universitaria di Ferrara, Ferrara; ²³U.O.C. Ematologia e Trapianto di Midollo Osseo, A.O.S.G. Moscati, Avellino; ²⁴UOC Ematologia e Centro Trapianti di Midollo Osseo, Azienda Ospedaliero Universitaria di Parma; ²⁵U.O.C. Ematologia, Azienda Ospedaliera di Caserta; ²⁶S.C. Ematologia, Ospedale Vito Fazzi, Lecce; ²⁷S.C. Ematologia, Ospedale S.G. Moscati, Taranto; ²⁸Ematologia e Trapianto di Midollo Osseo, Dipartimento di Medicina, Università di Perugia, Italy

Background: The overall outcome of relapsed/refractory (R/R) T-Lymphoblastic leukemia/lymphoma (T-ALL/T-LBL) in adults is poor, with less than 10% of patients alive at 5 years. There is no agreed standard of care and Nelarabine is the only recent drug that is specifically approved for R/R T-ALL/T-LBL, but there are limited data to support its approval.

Methods: This observational, phase 4, study provides recent additional data on the efficacy and safety of Nelarabine in adults with R/R T-ALL/T-LBL and evaluates the feasibility and outcome of Allo-SCT after Nelarabine salvage therapy. Primary endpoints were ORR and probability of OS. Additional endpoints were safety, Allo-SCT rate and post-transplant OS. We included, 118 patients with R/R, T-ALL/T-LBL that received salvage therapy with Nelarabine in 27 Italian hematologic sites between May 2007 and November 2018.

Results: Median age was 38 years (range, 18-81), 77 were T-ALL and 41 T-LBL, 65/118 (55%) had received > 2 prior chemotherapy lines and 18/118 (15%) relapsed after Allo-SCT. Median number of Nelarabine cycles was 2 (range 1-4). ORR was 50%. In detail, 43/118 (36%) patients achieved a CR, 16 a PR (14%), and 59 (50%) were refractory; 47 (40%) patients received an Allo-SCT after salvage. The OS at 1 year from the first dose of Nelarabine for the entire population was 37% (18% at 5 years) with a median OS of 8 months. The OS at 1 year was significantly better for patients who underwent Allo-SCT after Nelarabine (58% vs 22%, log-rank p=0,0001). In multivariate analysis the favorable independent predictive factors for OS were: age < 55 yrs and Allo-SCT after Nelarabine. OS at 2 and 5 years from Allo-SCT were 46% and 38%, respectively. Seventy-five patients (64%) experienced one or more drug related AE. Grade 3-4 neurologic toxicities were observed in 9/118 (8%) of patients, grade 3-4 thrombocytopenia and neutropenia were reported in 41% and 43% of cases, respectively. There were 3 cases of AE related deaths (2 septic shock and 1 aspergillosis).

Conclusions: This is one of the largest cohort of adult patients with relapsed or refractory T-ALL/T-LBL treated in realworld with Nelara-

bine. Taking into account the poor prognosis of this population Nelarabine can be considered as an effective option providing an ORR of 50% and a CR rate of 36%. In addition, 40% of cases who received Nelarabine salvage therapy underwent an Allo-SCT with an expected OS at 2 and 5 years of 46% and 38%, respectively. Overall, the safety profile of Nelarabine was acceptable with only 8 % of cases with grade III-IV neurological AE.

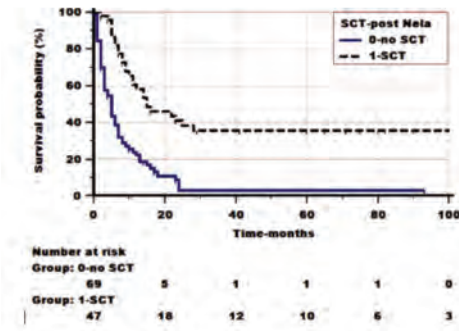


Figure 1.

P087

ABSTRACT WITHDRAWN

P088

GERIATRIC ASSESSMENT-BASED TREATMENT OF ELDERLY PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH-ALL) PATIENTS. RESULTS OF THE GIMEMA LAL1104 PROTOCOL

L. Cesini, S. Chiaretti, F. Di Raimondo, E. Angelucci, M. Luppi, M. Bonifacio, N. Cascavilla, C. Fozza, E. La Sala, S. Soddu, P. Fazi, A. Vitale, R. Foà, F. Ferrara

Department of Translational and Precision Medicine Sapienza University of Rome, Department of Translational and Precision Medicine Sapienza University of Rome, Division of Haematology, Azienda Policlinico-OVE, Hematology and Bone Marrow Transplantation Unit, IRCCS Ospedale Policlinico San Martino, Hematology Unit, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, Institute of Stem Cells Biology, Regenerative Medicine and Innovative Therapies (ISBRMIT), Fondazione IRCCS "Casa Sollievo della Sofferenza-IRCCS", Department of Medical, Surgical and Experimental Sciences, University of Sassari, Fondazione GIMEMA – Franco Mandelli Onlus, Fondazione GIMEMA – Franco Mandelli Onlus, Fondazione GIMEMA – Franco Mandelli Onlus, Department of Translational and Precision Medicine Sapienza University of Rome, Department of Translational and Precision Medicine Sapienza University of Rome, Division of Hematology, Cardarelli Hospital

The outcome of acute lymphoblastic leukemia (ALL) in the elderly is poor and many patients (pts) are not included in clinical trials because of comorbidities. To overcome these issues, the GIMEMA designed a front-line trial (LAL1104) for Ph-negative ALL pts >60 years. The primary endpoint was the overall survival (OS) of these pts, treated according to a geriatric assessment. Pts were stratified into 3 categories: fit if they had normal activities of daily living (ADL), no grade (G) 2 comorbidities and no geriatric syndromes; intermediate if they had a normal ADL, G2 comorbidities ≤ 2 and absence of geriatric syndromes; frail if they were >85 yrs, had an abnormal ADL, G2 comorbidities ≥ 3 or G3 comorbidities ≥ 1 and ≥ 1 geriatric syndromes. Treatment included vincristine (d 1, 8, 15, 22) and daunorubicine (d 1-3, 22-24) in induction, intermediate dose methotrexate (MTX) and cytarabine as consolidation, autologous transplantation (autoSCT) and maintenance with MTX, 6-mercaptopurine (6MP) and monthly vincristine for fit and intermediate pts; frail pts received vinblastine (d 1, 8, 15, 22) as induction, and MTX and 6MP as maintenance; pts underwent CNS prophylaxis.

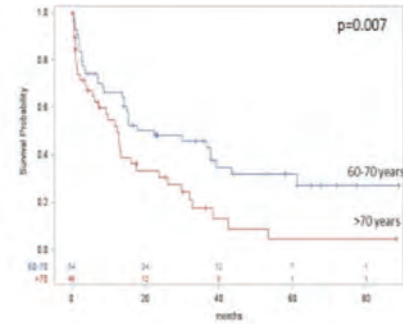


Figure 1.

From October 2007 to December 2016, 102 newly-diagnosed elderly pts were enrolled. Median age was 69.6 yrs (60.5-84.6); 85% were B-lineage ALL; 7% carried the t(4;11). 49 pts (51%) were fit, 28 (29%) intermediate and 19 (20%) frail; 21% had a PS >2. A response was achieved in 63 pts (63.5%); 17 (18%) died in induction. The 4-yr OS and disease-free survival (DFS) are 38.5% and 19.5%; there were no differences in 4-yr OS and DFS according to the geriatric assessment (fit vs intermediate vs frail): 41% vs 32% vs 44% and 24% vs 19% vs 0%; contrariwise, younger pts (<70 yrs) showed better 4-yr OS than older ones (32% vs 9%, p=0.007, Figure 1). 15 pts (14%) underwent autoSCT, which impacted in OS (p=0.02). Infections were recorded in 41% pts (sepsis and pneumonia: 20% and 15%). 10 pts (10%) developed paralytic ileus; hepatic, cardiac and neuropathic toxicities affected 6% of pts. Though less intensive than other trials designed for elderly ALL, the GIMEMA LAL1104 protocol compared favorably with the literature. Age was the most important prognostic factor. Geriatric assessment was effective in reducing early fatal events in unfit pts and abolished differences in OS. Enrollment in ad hoc for elderly trials, including new drugs and geriatric assessment, will help to improve the outcome of these difficult cases.

P089

HOW TO DETECT "3C-UP", A NEW ADULT PHILADELPHIA NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA SUBGROUP

A. Ferrari¹⁻³, S. Vitali², V. Robustelli³, A. Ghelli¹, C. Baldazzi³, S. Righi⁴, E. Fonzi³, S. De Matteis¹, M. Ghetti¹, R. Napolitano¹, M. Tebaldi¹, S. Salvi¹, C. Papayannidis³, G. Marconi³, S. Paolini³, G. Ferrari³, M.C. Fontana³, E. Imbrogno¹, A. Padella³, G. Simonetti¹, G. Pasquini², D. Cangini¹, B.M. Giannini¹, C. Cerchione¹, A. Santoro⁵, J.M. Hernández-Rivas⁶, D. Calistri¹, G. Castellani², E. Sabattini⁴, N. Testoni³, D. Remondini², G. Martinelli⁷

¹Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS; ²Dipartimento di Fisica e Astronomia, INFN; ³Dipartimento di Medicina Specialistica Diagnostica e Sperimentale (DIMES), Ist. Di Ematologia; ⁴Hematopathology Unit, DIMES; ⁵Hematology department A.O. Ospedali Riuniti Villa Sofia-Cervello; ⁶Fundación de Investigación del Cáncer de la Universidad de Salamanca; ⁷Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS

Background: The adult "triple negative" [Ph-/-/-; neg for t(9;22); t(1;19); t(4;11); 61% of adult B-ALL] B-ALL group is characterized by an high genetic heterogeneity, lack of innovative target therapies and poor survival. Many chimeric genes have been recently identified (Gu, Nat Genet. 2019), that led to a refined classification of B-ALL and to, in some cases, tailored therapies; but identified them (also Ph-like) is challenging. CRLF2 is frequently altered in adult B-ALL, especially in Ph-like and Ph- pts. Alterations that often lead to a CRLF2 overexpression. Adult pts with CRLF2 upregulated have poor outcome and novel strategies are needed to improve it.

Aims: We focused to CRLF2 overexpression event to clustering Ph-/-/-, in order to assess biomarkers in these subgroups to test new drugs and to easily/quickly/economically identify them.

Patients and Methods: GEP were performed on 55 Ph-/-/- ALL, 29 B-ALL Ph+ and on 7 donors. We cluster triple negative GEP data with a robust pipeline, based on CRLF2 upregulation and in a top ten-gene list.

Ph-/- ALL samples were then characterized for the presence of gene fusions, Copy Number Alterations and mutations using different approaches.

Results: The Ph-/- fusions rate is of 43% (23/52). Combining our new Ph-/- GEP clustering to SNVs, Fusion and CNA results we identify a defined 2-clusters-subdivision (Gr1 and Gr2). The Gr2 is characterized by CTGF, CRLF2 and CD200 (Gr2=3C-up) co-overexpression and it represents 14.1% of all B-ALL. The Gr2 GEP is similar to Ph+ one and similar to Ph-like. Fusion copy number alteration and mutational screening done, detected that 3C-Up group has a higher frequency of Ph-like associated lesions. Gr1 represents 46.9% of all B-ALL and RAS pathway genes are highly affected in this group. p53 pathway is enriched in both groups. Other available targets were identified. Lastly, we develop a cytofluorimetric (IF) a B-ALL panel to quickly detect 3C-Up at diagnosis/relapse. With this test, we confirm that 3C marker co-overexpression is confirmed at protein level in pt mononuclear cells.

Conclusions: We identified a new signature, related to CRLF2 high expression, to classify Ph-/- ALL B in 2 subgroups. 3C-Up has Ph-like related alterations and high co-expression of CRLF2, CTGF and CD200 that we could easily identify with IF. This new Ph-/- subclassification identify new potential therapeutic targets with available drug to test. Supported by: AIL, FP7 NGS-PTL project, Harmony.

P090

MORE OR LESS? IMPACT OF DOSE NUMBER ON OUTCOMES OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH INOTUZUMAB OZOGAMICIN

G. Martinelli, R. Cassady, W. Stock, E. Jabbour, M. Stelljes, A. Advani, D. Marks, S. O'Brien, T. Wang, A. Neuhof, E. Vandendries, H. Kantarjian, D. DeAngelo

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori; University of Washington School of Medicine and Fred Hutchinson Cancer Research Center; University of California Irvine Medical Center; University of Texas MD Anderson Cancer Center; Universitätsklinikum Münster; Cleveland Clinic Taussig Cancer Institute; University Hospitals Bristol; University of California Irvine Medical Center; Pfizer Inc.; Pfizer Pharma GmbH; Pfizer Inc.; University of Texas MD Anderson Cancer Center, Dana-Farber Cancer Institute

Background: In INO-VATE (NCT01564784), patients with relapsed/refractory acute lymphoblastic leukemia who were treated with inotuzumab ozogamicin (InO) vs standard chemotherapy had significantly greater remission rates and longer overall survival (OS), with more patients proceeding to stem-cell transplant (SCT).

Aims: Here we report outcomes by number of InO cycles (Cyc) received.

Methods: Methods are detailed in Kantarjian, NEJM 2016. Outcomes are reported based on number of InO Cyc received (≤ 3 or > 3 Cyc with no SCT and ≤ 2 or > 2 with SCT [consistent with current recommendations]) in patients who achieved complete remission (CR)/CR with incomplete hematologic recovery. Data cutoff: Jan 4, 2017.

Table 1. Outcomes.

	NRM at 100-d, % (95% CI)	Median PFS, mos (95% CI)	Post-transplant survival at 18-mos, % (95% CI)	OS at 24-mos, % (95% CI)	Median OS, mos (95% CI)	Patients with VOD, n (%)
W/o SCT						
≤ 3 Cyc (n=18)	N/A	3.2 (2.5-3.4)	N/A	11.9 (2.0-31.5)	5.3 (3.4-9.4)	2 (11.1)
> 3 Cyc (n=32)	N/A	6.2 (4.8-8.7); HR 0.33, P=0.0002	N/A	13.5 (4.3-28.0)	8.0 (5.8-13.3); HR 0.63, P=0.072	1 (3.1)
With SCT						
≤ 2 Cyc (n=15)	14.3 (5.1-28.0)	9.2 (5.6-NE)	57.1 (39.3-71.5)	34.3 (36.6-69.0)	29.6 (7.7-43.6)	6 (17.1)
> 2 Cyc (n=36)	30.6 (16.4-46.0)	8.4 (6.0-9.6); HR 1.37, P=0.849	25.0 (12.4-39.8)	25.0 (12.4-39.8)	11.4 (8.6-14.3); HR 1.65, P=0.0954	12 (33.3)

CI, confidence interval; Cyc, cycles; HR, hazard ratio; N/A, not applicable; NE, not evaluated; NRM, non-relapse mortality; OS, overall survival; PFS, progression-free survival; SCT, stem-cell transplant; VOD, veno-occlusive disease

Results: Among patients who responded but did not proceed to SCT, MRD-negativity was achieved by 88% of 32 patients receiving > 3 Cyc vs 61% of 18 patients receiving ≤ 3 Cyc (P=0.037). Patients who had > 3 Cyc had a longer duration of remission (HR 0.35, P=0.0005) and improved survival outcomes vs those who had ≤ 3 Cyc (Table 1). The rate of serious adverse events (AE) and the percentage of patients discontinuing treatment due to AEs was numerically lower with > 3 Cyc vs ≤ 3

Cyc. Among patients who responded and proceeded to SCT, the percentage achieving MRD-negativity (81% vs 69%, P=0.123) and the duration of remission (HR 1.37, P=0.848) were similar for those who had > 2 Cyc (36 patients) vs ≤ 2 Cyc (35 patients). Patients with ≤ 2 Cyc had improved survival outcomes and non-relapse mortality (NRM) vs those with > 2 Cyc (Table 1). 17.1% of patients receiving ≤ 2 Cyc and 33.3% of patients receiving > 2 Cyc developed veno-occlusive disease (Table).

Conclusions: These results are in agreement with current recommendations that patients proceeding to SCT should receive ≤ 2 Cyc, whereas those not proceeding to SCT may benefit from receiving up to 6 cycles of therapy.

P091

THE MRDITALLAB PROJECT: BRINGING MINIMAL RESIDUAL DISEASE INTO REAL-LIFE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

S. Chiaretti, I. Della Starza, A. Santoro, O. Spinelli, L. Elia, R. Cucci, M. Vignetti, F. Fabbiano, R. Bassan, A. Rambaldi, R. Foà

Department of Translational and Precision Medicine, Sapienza University of Rome, Fondazione GIMEMA – Franco Mandelli Onlus, Hematology Unit, Division of Hematology and Bone Marrow Transplantation, Ospedali Riuniti Villa Sofia-Cervello, Hematology and Bone Marrow Transplant Unit, ASST-Papa Giovanni XXIII, Department of Translational and Precision Medicine, Sapienza University of Rome, Fondazione GIMEMA – Franco Mandelli Onlus, Hematology Unit, Division of Hematology and Bone Marrow Transplantation, Ospedali Riuniti Villa Sofia-Cervello, Hematology Unit, Ospedale dell'Angelo and Ospedale Ss Giovanni e Paolo, Hematology and Bone Marrow Transplant Unit, ASST-Papa Giovanni XXIII, Department of Translational and Precision Medicine, Sapienza University of Rome

Acute lymphoblastic leukemia (ALL) is a heterogeneous hematologic malignancy which requires a complex and highly specialized management. Although most adult patients with ALL experience a complete hematologic remission (CR) and long-term survival rates are now out-reaching 50%, a substantial proportion of patients remains minimal residual disease (MRD)-positive after induction/consolidation chemotherapy. MRD is presently regarded as the most important prognostic factor and is predictive of relapse. Among the various methods used for MRD detection, the most sensitive is real-time quantitative polymerase chain reaction (RQ-PCR) analysis based on the use of patient specific primers; though time consuming, this assay ensures sensitivity and accuracy. MRD is currently used in national and international trials for patients' stratification, but is not routinely offered outside of clinical trials. In order to fill this gap, the GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto) - in partnership with Amgen - has given birth to the MRDitaLLab project, a centralized network comprising highly skilled laboratories, whose final goal is to guarantee an appropriate MRD evaluation according to the EuroMRD guidelines. Through a dedicated IT platform, MRDitaLLab allows Italian hematologists to offer their ALL patients, both Ph- and Ph+, a standardized molecular MRD testing. MRD analysis will be performed by three hub laboratories certified by the EuroMRD Consortium, for those patients who are not enrolled in clinical trials but are treated either with: 1) a MRD-oriented backbone for Ph- ALL, 2) elderly-oriented schemes, or 3) a chemo-free regimen (i.e. a tyrosine kinase inhibitor plus steroids) for Ph+ ALL cases. MRD analysis will be carried out at two decisional time-points. MRD results will be then provided directly to the clinicians in compliance with patients privacy protection. Finally, at clinician discretion, data will be collected in the platform. In parallel, the MRDitaLLab platform will also provide additional training and educational activities. The MRDitaLLab service has been activated in March 2019 and will support Italian hematologists in monitoring MRD levels in line with European standards to refine risk stratification and optimize therapeutic choices with the aim of improving the clinical management of adult ALL patients. So far, 7 Italian centers have adhered to this project and 6 are being activated.

P092

OUTCOME OF PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN ELDERLY AND NOT-TRANSPLANT-ELIGIBLE PATIENTS: REPORT FROM A REAL LIFE MULTICENTRIC COHORT TREATED OUTSIDE CLINICAL TRIALS.

F. Grimaldi¹, M. Annunziata², D. Nappi², I. Zacheo¹, C. Califano³, A. Camera⁴, G. De Falco⁵, B. Serio⁶, M.R. Villa⁷, F. Pane¹, F. Ferrara²

¹AOU Federico II; ²Azienda Ospedaliera "Cardarelli"; ³Oncoematologia Ospedale "A. Tortora"; ⁴Ematologia Ospedale "S. Anna e S. Sebastiano"; ⁵Ematologia Ospedale "S.G. Moscati"; ⁶Ematologia e trapianti di midollo, Ospedale "San Giovanni di Dio e Ruggi D'Aragona"; ⁷Ematologia ASL NA 01 Loreto Mare, Italy

Introduction: Outcome of Philadelphia positive Acute Lymphoblastic Leukemia (Ph-ve ALL) has dramatically improved with introduction of tyrosine kinase inhibitors (TKi). However less is known about impact of TKi therapy in elderly ALL patients. With this aim, we analyzed outcome of Ph-ve ALL patients from 7 different Haematological Institutions in Campania, treated with TKi, older than 60 years, and considered not-transplant-eligible due to age and comorbidities.

Patients and Methods: Data from 23 patients (Male/Female 10/13; Median age 69 years, range 60-81), from August 2012 to February 2019 were analyzed. Co-morbidities were present in 18 of 23 (78%) patients (Median number 2; range 0-6) [Previous cancer history in 4 and cardiovascular disease in 6 patients]. Median number of concomitant medications was 3 (range: 0-11). Protein p190 was detected in 73,9% of cases; p210 in 21,7%; p190/p230 in 4,4%. B-common phenotype was present in 90% of cases. Additional cytogenetic abnormalities featured 30% of evaluable karyotype at diagnosis. Induction consisted of Dasatinib 140mg, steroid and 4 doses of weekly Vincristine in 34,7% of cases, while 65,3% were treated with TKi plus steroid alone (26,6% Imatinib; 73,4% Dasatinib). Globally Dasatinib, alone or in combination, was first line therapy in 82,6% of patients. Median days of hospitalization was 29 (range 0-45), with 3 patients treated in out-patient regimen. All but one patient were evaluable for response at d+28, with Complete Haematological Remission achieved in all cases. Major Molecular Response (MMR) and Complete Molecular Response (CMR) was observed at d+30 in 9% and 4,5% respectively; at d+90 rate of MMR and CMR improved with 36,3 and 22,7% observed respectively. In 9 patients in molecular relapse, second line TKi restored MMR in 55,5%, and CMR in 33,3% of cases. Cumulative incidence of MMR and CMR in the whole cohort was 77,3% and 50% respectively. Median overall survival was of 18 months (Range 1-78), median disease-free survival was of 13 months (range: 1-77). At the time analysis stopped, 15 patients remain alive. Meningeal prophylaxis was feasible in 86% of cases, with only 1 meningeal relapse observed. Pleural effusion (Max grade 2) was the most common adverse event, occurring in 22% of Dasatinib patients. Incidence of infection was very rare.

Conclusions: Our data show how, despite older age and higher number of comorbidities, TKi therapy grants excellent results in Ph-ve ALL elderly patients.

P093

IN ACUTE LYMPHOBLASTIC LEUKEMIA, STEROID INDUCED HYPOFIBRINOGENEMIA IS ASSOCIATED WITH BCR/ABL REARRANGEMENT

E. Buzzatti¹, C. Sarlo², F. Esposito¹, R. Palmieri¹, G. Paterno¹, L. Mercante¹, P. Casciani¹, R. Laureana¹, E. De Bellis¹, C. Gurnari¹, V. Forte¹, F. Buccisano¹, L. Maurillo¹, W. Arcese¹, A. Venditti¹, M.I. Del Principe¹

¹Cattedra di Ematologia, Dipartimento di Biomedicina e Prevenzione Università degli Studi di Roma "Tor Vergata"; ²Ematologia, Policlinico Universitario-Campus Biomedico, Italy

Introduction: Hypofibrinogenemia in adult acute lymphoblastic leukemia (ALL) is typically associated with asparaginase (ASP) delivery; however, also steroid therapy seems to interfere with plasma levels of fibrinogen (FBG). Since the reasons underlying the fluctuations of plasma levels of FBG in B-ALL have not been carefully investigated, we aimed at 1) assessing the plasma levels of FBG during the therapeutic phase before the first ASP dose; 2) exploring if the propensity of FBG to fluctuate correlated with biologic features of B-ALL.

Methods: We retrospectively analyzed FBG levels in 36 B-ALL patients

(pts). Nineteen (53%) were females and 17 (47%) were males, median age was 44 years (range 18-76), median bone marrow (BM) blast cell infiltration was 90% (range 33-98), median white blood cell count (WBCc) was $18.6 \times 10^9/L$ (range 0.840-230). Sixteen (44%) pts had a BCR/ABL + ALL and were given tyrosin kinase inhibitors plus steroids, with or without chemotherapy, 20 (56%) had a BCR/ABL- ALL and were treated with intensive chemotherapy, according to current GIMEMA protocols. FBG fluctuations were graded according to the Common Toxicity Criteria for Adverse Events version 4.

Results: During the steroid pre-phase, we observed a decrease in levels of FBG in 12/16 (75%) pts with BCR/ABL+ ALL, with 4 (33%) experiencing a hypofibrinogenemia grade(G)=3-4 and the remaining 8 (67%) showing a hypofibrinogenemia G1-2. During the steroid pre-phase, only 4 (25%) pts among the BCR/ABL- cohort had a hypofibrinogenemia G1-2 with none experiencing a G3-4 decrease of FBG. During the induction phase and before the administration of ASP, all pts with BCR/ABL+ B-ALL had at least hypofibrinogenemia G1-2 that lasted a median time of 13,5 days (range 1-35) whereas only 9 (45%) with BCR/ABL- B-ALL showed hypofibrinogenemia G1-2. In univariate analysis, hypofibrinogenemia correlated with a BCR/ABL positivity (p=.001), advanced age (p=.007) and hyperleucocytosis (WBCc> $10 \times 10^9/L$) (p=.005). No significant alteration of other coagulation tests, of liver function or a significant decrease in the platelet count during the steroid pre-phase were observed. The same was during the induction phase except for platelet count.

Conclusions: Our retrospective study shows that during the steroid pre-phase, hypofibrinogenemia is more frequent in BCR/ABL+ pts than in those negative, especially if aged>60 years. Further studies are needed to clarify the mechanisms of acquired hypofibrinogenemia in B-ALL.

P094

RETROSPECTIVE ANALYSIS OF SAFETY AND EFFICACY OF PEGYLATED ASPARAGINASE IN PEDIATRIC AND ADULT PATIENTS.

C. Alati¹, G. Praticò¹, B. Greve¹, G. Iaria², F. Ronco², B. Martino¹

¹U.O.C. Ematologia - G.O.M. Bianchi Melacrino Morelli; ²U.O.S.D. Onco-Ematologia Pediatrica - G.O.M. Bianchi Melacrino Morelli

In the last few years pediatric-inspired therapy improved the remission rate and overall survival in adult patients. The introduction of these intensified regimens of poli-chemotherapy led to an increase to the use of asparaginase, one of the most effective agents against ALL (ALL). Pegylated asparaginase (PEG-ASP) has a more favorable toxicity profile and an easier administrative schedule. We retrospectively analyzed a cohort of 25 pediatric and adult patients treated with PEG-ASP in the Division of Hematology and in the Division of Pediatric Onco-Hematology in our hospital. From 2012 to 2019, 19 pediatric patients (median age 8, range 2-16) were treated with PEG-ASP for ALL, for a total of 47 administrations (induction or consolidation phase) at the dosage of 2500 U/m² with the exception of a treatment at 1000 U/m² in a children with Down Syndrome. During the first administration four patients experienced grade III hepato-toxicity, 1 pancreatitis, 5 coagulation alterations, 1 maxillary sinus thrombosis, 1 allergic reaction. At subsequent administrations, 2 patients had allergic reaction and 2 patients coagulation alterations. No correlations with phase of treatment or type and dosage of chemotherapy were identified. Screening for thrombophilia was performed in all patients: one children presented homozygous mutation of MTHFR. From 2017 to 2019 6 adult patients (median age 43, range 23-65) were treated with peg-asparaginase, 5 for ALL (3 ALL T, 2 ALL B, 3 with hyperleucocytosis, 2 with bulky mediastinal mass, 1 with bulky mediastinal mass and localization into the scalp) for a total of 10 administrations (induction or consolidation phase) at the dosage of 2000 U/mq and 1 for relapsed and refractory lymphoblastic crisis of chronic myeloid leukemia (with T315I and E255F mutations) at the dose of 1000 U/mq. Three patients didn't experience any toxicity, 1 patient experienced grade III hepato-toxicity and hyperglycemia, 1 hyperglycemia, 1 allergic reaction. At subsequent administrations, 1 patient had allergic reaction. No correlations with phase of treatment or type and dosage of therapy were identified. All adult patients obtained complete remission, 5 patients are alive and in remission and the last one died for late relapse and refractory disease. These data suggest that use of PEG-ASP is safe and manageable in adult and pediatric patients with complete and rapid resolution of toxicity and its use should be more widespread in adult patients.

Quality of Life, Pain Therapy and Home Care, Supportive Care 1

P095

PHASE II MULTICENTER, NOT COMPARATIVE, STUDY OF MULTIPLE DOSES OF NEPA (NETUPITANT + PALONOSETRON) IN PREVENTING CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING IN NON-HODGKIN LYMPHOMA PATIENTS ELIGIBLE FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION RECEIVING MULTIPLE DAY / HIGH DOSE CHEMOTHERAPY REGIMENS

N. Di Renzo¹, M. Musso², R. Scimè³, F. Benedetti⁴, P. Chiusolo⁵, L. Cupelli⁶, R. Foà⁷, A. Guarini⁸, P. Mazza⁹, D. Pastore¹⁰, A. Mengarelli¹¹, G. Milone¹², V. Pavone¹³, G. Specchia¹⁴

¹UOC di Ematologia e Trapianto di Cellule Staminali-PO "Vito Fazzi" - Lecce, ²Dipartimento Oncologico "La Maddalena"Palermo, ³UTMO- Ospedale V. Cervello- Unità Operativa di ematologia; ⁴Centro Trapianto di Midollo Osseo "Giuseppe Perona"; ⁵Divisione Ematologia Policlinico Agostino Gemelli; ⁶Unità di Ematologia - Ospedale S. Eugenio; ⁷Dip. Di Biotecnologie cellulari ed Ematologia. Università "La Sapienza"; ⁸UOC Ematologia - IRCCS "Giovanni Paolo II"; ⁹UOC di Ematologia -PO "S.G. Moscatti"; ¹⁰UOC Ematologia PO "Perrino-Di Summa"Brindisi; ¹¹USOD Ematologia e Trapianti - Istituto Nazionale Tumori Regina Elena; ¹²Programma Trapianto-Divisione Ematologia- Ospedale Ferrarotto; ¹³UOC di Ematologia e Trapianto - Azienda "Cardinale G. Panico"; ¹⁴UOC Ematologia con Trapianto Azienda Ospedaliera Policlinico

Cancer chemotherapy may be associated with a high incidence of nausea and vomiting (CINV), which may occur acutely within 24 hours after the start of chemotherapy or following a delay of 24 to 48 hours after chemotherapy. Despite the availability of several antiemetics, clinical findings show that control of nausea and vomiting continue to be a serious concern for hematological patients, mainly for those receiving multiple-day (MD) and high-dose (HD) chemotherapy (CT). NEPA is the first antiemetic developed as an oral fixed dose combination of two antiemetic agents, a new highly selective NK1-RA, netupitant, and a second generation 5HT3-RA, palonosetron, that simplify the antiemetic regimen allowing for a lower number of capsules and days of treatment. The aim of our study was to assess in the field of both MD-CT and HD-CT the administration of multiple doses of NEPA in non Hodgkin's lymphoma (NHL) patients eligible for autologous hematopoietic stem cell transplantation.

Table 1. Complete Control and Response, Emesis and Rescue during the Study Phase.

Assessment	Complete Control	Complete Response	No Emesis	No Rescue Therapy
Day 1	68 (97.1%)	68 (97.1%)	69 (98.6%)	68 (97.1%)
Day 2	68 (97.1%)	68 (97.1%)	68 (97.1%)	70 (100%)
Day 3	68 (97.1%)	68 (97.1%)	68 (97.1%)	68 (97.1%)
Day 4	68 (97.1%)	68 (97.1%)	68 (97.1%)	69 (98.6%)
Day 5	68 (97.1%)	69 (98.6%)	69 (98.6%)	69 (98.6%)
Day 6	68 (97.1%)	69 (98.6%)	69 (98.6%)	70 (100%)
Day 7	69 (98.6%)	70 (100%)	70 (100%)	70 (100%)
Day 8	67 (95.7%)	69 (98.6%)	69 (98.6%)	70 (100%)
Day 9	67 (95.7%)	68 (97.1%)	69 (98.6%)	69 (98.6%)
Day 10	68 (97.1%)	69 (98.6%)	69 (98.6%)	70 (100%)
Day 11	65 (92.9%)	67 (95.7%)	68 (97.1%)	69 (98.6%)
Day 12	62 (88.6%)	64 (91.4%)	64 (91.4%)	68 (97.1%)
Day 13	65 (92.9%)	67 (95.7%)	68 (97.1%)	69 (98.6%)
Day 14	65 (92.9%)	66 (94.3%)	68 (97.1%)	68 (97.1%)
Day 15	66 (94.3%)	67 (95.7%)	69 (98.6%)	68 (97.1%)
Acute Phase	62 (88.6%)	62 (88.6%)	63 (90%)	66 (94.3%)
Delayed Phase	67 (95.7%)	69 (98.6%)	69 (98.6%)	70 (100%)
Overall Phase	60 (85.7%)	61 (87.1%)	62 (88.6%)	66 (94.3%)

The absolute frequency and the percentage of evaluable patients are reported for each time assessment and for each efficacy variable. Complete Response is defined as no emetic episode and no use of rescue medication after chemotherapy administration. Complete Control is defined as no emetic episode, no use of rescue medication and no more than mild nausea.

The chemotherapy regimen (BEAM/FEAM) was administered for 5 days, NEPA was taken on day 1, 3 and 5, and nausea and vomiting were

monitored up to day 15. The primary endpoint was the percentage of patients achieving a Complete Response (CR; no vomiting and no use of rescue medication) during the overall phase, defined as the period from day 1 (first day of chemotherapy) until 48 hours after the last dose of chemotherapy. On a total of 82 patients enrolled, 70 patients concluded the study phase. According to the one-stage Fleming design of the study, the primary endpoint (set as CR during the overall phase in more than 42 patients) was achieved. Indeed, 61 patients (87.1%) were complete responders. Moreover, 62 patients (88.6%) did not have any emetic episode and 66 (94.3%) did not need any rescue therapy during the overall phase. Likewise, the control of nausea was high and only 5 patients (7.1%) experienced more than mild nausea. Indeed, the global satisfaction of the antiemetic therapy (VAS) among the patients was substantial with a mean of 9.13 over 10. In conclusion, our study demonstrated for the first time in this challenging setting that multiple doses of NEPA can efficiently manage nausea and vomiting associated with MD/HD-CT.

P096

A GIMEMA STUDY TO SUPPORT THE VALIDITY OF THE EORTC QLQ-C30 ACROSS PATIENTS WITH HEMATOLOGIC MALIGNANCIES

K. Sommer, F. Cottone, P. Fazi, M. Vignetti, F. Efficace, On behalf of the GIMEMA WP Quality of Life

Italian Group for Adult Hematologic Diseases (GIMEMA), Data Center and Health Outcomes Research Unit

The European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) is a cancer-specific health-related quality of life (HRQoL) questionnaire. However, validation data have been mainly limited to patients solid tumors. This questionnaire is commonly used to make inferences based on group comparisons or to evaluate the change in HRQoL patterns over time. To ensure that differences observed in the EORTC QLQ-C30 scores reflect true differences in HRQoL, rather than measurement artifacts, measurement invariance (as an important validation criteria) needs to be established. This study tested the assumption of measurement invariance of the EORTC QLQ-C30 in hematological malignancies, focusing on age, sex, comorbidity, disease, and time. Baseline EORTC QLQ-C30 data of 2,134 patients suffering from hematological malignancies were included in the analyses. Measurement invariance (metric and scalar invariance) across groups (age, sex, comorbidity, disease) and time (baseline, 1 month and 2 months follow-up) was examined using the structural equation modeling approach. Measurement invariance across time (i.e., longitudinal invariance) was tested in a subgroup of patients diagnosed with myelodysplastic syndromes. The results demonstrated metric and scalar invariance for age and comorbidity and the final models showed good fit to the data (age: CFI = 0.99, TLI = 0.99, RMSEA = 0.05; comorbidity: CFI = 0.99, TLI = 0.99, RMSEA = 0.05). In addition, longitudinal invariance (metric and scalar) was supported and fit indices indicated good model fit for the final model (CFI = 0.99, TLI = 0.98, RMSEA = 0.03). Confirmatory factor analyses indicated partial scalar invariance for sex and disease. The thresholds of items 1, 2, and 23 of the physical functioning scale and the emotional functioning scale were noninvariant with regard to sex, while the thresholds of items 4, 23, 24, and 25 of the physical functioning scale, emotional functioning scale, and the cognitive functioning scale were noninvariant with regard to disease type. Still, the final models for showed good fit to the data (sex: CFI = 0.99, TLI = 0.99, RMSEA = 0.05; disease; CFI = 0.99, TLI = 0.99, RMSEA = 0.05). Overall, the current findings support measurement invariance of the EORTC QLQ-C30 across patients with hematologic malignancies adding novel validation data of this measure in this cancer population.

P097

MALNUTRITION AND GVHD IN ALLOGENIC BONE MARROW TRANSPLANTATION: A PROSPECTIVE STUDY

E. Morello, F. Arena, M. Malagola, A. Turra, N. Polverelli, T. Zollner, E. Cavagna, M. Farina, V. Cancelli, L. Gandolfi, M. Andreoli, D. Russo

Dpt of Clinical and Experimental Sciences, University of Brescia, Unit of Blood Diseases and Bone Marrow Transplantation, ASST-Spedali Civili di Brescia, Italy

Background: Allo-SCT conditioning is associated with multiple side effects that can affect food intake and nutritional status. Malnutrition is a frequent complication of allo-SCT, but no clear correlation and data are available on impact of this metabolic syndrome on transplant outcome.

Methods: We performed an observational prospective study on 30 adult patients consecutively submitted to allo-SCT for hematological malignancies at our institution (August 2017-August 2018). The nutritional status was weekly assessed by a dietitian from the day of admission to day +30. Nutritional status was classified according to Patient-Generated Subjective Global Assessment (PG-SGA) score. The higher the score, the worse is the nutritional status. GVHD was classified according Glucksberg System.

Results: Mean patient age was 50.7 ± 20.7 years and 14/30 (47%) patients were male. Acute myeloid leukemia was the most frequent diagnosis (47%). The donor was matched unrelated, HLA sibling and haploidentical in 17/30 (57%), 8/30 (27%) and 5/30 (16%) cases, respectively. Conditioning was non-myeloablative in 19/30 (63%) and myeloablative in 11/30 (37%). TBI was used in 8/30 cases (27%). Most of the patients (74%) were well nourished at admission (Stage A). From day +21, all the patients progressively developed a deterioration of their nutritional status. Patients who had a higher PG-SGA score at +7 had a higher risk of developing GVHD than well nourished patients (PG-SGA score 14, no-GVHD vs 20 in GVHD group; $p=0.023$). The higher the score, the worse is the nutritional status and higher the incidence of aGVHD. The cumulative incidence of acute GVHD was 36.7% at 100 days. Three patients developed a Grade 1 acute GVHD, seven Grade 2 and one Grade 3. The PG-SGA score had a statistically significant correlation with the incidence of aGVHD, in particular at day zero ($p=0.050$), at day +7 ($p=0.024$) and at +14 ($p=0.047$) (Figure 1).

Conclusions: Early worsening of nutritional status during allo-SCT hospitalization seems to be associated with higher incidence of aGVHD and maintaining a good nutritional status during hospital stay represents a protective factor for aGVHD. The incidence of aGVHD was higher in patients who were malnourished at day zero, +7 and +14. Considering these results, nutritional support in transplanted patients should be part of the clinical routine and strategies to reduce the burden of symptoms due to toxicity of the conditioning regimens are warranted.

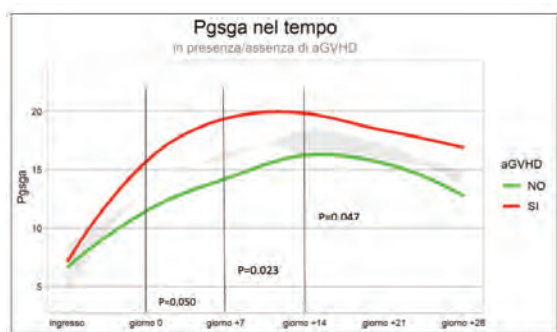


Figure 1.

P098

LYMPHOMA SURVIVOR PATIENTS: AN EVALUATION ON THE CLINICAL MANAGEMENT IN CENTERS AFFILIATED TO FONDAZIONE ITALIANA LINFOMI (FIL)

C. Minoia, A. Bari, L. Nassi, R. Banzi, C. Gerardi, V. Lenti, M. Calabrese, M. Spina, A. Guarini

Haematology Unit IRCCS Istituto Tumori "Giovanni Paolo II" Bari Italy, Department of Oncology and Hematology Modena Cancer Center Unit of Target Therapy in Onco-Hematology and Osteoncology University of Modena and Reggio Emilia Modena Italy, Ematologia Azienda Ospedaliero Universitaria Maggiore della Carità Novara Italy, Mario Negri Institute for Pharmacological Research IRCCS Milan Italy, Mario Negri Institute for Pharmacological Research IRCCS Milan Italy, Fondazione Italiana Linfomi Alessandria Italy, Fondazione Italiana Linfomi Alessandria Italy, Division of Medical Oncology Centro di Riferimento Oncologico Aviano National Cancer Institute Aviano Italy, Haematology Unit, IRCCS Istituto Tumori "Giovanni Paolo II" Bari Italy

Lymphoma survivors could develop late toxicities for which a multi-specialistic assessment is needed. The aim of the study was to analyze the follow-up modalities of lymphoma survivors among the Hematology Centers affiliated to the Fondazione Italiana Linfomi (FIL) and identify areas for improvement of the clinical practice and research activity. Since November 2018 to January 2019, the FIL launched an online survey among its affiliated centers (n. 154). The survey included 4 short questions about the daily clinical practice, aimed to evaluate: 1) length of follow-up (cHL and DLBCL); 2) existence of an out-patient program dedicated to lymphoma survivors; 3) multi-disciplinary approach; 4) existence of a research program on survivorship. 54 Hematology Centers participated to the survey. The majority (77.7%, n. 42) still continue to follow the patients beyond the 5th year of remission (the 37% till the 10th year and the 42.5% beyond the 10th year), while the 18.5% (n. 10) of Centers address the patient to the general practice physician. The 40.7% of Centers (n. 22) have an out-patient clinical activity specifically dedicated to lymphoma survivors. It consists exclusively of the onco-hematologic evaluation for the 36.6% of Centers (n. 8) and of a multi-disciplinary approach in the remaining ones (63.6%, n. 14). The multi-specialistic assessment was: cardiologic (100%, n. 14), endocrinologic (57.1%, n. 8), gynecologic (35.7%, n. 5), psycologic (35.7%, n. 5), nutritional (28.5%, n. 4), neurologic (28.5%, n. 4), radiotherapeutic (28.5%, n. 4), pneumologic (21.4%, n. 3), others (7.1%, n.1): a symptom oriented approach, oculistic, radiologic, internistic, orthopedic, psychiatric. 5 Centers (37.5%) offer 1-2 specialistic evaluations other than onco-hematologic, 5 Centers (37.5%) 3-4 and 4 Centers (28.5%) 4-8 specialistic evaluations. Only 6 Centers (11.1%) are involved in a research activity on lymphoma survivorship. Our data document that the majority of patients continue the follow-up at the Center in which they had been cured also after the 5th year of remission, but in only about the 40% of them there is a dedicated out-patient program for survivors and in less than one third the possibility to receive a multi-disciplinary approach. In this context, a need for recommendations for the follow-up of late toxicities emerged, with the finality to plan a multi-disciplinary approach equally organized at Italian Hematologic Centers and increase the research activity.

P099

ROLE OF SEDOANALGESIA FOR PAIN ASSOCIATED WITH BONE MARROW ASPIRATION AND BONE BIOPSY

C. Cerchione¹, G. Martinelli¹, L. Catalano², N. Pugliese², A. Gravetti², D. Nappi², S. Ronconi¹, D. Cangini¹, M.B. Giannini¹, M. Altini¹, F. Pane², V. Martinelli²

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola; ²Hematology, AOU Federico II, Italy

Bone marrow aspiration and biopsy (BMAB) is a painful procedure, and the commonly adopted local infiltration anesthesia (LIA) with lidocaine is unable to relieve the pain during the most uncomfortable phases, or the anticipatory anxiety related to pain recalling thereafter. As there are no formal guidelines for adding a sedoanalgesic premedication before beginning the BMAB, many combinations have been adopted by several authors. Our randomized and patient blinded trial aimed to evaluate, as primary end point, the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who

underwent BMAB for hematological malignancies. Two secondary end points were: 1) to define if patients who already underwent to BMAB without LIA prefer sedoanalgesia; 2) to demonstrate if sedoanalgesia can influence the quality of the biological specimen harvested. Patients were randomly assigned into two arms for receiving either placebo plus LIA (standard group, 48,6%) or oral fentanyl citrate 200 mcg + oral midazolam 5 mg in addition to LIA (combo-group, 51,4%) during BMAB. Pre-procedural anxiety and procedural pain were assessed according to the Numered Rating Scale (NRS: 0-10), dividing the time of the procedure into five intervals (T0, T1, T2a, T2b, and T3) and evaluating discomfort grade during each moment of procedure in both groups. Cognitive function was measured before and 30 minutes after the procedure. Possible side effects were recorded, as well as the adequacy of tissue samples harvested. A total number 116 patients were enrolled in the study. 9 patients did not meet inclusion criteria and were excluded. 52 patients were randomized and assigned to standard group and 55 to combo group. At T2b and T3 (corresponding to the biopsy time and time after the biopsy, respectively) there was a significantly lower ($p < 0.05$) perception of pain in the patients who received sedoanalgesia (combo-group) compared to those who did not (standard group). Moreover, 100 % of the patients in combo group who had previously undergone this procedure without premedication reported that they would prefer sedoanalgesia for the subsequent procedures. Administration of oral analgesia and anxiolysis is a safe and feasible option to be used in outpatient setting; sedoanalgesia is very effective in reducing pain during the biopsy and it diminishes the anticipatory anxiety related to a painful procedure. Patients should have the possibility to choose between local anesthesia alone or sedoanalgesia plus local anesthesia.

P100

ASSESSMENT OF HM-PRO ITALIAN VERSION IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES IN CLINICAL PRACTICE

E.N. Oliva¹, P. Cufari¹, A. Andriani², C. Stelitano¹, S. Frongia², C. Alati¹, V. Martini¹, D. Gangemi¹, F. Rodà¹, B. Greve¹, G. Praticò¹, S. Salek³, T. Ionova⁴, B. Martino¹

¹Grande Ospedale Metropolitano Bianchi Melacchino Morelli; ²Ospedale F. Spaziani; ³School of Life and Medical Sciences University of Hertfordshire; ⁴Multinational Centre for Quality of Life Research St. Petersburg State University Medical Center

Background: HM-PRO is a new tool for the evaluation of patient reported outcomes (PROs) in haematological malignancies (HMs). It consists of two scales: quality of life and signs and symptoms.

Aims: To evaluate the correlates of Italian HM-PRO scores in clinical practice.

Methods: 200 patients with HMs will complete the HM-PRO. Demographics and disease characteristics are collected. Higher scores represent higher negative impact of disease and/or treatment and symptoms.

Results: 49 patients (36 males), mean age 65 (SD 12) years, have been included. Twenty-two were ambulatory, 10 in Day Hospital and 17 hospitalised. HM duration was median 1.3, interquartile range (IQR) 0.3 – 5.6 years. Diagnoses: 15 acute myeloid leukemia (all undergoing active treatment), 12 myelodysplastic syndrome, 7 aggressive Non Hodgkin Lymphoma (NHL), 4 acute lymphoblastic leukemia, 3 indolent NHL, 3 myeloma, 2 aplastic anemia, 1 MDS/myeloproliferative neoplasm, 1 chronic lymphocytic leukemia. Sixteen were in remission, 17 stable and 16 progressive. Mean Hb was 10.6 (SD 2.5) g/dL, PLT 137 (SD 107) Gi/L, and 20% had severe cytopenias requiring red blood cell (N=16) and platelet (PLT) transfusions (N=12). Standardized Chronbach Alpha was 0.86 for physical (PB), 0.82 for emotional behaviour (EB), 0.63 for social wellbeing (SW), 0.75 for Eating and Drinking impact (ED), 0.73 for symptom score (SS). Median scores: PB 29 (IQR 11-77), SW 17 (IQR 0-50), EB 27 (IQR 14-45), ED 25 (IQR 0-75), SS 22 (IQR 13-35). Age correlated with PB ($r=0.389$, $p=0.006$) and SW ($r=0.497$, $p<0.0001$). Time from diagnosis correlated with ED ($r=-0.326$, $p=0.022$) and SS ($r=-0.293$, $p=0.041$). Median EB score was better in cases in remission (14, IQR 5-27) than those that were stable (37, IQR 15-55) or in progression/recurrence (30, IQR 19-46, $p=0.021$). However, in patients with acute leukemia, PB, EB and SS scores were worse in those in remission or stable versus those resistant/refractory. Patients on active treatment perceived a significant impact on ED, with median 50

(IQR range 0-75) versus 0 (IQR 0-25) in patients off treatment ($p=0.004$). Hb correlated with PB ($r=0.369$, $p=0.009$) and ED ($r=-0.401$, $p=0.004$); PLT counts with PB ($r=-0.397$, $p=0.005$), SW ($r=0.377$, $p=0.008$) and SS ($r=-0.326$, $p=0.022$); and neutrophils with PB ($r=0.373$, $p=0.008$).

Conclusions: HM-PRO is valid to capture PROs in patients with HMs. Disease status, treatment and peripheral blood counts are associated with health-related QoL and symptoms.

P101

THE CAREGIVERS' EXPERIENCE IN PROVIDING CARE TO A FAMILY MEMBER AFFECTED BY BLOOD MALIGNANCIES: A PHENOMENOLOGICAL STUDY IN THE HOME CARE SETTING

I. Capodanno¹, P. Alfieri¹, C. Pedroni¹, R. Prandi², M. Rocchi³, E. Tamagnini¹, F. Merli¹, L. Ghirotto¹

¹Azienda USL - IRCCS Reggio Emilia; ²Servizio Infermieristico Domiciliare Distretto di Carpi - Azienda USL Modena; ³Hospice Casa Madonna dell'Uliveto Albinea

In literature there is no research reporting the experience of caregivers assisting at home patients with hematological malignancies until the end of life. While understanding the caregiver's experience is a difficult task to define in terms of quantitative data, the qualitative research methodology is more suitable to deepen such a phenomenon and to assess responsive models to the practical, psychological and relational needs of families. The objective of the study was to comprehend the experience of caregivers as pictured by their own perspective, where a domiciliary program of supportive and palliative care is routinely offered to frail patients with leukemia, lymphoma and myeloma. This was carried out as a descriptive phenomenological study, in order to investigate, enhance and describe the subjective meaning of the participants' lived-experiences as well as deeply explore insights into people's motivations and actions. The study was conducted between June 2016 and July 2018 in a cohort of 17 caregivers recruited by identifying and selecting information-rich cases according to the principle of purposive sampling (in our centre more than 300 patients have been assisted at home since 2012). Data were collected by 14 in-depth interviews carried out by two trained researchers using a pre-planned guide for interviews that guaranteed the inter-rater consistency. Caregivers were asked to describe and comment on their experience throughout the patient's illness journey. When necessary researchers posed probing questions in attempt to obtain detailed narratives. Data collection ended when data saturation was reached. Five core themes emerged from the analysis: a) feeling connected with the patient (feelings of being connected to the desires, behaviors, awareness of the patient); b) experiencing the path with fatigue (perceptions of providing care to the patient at home); c) perceiving home care as an opportunity (aspects linked to home care and caregiving within a privacy-sensitive situation); d) feeling the support from health professionals (qualitative evaluation of caregivers towards home care professionals); e) developing a sense of self-efficacy (experiences of strength, skill and determination in taking on the role of caregiver). These findings suggest the importance to provide training and empowerment to the caregivers as part of the global management of hematological patients assisted at home during the advanced phases of disease and end-of-life period.

P102

SUBCUTANEOUS IMMUNOGLOBULIN ADMINISTRATION DECREASES THE INFECTION RATE COMPARED TO INTRAVEOUS IMMUNOGLOBULIN IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND SECONDARY IMMUNODEFICIENCY

A. Visentin A, F.R. Mauro, S. Rosati, S. Imbergamo, E. Scmazzone, S. Pravato, L. Romano Gargarella, M. Facco, F. Frezzato, F. Severin, R. Foà, G. Semenzato, L. Trentin

Hematology and Clinical Immunology Unit, Department of Medicine, University of Padua, Padua, Italy. Venetian Institute of Molecular Medicine, Padua, Italy; Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy

Secondary immunodeficiency (SID) is a common complication in chronic lymphocytic leukemia (CLL) which favors the development of life-threatening infections. Subcutaneous immunoglobulins (IG) (SCIG) have been proved to be as effective as intravenous immunoglobulin

(IVIg) in primary immunodeficiencies. Since, only a few studies investigated SCiG in SID, the aim of this study was to assess the efficacy of SCiG in CLL. Igs were administered to CLL patients with symptomatic hypogammaglobulinemia. Serum Ig levels (IgG, IgA and IgM) were recorded within 3 months before starting IG therapy (baseline), after 3 and 6 months of therapy and within three months from the last available follow-up. We collected data from 116 CLL patients who received IG: 63% males, the median age was 68 years, 91% received at least one therapy (range 0-9), 48% harbored unmutated IGHV gene and 16% TP53 disruptions. 49 patients received IViG and 88 SCiG. Despite similar basal and +3 months IgG levels, patients receiving SCiG achieved higher IgG at +6 months and at the last follow-up (3.9, 5.0, 5.5 and 5.4 g/L vs 3.8, 5.2, 6.1 and 6.2 g/L for IViG and SCiG, $p=0.001$). 29 patients shifted from IViG to SCiG, while none to the opposite. We observed that patients who shifted from IViG to SCiG were able to achieve higher IgG levels (median IgG 4.8 vs 6.6g/L, $p<0.0001$). While all patients experienced G3-4 infections (mainly pneumonia or sepsis) before IG, the rate of infections decreased to 80% with IViG but to 64% with SCiG ($p=0.0019$). The incidence of all and G3-4 infections decreased to 0.59 and 0.17, and 0.30 and 0.13 events/people/year with IViG and SCiG, respectively. Moreover, the incidence of infections in patients receiving SCiG and kinase inhibitors (i.e. ibrutinib or idelalisib) was not higher than in untreated patients. Adverse events during SCiG infusions occurred in 11% subjects, usually local mild skin reaction or pruritus. Bruising did not increase during concomitant SCiG and ibrutinib. After a median duration of IG of 3.7 years, 71% of subjects discontinued IViG therapy as compared to 36% with SCiG ($p<0.0001$). Overall, 42 patients died during the follow-up mainly due to infections (52%) and disease progression (21%). In this study we described the clinical features of a large cohort of CLL with SID receiving IG. We demonstrated that SCiG are active and well tolerated drugs that allows to reach higher IgG levels and to decrease the incidence of severe infections better than IViG.

P103

OBESITY AND METABOLIC SYNDROME AMONG ADULT LYMPHOMA SURVIVORS

A. Daniele, S. De Summa, A. Sciacovelli, A. Rana, S. Ciavarella, A. Paradiso, R. Divella, E. Savino, A. Guarini, C. Minoia

S.C. Ematologia; S.D.D. Patologia Clinica, S.C. Oncologia Sperimentale e Gestione Biobanca - IRCCS Istituto Oncologico "Giovanni Paolo II"

Background: Metabolic dysfunction, including the metabolic syndrome (MetSyn) and insulin resistance, is a long-term complication of curative treatment for many cancer patients including lymphoma survivors. In these patients, prognosis and quality of life can be adversely influenced by obesity, physical inactivity and metabolic dysfunction so, preventive measures, including dietary counseling and tailored exercise should be initiated early in the course of survivorship.

Patients and Methods: The study was conducted analyzing, in relation to the diagnosis of diffuse large B-cell lymphoma (DLBCL) vs Hodgkin lymphoma (HL) and therapy, the presence of MetSyn, obesity, sarcopenia, and type A and B dyslipidemia. We enrolled 114 lymphoma survivors (65 women and 49 men) aged between 24 and 82 years in continuous remission of lymphoma for at least 3 years within the "CCM2014 project supported by the Italian Ministry of Health. Each of them underwent an anthropometric and plicometric evaluation and measurement of metabolic parameters (glycaemia, total cholesterol and HDL, triglycerides). For each of them a personalized food plan was developed.

Results: Our preliminary results showed a lower risk of developing MetS in patients with HL vs DLBCL ($p<0.001$), while steroid use during therapy significantly increased the risk of MetS and sarcopenia in DLBCL patients. Concerning the dyslipidemia A, multivariate analysis showed that the HL group had a significantly lower risk than the DLBCL of developing this conditions. Dyslipidemia B showed instead of being related to smoking. Particularly in the univariate analysis both ex-smokers and smokers have a significantly higher risk of developing this metabolic disorder. Anova test, showed in the DLBCL group a statistically significant correlation concerning the waist circumference in both women and men with MetS vs no MetS with $p>0.05$ and $p=0.005$ respectively; statistically significant association was also observed in the percentage of lean mass; DLBCL men in the group with MetSyn

had a significant sarcopenia compared to those without metabolic syndrome ($p=0.04$).

Conclusions: These preliminary data suggest that DLBCL patients have a higher risk of developing metabolic syndrome and sarcopenia compared to HL most likely as a result of taking steroids, so an early nutritional intervention associated with adequate physical activity could reduce the risk of onset of both complications in lymphoma survivors.

Chronic Lymphatic Leukemia and Lymphoproliferative Syndromes

P104

SEVERE INFECTIONS AND PNEUMONIA IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) TREATED WITH KINASE INHIBITORS (KIS) IBRUTINIB OR IDELALISIB IN THE REAL WORLD

F.R. Mauro¹, D. Giannarelli², A. Visentin³, G. Reda⁴, M. Coscia⁵, A. Tedeschi⁶, P. Sportoletti⁷, A. Chiarenza⁸, S. Ciolli⁹, L. Levato¹⁰, M. Gentile¹¹, L. Laurenti¹², G.M. Rigolin¹³, C. Vitale⁵, A.M. Giordano¹⁴, R. Murru¹⁵, L. De Paoli¹⁶, G. Del Poeta¹⁷, S. Rosati¹, C. Riemma¹, R. Cassin⁴, A.M. Frustaci⁶, C. Stelitano¹⁸, A. Cuneo¹³, S. Molica¹⁰, C. Girmenia¹, R. Foà¹, L. Trentin³

¹Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome; ²Department of Experimental Clinical Oncology, Istituto Nazionale Tumori Regina Elena, Rome; ³Hematology, Department of Medicine, University of Padua; ⁴Hematology Department, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan; ⁵Division of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino; ⁶Department of Hematology, Niguarda Cancer Center, Milano; ⁷Institute of Hematology-Centro di Ricerca EmatoOncologica (CREO), University of Perugia; ⁸Division of Hematology, Ferrarotto Hospital, Catania; ⁹Department of Hematology, Firenze University; ¹⁰Department of Hematology, Pugliese Ciaccio Hospital, Catanzaro; ¹¹Hematology Section, Cosenza Hospital, Cosenza; ¹²Fondazione Policlinico Universitario A. Gemelli, Roma; ¹³Hematology Section, St. Anna University Hospital, Ferrara; ¹⁴Department of Emergency and Transplantation, Hematology Section, University of Bari; ¹⁵Division of Hematology and CTMO, Businco Hospital, Azienda Ospedaliera Brotzu, Cagliari; ¹⁶Division of Hematology, Dept. of Translational Medicine, Università del Piemonte Orientale, Novara; ¹⁷Division of Hematology, S. Eugenio Hospital and University of Tor Vergata, Rome; ¹⁸Department of Hematology, Azienda Ospedaliera Bianchi Melacchino Morelli, Reggio Calabria, Italy

Kinase inhibitors (KIs) have significantly improved the outcome of patients with CLL and are widely used in clinical practice. Nonetheless, infections are still an important cause of morbidity and mortality in patients treated with these agents. A retrospective study was carried out to define the clinical characteristics and outcome of CLL patients who developed severe infections while on ibrutinib or idelalisib given in the real world. This study included 612 patients, from 18 Italian institutions, who were treated with KIs. The median follow-up was 16.5 months for the 485 patients treated with ibrutinib (461 R/R and 24 TN patients) and 16 months for the 127 R/R patients treated with idelalisib. Binet stage C was present in 49% of patients, 71.9% were IGHV unmutated and 47.5% showed a TP53 disruption. The median number of prior treatments was 2 (0-9). Severe infections were recorded in 177/612 (30%) patients, in 129/461 (26.6%) treated with ibrutinib and in 48/127 (37.8%) treated with idelalisib ($p=0.01$). The two most common types of infection were pneumonia (131/612 patients, 21.4%) and sepsis (16/612 patients, 2.6%). Five patients treated with ibrutinib developed a neurologic infection (encephalitis, 4; meningitis, 1). A fungal infection, in most cases due to *Aspergillus*, was recorded in 11 patients, 8 treated with ibrutinib and 3 with idelalisib. The infection-free survival (IFS) was significantly shorter for patients treated with idelalisib (36 months IFS, idelalisib vs ibrutinib, 23.1% vs 60.4%; $p<0.0001$). Infections were the reason for treatment discontinuation in 26/461 (5.6%) patients treated with ibrutinib and 18/127 (14.2%) with idelalisib. Deaths due to infection occurred in 5.5% of patients, to CLL progression in 3.7% and to Richter syndrome in 2.8%. The occurrence of severe infections had a significant adverse impact on survival in patients treated with ibrutinib ($p=0.001$) and in those treated with idelalisib ($p=0.01$). In conclusion, infections represented a relevant cause of morbidity and mortality in CLL patients treated with KIs in the real world. Awareness of the risk of infections, in particular in heavily pre-treated patients, appropriate prophylaxis and prompt diagnosis are factors of crucial importance for a successful management of CLL patients treated with KIs.

P105

DROPLET DIGITAL PCR IS A SENSITIVE TOOL FOR DETECTION OF TP53 DELETIONS AND POINT MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

R. Frazzi¹, V. Bizzarri², L. Albertazzi³, V.Y. Cusenza¹, L. Coppolecchia⁴, S. Luminari⁴, F. Ilariucci⁴

¹Laboratory of Translational Research; ²Laboratory of Genetics; ³Laboratory of Chemical Chemistry; ⁴Hematology, Azienda Unità Sanitaria Locale IRCCS di Reggio Emilia

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in adults and has an average incidence rate of 4.3 new cases / 100,000 people per year. Among the several prognostic parameters available today, the inactivation of TP53 gene is considered one of the most relevant. The positivity to del17p by fluorescence-in situ hybridization (FISH) is the currently accepted method for TP53 evaluation of patients diagnosed with CLL but this technique is characterized by low sensitivity. Availability of highly sensitive and specific techniques to detect del17p would allow to evaluate minimal residual disease (MRD) during patient management and to identify novel surrogates for progression free survival evaluation. In this work, we present a droplet digital PCR (ddPCR)-based approach aimed at discovering TP53 deletions or point mutations at the time of diagnosis. We recruited 46 patients diagnosed with CLL with Azienda Unità Sanitaria Locale IRCCS di Reggio Emilia (years 2017-2018), who underwent FISH for the assessment of del17p. We designed ddPCR probes on exons n°5, 6 and 7 of the TP53 gene and used RRP30 probes as reference gene. We first compared copy number variation results by ddPCR for each single exon to FISH responses, in order to check the diagnostic accuracy of our approach. The samples with undefined results were further characterized through specific probes for exons n°1, 2 and 4. Receiver operating characteristic (ROC) curve analysis was performed to assess quantitative markers diagnostic accuracy. The Area under the ROC curve (AUC) was estimated with its confidence interval (CI). Diagnostic cut-off was located by Youden-Index maximization. Diagnostic accuracy performance indexes (sensitivity, specificity) of the calculated threshold were accompanied by CI. Each single exon was characterized by high specificity (range 91-97%) and high sensitivity (range 82-100%). We next investigated a few patients for whom pathogenic point mutations had been previously discovered by Sanger sequencing. We found that ddPCR can quantify the point mutations in samples collected at different time-points reflecting the mutational load of the patient. Our data demonstrate that ddPCR is a reliable and sensitive technique for the detection of TP53 deletions and point mutations. This technique can represent an useful approach for MRD assessment. It can be also possibly extended to other lymphomas, namely diffuse large-B cell, follicular and marginal zone lymphomas.

P106

COMBINED ANTIBODY DEFICIENCY AND INFECTIOUS FREE SURVIVAL IN HYPOGAMMAGLOBULINEMIA PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A LONG TERM FOLLOW UP MONOCENTRIC REAL-LIFE COHORT ANALYSIS

R. Murru, G. Caocci, A. Galitzia, S. Uda, F. Culurgioni, S. Oppi, G. La Nasa

Hematology and Stem Cell Transplantation Unit, Ospedale Oncologico A. Businco, Azienda Ospedaliera Brotzu, Cagliari, Italy; Department of Medical Sciences and Public Health, Università degli Studi di Cagliari, Italy

Background: Infections represent a major contributor to morbidity and mortality in chronic lymphocytic leukaemia (CLL). Hypogammaglobulinemia (HGG) is a common immune dysfunction in CLL, associated with an increased risk of infections.

Aims: To analyze impact of HGG on infection free survival (IFS), infectious risk and major infections in a monocentric real life cohort of CLL patients followed for 25 years; to determine impact of IgA deficiency, combined antibody deficiency (CAD) and HGG late onset on infectious death risk.

Methods: We retrospectively evaluated 211 unselected CLL patients, diagnosed from 01/1988 to 03/2018, median age 64 years (37-88). HGG was evaluated at diagnosis (n=175), before first line therapy (n=100) and 12 months after therapy (n=92).

Results: During the course of disease 126 patients (60%) underwent

at least one infectious event, regardless of severity. Of these, 51 (24%) reported at least one major infectious event (grade ≥3 CTCAE), mainly pneumonitis, skin and soft tissue infections. IgA levels at diagnosis were significantly lower in B and C Binet (p=0.002); IgG (p<0.001) and IgA levels (p=0.03) were reduced in patients who experienced infections within 12 months after diagnosis (Figure 1A); 51 patients (24%) reported major infections and showed reduced IgG and IgA levels at diagnosis (p=0.01, p=0.006). 25-year IFS for major infections was 67.2% for the entire cohort. C Binet patients showed significantly lower IFS in comparison with A and B stage (21.5% vs 73.5% and 73.3%; p<0.001) (Figure 1B). In multivariate analysis IGHV unmut was associated with lower IFS (p=0.009). CAD was observed at diagnosis in 60% of patients with severe infections versus 27% of patients who did not experience events (p<0.001). Furthermore, 20.4% of patients developed a late onset CAD with significant reduction of IFS and increase of serious infectious events. OS was heavily reduced by early infectious events occurred during the first year from diagnosis, with higher mortality in patients with CAD at diagnosis or late onset; in both cases, risk of infectious death was 10 times higher compared to remaining cohort (p=0.004).

Conclusions: Our results confirmed the negative impact of HGG on infectious risk and major infections; IgA deficit and CAD were strong predictors for development of these events. Early identification of clinical profile of high risk infections (selective IgA deficit, CAD, late onset HGG) is useful in optimal management of CLL patients.

tions and with a satisfactory hematological response, and “Non-Responder” (n= 4) as being resistant to Ibrutinib and switched to another type of therapy. We observed that both HSP70 and HSF1 were reduced after therapy in only those patients responsive to Ibrutinib (86±2% for HSP70 and 80±13% for HSF1 from the beginning of the therapy until the end of the observation). When we analyzed the two proteins in patients who failed Ibrutinib, we observed an increase when the treatment was failing and thus the disease was progressing (4.4±2.0-fold for HSP70 and 3.3±0.5-fold for HSF1 from the beginning of the therapy to progression). Our findings may suggest an involvement of HSP70/HSF1 axis in mechanisms of therapy-resistance in CLL cells as described for other cancer types in literature. Considering our previous demonstration that HSP70/HSF1 axis is a druggable target in CLL patients, independently from their prognosis, the use of HSP70 and HSF1 inhibitors could represent a novel rational therapeutic approach to overcome Ibrutinib resistance in those patients who relapsed after this type of treatment.

P108 ASSESSMENT AND USE OF IMMUNOGLOBULINS IN THE REAL LIFE MANAGEMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). A GIMEMA SURVEY

C. Riemma¹, A. Piciocchi², E. Crea², R. Foà¹, F.R. Mauro¹ on behalf of the GIMEMA Working Party Malattie Linfoproliferative Croniche*

¹Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome; ²Italian Group for Adult Hematologic Diseases (GIMEMA), Rome, Italy

Hypogammaglobulinemia (HGG) is frequently observed in patients with chronic lymphocytic leukemia (CLL), in particular, in heavily treated patients and in those with adverse biologic features. Several guidelines recommend the infusion of immunoglobulins (Igs) in CLL patients with HGG and recurrent infections. However, the clinical relevance of serum IgG levels and Ig support in CLL is uncertain.

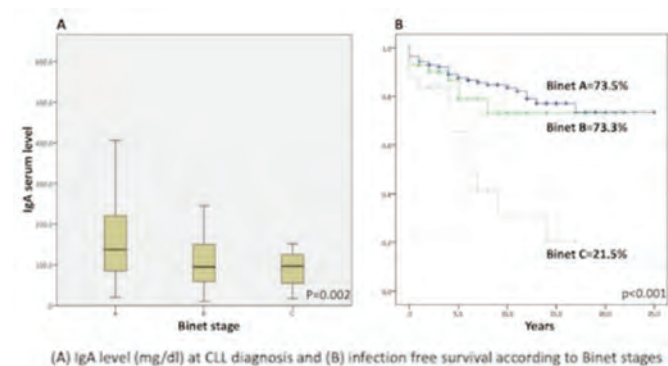


Figure 1.

P107 HSP70/HSF1 AXIS IS INVOLVED IN IBRUTINIB-RESISTANCE MECHANISMS OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

F. Frezzato, A. Visentin, M. Bragagnolo, F. Severin, S. Pizzo, C. Lista, M. Facco, F. Piazza, G. Semenzato, L. Trentin
Department of Medicine Padua University School of Medicine, Veneto Institute of Molecular Medicine (VIMM), Italy

The management of patients with Chronic Lymphocytic Leukemia (CLL) has been significantly changed by the introduction of the Bruton's tyrosine kinase (BTK) inhibitor Ibrutinib. Although responses are long lasting in most patients, relapses occur and outcomes after Ibrutinib failure are dismal due to a lack of effective agents. Mutations affecting BTK and PLC 2 are involved in resistance phenomena, but these mutations are not common and alternative mechanisms supporting the development of Ibrutinib-resistance remain to be clarified. We previously demonstrated that Heat Shock Protein of 70kDa (HSP70) plays a pivotal role in mediating survival and progression of CLL. Moreover, studies performed on various cancers, including hematological, reported a correlation between HSP70 overexpression and therapy resistance. For this reason, we are aimed at addressing whether HSP70 and its main regulator HSF1 (Heat Shock Factor 1) are involved in the development of Ibrutinib-mediated resistance. By Western blotting we analyzed HSP70 and HSF1 proteic levels in purified B lymphocytes from 8 CLL patients following *in vivo* Ibrutinib-containing regimen. Cells were collected before therapy and over several months of treatment (about 30 months with a time-point of 4 months). At the end of the collection, patients have been divided in “Responder” (n=4), when in good general condi-

Table 1. Investigators included in the survey.

Investigator	Center	Genova
A. Tassinari	UDC Ematologia 1 e 2 Centro Trapianti di Midollo del midollo dell'IRCCS AGO San Martino-IGT	Genova
A. Tassinari	Ospedale Regionale "La Salluzia" - S. Emidio	Imperia
A. Maggi	UDC Ematologia - A.O. "S. Annunziata" - P.O. S.G. Muscati	Taranto
A. Pizzi	ASL22 - Birela Ospedale degli Infermi - Osp. S. Spirito - Osp. S. Maria della Pace	Teramo
A. Cazzaniga	Fond. Ospedaliera di Ricovero - Osp. di Medicina Specialistica UDC Ematologia	Vercelli
A. Miele	Divisione di Ematologia-Diagnosi "Antonio"	Brescia
A. Chiarenza	Università di Catania - Ospedale di Ematologia - Ospedale "Materassi"	Catania
A. Milla	Risparmio di oncologia - Istituto Fondazione e Riferimento di carenze di carenze	Sestri
A. Cassatella	IRCCS AGO San Martino-IGT - UDC Ematologia	Genova
A. Pileri	Ospedale "S. Maria Goretti"	Parma
E. Bellizzi	Clinica Medica II - Azienda Ospedaliera Università San Martino	Genova
S. Biagini	Dipartimento Oncologico Ospedale di Sassari	Sassari
C. Pizzardi	Ospedale Maggiore - Ospedale Maggiore	Bologna
C. Cazzaniga	Sezione di Ematologia - Cancer Center Humanitas	Rozzano
F. Suardi	Dipartimento Ematologia-Oncologia A.B. "Bianchi-Malerba-Orsini"	R. Cassina
C. Benatti	Azienda Ospedaliera "S. Gerardo"	Brescia
S. Corbelli	Divisione di Ematologia - ADD - Lunigiana	Firenze
C. Baraldi	Divisione di Ematologia - Università di Pisa	Pisa
C. Sica	Azienda Ospedaliera di Ricerca Nazionale "A. Castelli"	Trieste
G. Di Fronzo	UDC Oncologia Ospedale di Niguarda-Casaticcia "M. Minelli"	Milano (Sesto)
G. Persechini	UDC Ematologia - Azienda Ospedaliera "S. Antonio e Biagio e Cesare Arrigo"	Alessandria
G. Fratantuono	UD Ematologia - Centro Oncologico Bassilata	Roma (Via Vittoria)
E. Cazzaniga	Centro Asistiale di Ematologia - ASB	Livorno
T. De Rosa	UDC Ematologia - Ospedale di Carpi-Ospedale Civile di Prato	Carpi
F. Corò	Azienda Ospedaliera "Sant'Andrea" - Università Sapienza - Seconda Facoltà di Medicina e Chirurgia	Roma
F. Cazzaniga	Azienda Ospedaliera degli Studi di Napoli "Fedele II" - Facoltà di Medicina e Chirurgia	Napoli
M. Di Fronzo	M.U. di Ematologia e Trapianti - I.C. Istituto Nazionale Tumori Regina Elena	Roma
F. Colom	UDC di Ematologia - Ospedale Carlo Poma	Parma
F. Geronzi	UDC di Ematologia - Ospedale "S. Eusebio"	Aversa
G. Maurino	Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IGT	Mantova
G. Rossi	UDC Ematologia - Ospedale IRCCS di Ricerca Oncologica Maggiore Poma	Mantova
G. Minio	UDC Ematologia Generale P.O. S. Vincenzo	Torino
G. Pileri	UDC Ematologia - Medicina Specialistica e Oncologia Medica - ADD Policlino G. Martino Messina	Messina
A. Giacchi	UDC Ematologia - ADD "Santeramo"	Siena
R. Pileri	Spazio Clinico di Ematologia Ospedale "S. Agostino" - Azienda Ospedaliera-Università	Foggia
I. Spadoni	Azienda Ospedaliera - Università Degli Studi di Bari - Azienda Ospedaliera-Università	Bari
L. Argentei	ADP Santa Maria - IGT - S.C. Oncopatologica	Terzi
F. Pileri	Ospedale Civile "Vito San Carleolo"	Parma
L. Tassi	Divisione di Ematologia - Azienda Ospedaliera Ospedale Riuniti "Papa Pio"	Messina
L. Tassi	Ematologia ed Immunologia Clinica - Università degli Studi di Padova	Padova
L. De Paoli	SCD Ematologia - Università del Piemonte Orientale Amedeo Avogadro	Alessandria
G. Di Fronzo	UD Ematologia - I.C. Città della Salute e della Scienza di Torino - San Giovanni Battista	Torino
G. Lasio	UDC di Ematologia - Istituto Tumori - Giovanni Paolo II	Bari
L. Tassinari	Università Cattolica del Sacro Cuore - Policlinico A. Gemelli	Roma
L. Pileri	UDC di Ematologia e Trapianti - AGO San Giovanni di Dio e Ruggi d'Anagnina	San Marino
L. Tassinari	Unità Trapianti di Midollo di Ospedale Maggiore	Imperia
M. Mian	Ematologia Ospedale S. Maurizio - Ospedale S. Biagio - Azienda Ospedaliera "S. Matteo"	Bozzano
M. Valentini	UD Ematologia - Fondazione IRCCS "Istituto G. Pini"	Treviso
M. Di Fronzo	UDC di Ematologia - Ospedale Maggiore Poma	Mantova
M. Motta	UD Ematologia Ospedale Civile - Ospedale "S. Maria Goretti"	Brescia
M. Leuci	Divisione di Ematologia dell'Università degli Studi di Torino - "Città della Salute e della Scienza di Torino"	Torino
M. Gentile	UD Ematologia - P.O. Annunziata - A. di E. E. di E. E.	Cosenza
R. Nicolini	UDC Ematologia Ospedale "Antonio Ruffini"	Barletta
M. Forlani	Unità di Onco Ematologia - Azienda Ospedaliera "S. Gerardo"	Catania
M. Marchetti	SOC Medicina Interna B - Ospedale Civile "Messa di Anzi"	Anzi
M. Pileri	Struttura Specialistica Dipartimentale di Ematologia Ospedale San Giovanni Bosco	Torino
M. Tassinari	UDC di Ematologia Ospedale Civile	Catania
D. Aruffo	Divisione Ematologia - Università Campus BioMedico	Roma
D. Pileri	Ospedale "S. Biagio" - Ospedale "S. Maria Goretti"	Verona
P. Pileri	Divisione di Ematologia ed Immunologia Clinica - Ospedale S. Maria della Misericordia	Parma
R. Pileri	Centro di Riferimento Oncologico	Averara
P. Galloni	UDC Ematologia e Terapia Cellulare - Ospedale "S. G. Massimo" di Ascoli Piceno	Ascoli
R. Argentei	UDC Medicina Trasfusionale e Culture Spinali - Azienda Ospedaliera San Gerardo Torricani	Bari
R. Pileri	SOC Ematologia e Centro Trapianti Ospedale di Treviso - IGT UST	Treviso
G. Di Fronzo	Azienda Ospedaliera Santa Anna Dipartimento di Scienze Mediche Sezione di Ematologia	Taranto
R. Centurioni	UD di Medicina Interna - Ospedale Civile "Ascoli Piceno"	Ascoli Piceno
R. Motta	Struttura Complessa di Ematologia e CMD - Ospedale "A. Vesuvio"	Capri
R. Pileri	UD Ematologia - Ospedale Civile "S. Maria Goretti"	Verona
R. Pileri	Unità Operativa - Centro per le malattie del sangue - Ospedale Civile S. Giacomo	Castel Verone
R. Santoro	UD Ematologia - Ospedale di Pinerolo	Milano
R. Pileri	UD Dipartimento di Ematologia - Dipartimento Oncologico	Rovigo
R. Pileri	Divisione di Ricerca e Diagnostica Sperimentale IRCCS Ospedale San Raffaele	Milano
S. Cazzaniga	UDC Ematologia - Servizio di Immunohaematologia e Medicina Trasfusionale Azienda Ospedaliera Provinciale 2	Belluno
S. Motta	UD Ematologia - Azienda Ospedaliera Pugliese Girone	Canosa
G. Cazzaniga	UDC di Ematologia e Medicina Specialistica - Ospedale S. Maria Goretti	Catania
V. Vianello	UD Oncologia Ospedale "Poma" Ospedale "A. Turati"	Piacenza (SA)
V. Frazzetta	Unità Complessa di Ematologia - UDC di Medicina Generale - ASST Valpurga	Sondrio

To evaluate how Ig levels are considered in the real life clinical management of CLL, hematologists from 136 GIMEMA centers were invited to answer an anonymous online questionnaire developed on a RedCap platform. The questionnaire consisted of 20 multiple choice questions, 15 closed-ended and 5 ranking questions. The main investigated issues were: 1. The rate of hematologists who check routinely serum Ig levels. 2. The rate of hematologists who consider an Ig support in patients with HGG. 3. The criteria considered in the decision making process for Ig support. 4. The expected factors suggesting a clinical benefit of Ig treatment. 5. The preferred use, intravenous or subcutaneous, of Ig infusion. A response to the questionnaire was sent by 78 (57.35%) of hematologists. Igs were regularly checked at diagnosis and during the course of the disease by 87% of hematologists and in the majority of cases (92.3%) all Ig classes - IgG, IgA, IgM - were evaluated. HGG was considered as a prognostic factor influencing the outcome of CLL patients by 70% of responders. Patients with HGG were considered for Ig support by 95.9% of the interviewed hematologists in the presence of recurrent hospital admissions due to infections, radiologically proven pneumonia, comorbidities and prior chemoimmunotherapy. The decrease in the number of infections and hospital admission was the expected benefit of the Ig support. Intravenous Igs were the most common type of Igs used. Quality of life and the capacity of patients to self-administer Igs subcutaneously guided the choice between the use of intravenous or subcutaneous Igs. In conclusion, the survey's response rate (57.3%) is relatively high compared to the usual survey rates. The results of this survey show that the majority of Italian hematologists believe that Ig levels deserve substantive consideration in the clinical management of CLL and that HGG with recurrent infections requires Ig support. Quality of life and the capacity of patients to self-administer Igs guided the choice between subcutaneous or intravenous Ig administration.

P109

A PROGNOSTIC MODEL TO PREDICT THE RISK OF ATRIAL FIBRILLATION IN CHRONIC LYMPHOCYTIC LEUKEMIA AND ITS VALIDATION IN A COHORT OF 354 IBRUTINIB-TREATED PATIENTS

A. Visentin, M. Deodato, F.R. Mauro, F. Autore, G. Reda, C. Vitale, S. Molica, G.M. Rigolin, S. Imbergamo, E. Scmazzone, S. Pravato, F. Frezzato, M. Facco, F. Piazza, L. Cesini, A. Tedeschi, L. Laurenti, R. Cassin, M. Coscia, A. Cuneo, R. Foà, G. Semenzato, L. Trentin

Hematology and Clinical Immunology unit, Department of Medicine, University of Padua, Padua, Italy; Venetian Institute of Molecular Medicine, Centro di Eccellenza per la Ricerca Biomedica Avanzata, Padua, Italy; Hematology unit, Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; Hematology unit, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; Hematology Institute, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; Hematology unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore, University of Milan, Milan, Italy; Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino, University of Turin, Turin, Italy; Department Hematology-Oncology, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy; Hematology section, Department of Medical Sciences, University of Ferrara - Azienda Ospedaliera-Universitaria di Ferrara, Ferrara, Italy

The BTK inhibitor ibrutinib has improved the management of chronic lymphocytic leukemia (CLL) but it can trigger atrial fibrillation (AF) in 8-10% of patients. The aims of this study were to report the prevalence of AF in ibrutinib-naïve CLL, to define a predictive model for the development of AF and to test it in a cohort of ibrutinib-treated subjects. We retrospectively analyzed data from ibrutinib-naïve CLL patients referred to Padua hospital. Time to AF (TTAF) and overall survival (OS) were evaluated with Kaplan-Meier method. Univariate and multivariate Cox models were run to identify independent factors associated with AF. Then, risk values were obtained based on the hazard ratios (HR/2). The score for AF was calculated as the sum of each risk value. Subsequently, the model was validated in a cohort of 354 ibrutinib-treated patients referred from 8 hematological centers. Among the 860 patients from Padua hospital, a history of AF was reported in 21 patients (2.4%) at CLL diagnosis. Among the remaining 839 patients, 47 (5.6%) developed AF after a median follow-up of 9.4 years. The median OS for patients with AF was shorter than that of patients without AF (12 vs 22 years, $p < 0.0001$). Based on univariate and multivariate

analysis we assigned 1 point to age > 65 years ($p = 0.001$), male ($p = 0.003$), dysthyroidism ($p = 0.001$), chronic lung diseases ($p = 0.001$), diabetes ($p = 0.023$) and history of G3-4 infections ($p = 0.019$); 2 points to valvular heart diseases ($p = 0.001$); 3 points to non-valvular cardiopathies ($p < 0.001$). A predictive model was designed based on the sum of the above points. The 10-year TTAF were 0%, 6%, 12% and 29% for patients with score 0, 1-2, 3-4, and ≥ 5 respectively ($p < 0.001$). Subsequently, we applied our AF model to a cohort of 354 ibrutinib-treated patients. 44 (12%) subjects developed AF after a median observation of 25 months, with a 2-year TTAF of 12%. 16 patients (4%) were classified as AF score 0, 218 (62%) score 1-2, 73 (21%) score 3-4, and 46 (13%) at least score 5. The 2-year TTAF was 0%, 5%, 17% and 40% for patients with score 0, 1-2, 3-4, and ≥ 5 respectively ($p < 0.001$). Only 9/44 patients (20%) discontinued ibrutinib. The OS of ibrutinib-treated patients with and without AF was similar ($p = 0.1252$). In this study comorbidities associated with a higher risk of AF were identified and recapitulated into a scoring system. We suggest that patients with a score ≥ 5 should be carefully monitored during ibrutinib or alternative therapies should be considered

P110

PREDICTIVE VALUE OF PERYIPHERAL T LYMPHOCYTES AND CD4+ SUBPOPULATION COUNT ON PROGRESSION TOWARDS ACTIVE DISEASE IN EARLY STAGE, TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA.

M. Gambella, M. Bruzzone, M. Bergamaschi, A. Ibatici, A. Laudisi, E. Coviello, G. Rivoli, M. Miglino, M. Cea, A. Cagnetta, R.M. Lemoli

Chair of Hematology, Department of Internal Medicine (DiMI), University of Genoa, Ospedale Policlinico San Martino-IST; Clinical Epidemiology Unit, Ospedale Policlinico San Martino-IST, Genoa, Italy

CLL is a heterogeneous disease where a fraction of patients progresses to advanced stages rather quickly, and others experience an indolent course. Here we identified circulating lymphocytes (CD4+, CD8+, NK and NKT cells) as powerful biomarker of CLL evolution in newly diagnosed patients. We analysed 226 CLL pts diagnosed between 2000 and 2018. Next we selected 56 CLL pts with early disease (Binet A-B, no active disease) and widespread variability among lymphocytes subtypes (CD3+ T cells, CD16+ CD56+ CD3- NK cells, CD3+ CD4+ T cells, CD3+ CD8+ T cells, CD3+ CD16+ CD56+ NKT cells) at diagnosis, assessed by PB flow cytometry. Progression free survival (PFS) was estimated until development of progression or symptomatic disease occurrence. We retrospectively compared mean values of Lymphocytes Subpopulations (LS) among progressed and unprogressed pt. Then we assessed LS as a dichotomous variable through mean values (mv) of a comparable cohort of healthy individuals (Apoil *et al.*; mv/mcl, T cells: 1473; CD4 cells: 928; CD8 cells: 405; NK cells: 253) and evaluated their impact on PFS. Median follow-up was 24.8 months (range 2.5-287.3). Comparison of mv of LS between progressed and non-progressed CLL pts revealed significant differences only for T cells (3700 vs 2032 respectively, $p < 0.001$) and NK cells (816 vs 439 respectively, $p = 0.010$); no differences were observed for NKT cells. Importantly the dichotomous variable role of LS in term of PFS prediction emerged also for T cells [T cells ≥ 1473 ($n = 42$) vs T cells < 1473 ($n = 14$); Median PFS: 7.8 years vs not reached respectively, $p = 0.017$] but not for NK cells [NK cells ≥ 253 ($n = 38$) vs NK cells < 253 ($n = 14$); median PFS 7.8 vs 3.1 years respectively, $p = 0.713$].

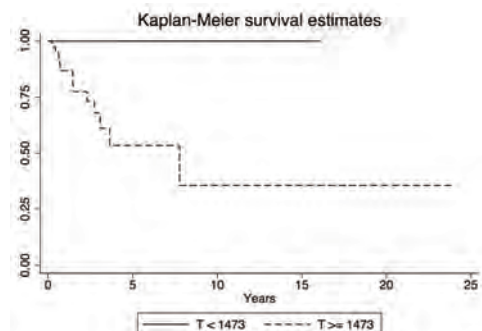


Figure 1.

Finally, analysis of CD4+ and CD8+ T subpopulations showed a significant impact of CD4+, but not of CD8+, on PFS [CD4+ cells \geq 928 (n=31) vs CD4+ cells < 928 (n=18); median PFS 7.8 years vs not reached respectively, p=0.016. CD8+ cells \geq 405 (n=38) vs CD8+ cells < 405 (n=11); median PFS 7.8 years vs not reached respectively, p=0.749], suggesting a different T-cells compartment behaviour. Overall our study suggests that higher T cells number at diagnosis has poor significance in term of CLL evolution from early stage to active disease. A specific T LS analysis suggest CD4+ levels as crucial, whilst CD8+ doesn't impact on disease progression. Although CD4+ LS still remain to be further investigated, here we hypothesize a specific involvement of these cells in CLL immune dysregulation.

P111

PURGING WITH CHLORAMBUCIL TO PREVENT INFUSION-RELATED REACTIONS BEFORE OBINUTUZUMAB ADMINISTRATION: A MONOCENTRIC PILOT EXPERIENCE.

A. Fresa¹, F. Autore¹, I. Innocenti³, A. Corbingi⁴, F. Morelli⁵, A. Tomasso⁶, L. Laurenti⁷

¹Institute of Hematology - Università Cattolica del Sacro Cuore; ²Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS; ³Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS; ⁴Institute of Hematology, Università Cattolica del Sacro Cuore; ⁵Institute of Hematology, Università Cattolica del Sacro Cuore; ⁶Institute of Hematology, Università Cattolica del Sacro Cuore; ⁷Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore, Italy

Background: Obinutuzumab-Chlorambucil (Chl-G) combination in untreated patients (pts) with chronic lymphocytic leukemia (CLL) and comorbidities, has proven to be safe and more effective than other treatments in the CLL11 study. The Chl dose used in CLL11 was lower than its use in associations with Rituximab in other European experiences; moreover, has emerged that the infusion of G is burdened by a high incidence of infusion-related reactions (IRRs). Reducing the disease burden before G infusion could be a solution to prevent the occurrence of these adverse events (AE).

Aims: Given the previous experience of our group, we used an increased dose of Chl in association with G, looking at safety and response.

Methods: We retrospectively analysed a pilot cohort of 10 untreated CLL pts (median age 73 years) with comorbidities, treated with G-Chl regimen. Chl 1 mg/kg every 28 days was administered at a standard daily dose of 10 mg for 8 cycles, with 2 cycles of purging before the start of the G administration. G was infused IV from cycle 3 onwards according to the manufacturer. An independent-samples t-test was conducted to compare complete blood counts (CBCs) before and after the first 2 cycles of Chl.

Table 1. Comparison of CBCs before and after purging treatment with Chlorambucil (Independent t-test).

Variable	Treatment time point	Mean (SD)	Mean difference (95% CI)	P-value
White Blood Cells (x10 ⁹ /l)	Baseline	58.908 (49.254)	38.642 (3.270 - 74.014)	0.034
	After Chl purging	20.266 (20.219)		
Lymphocyte count (x10 ⁹ /l)	Baseline	50.315 (44.199)	34.602 (2.866 - 66.338)	0.034
	After Chl purging	15.713 (18.118)		
Neutrophil count (x10 ⁹ /l)	Baseline	4.033 (2.225)	1.512 (-0.107 - 3.131)	0.065
	After Chl purging	2.251 (0.996)		
Platelet count (x10 ⁹ /l)	Baseline	94.3 (43.992)	-12.5 (-64.762 - 39.762)	0.621
	After Chl purging	106.8 (65.212)		
Haemoglobin levels (g/dl)	Baseline	12.87 (1.67)	0.7 (-1.36 - 2.76)	0.485
	After Chl purging	12.17 (2.62)		

Results: At 2 months after the end of the therapy (median observation time 10 months) the overall response rate was 80%, with 60% of partial responses and 20% of complete responses. In the safety analysis, grade 3-4 treatment-emergent AE occurred in 50% of pts. Three pts experienced grade 1-2 IRR (30%), but no grade 3-4 IRRs or clinical tumor lysis syndrome have been detected in our cohort. When comparing CBCs before and after the first 2 cycles of Chl, we observed a statistically significant reduction of lymphocytes from a mean of 50.310x10⁹/l to 15.710x10⁹/l (p=0.034), while no significant differences were detected in haemoglobin levels, neutrophil and platelet counts. Two pts had to permanently discontinue treatment for infections, 2 pts needed a dose reduction of Chl, and 1 of both G and Chl. Median dose of Chl used was 520 mg for each patient (210-640 mg) with median Chl of 78 mg for each cycle.

Conclusions: Our real-life pilot analysis confirmed that the use of a Chl purging regimen is a valid option to minimize the IRR, since no grade 3-4 were observed. Toxicities and response rates were like already published experiences. These results are encouraging, but further studies with wider cohorts are needed to confirm them.

P112

FRONT-LINE THERAPY FOR ELDERLY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: BENDAMUSTINE PLUS RITUXIMAB OR CHLORAMBUCIL PLUS RITUXIMAB? REAL-LIFE RETROSPECTIVE MULTICENTER STUDY IN THE LAZIO REGION.

F. Autore¹, I. Innocenti¹, F. Corrente¹, M.I. Del Principe², S. Rosati³, P. Falcucci⁴, E. Conte⁵, M.A. Limongiello⁶, D. Renzi, L. De Padua⁸, A. Andriani⁸, F. Pisani⁷, G. Cimino⁶, A. Tafuri⁵, M. Montanaro⁴, F.R. Mauro³, G. Del Poeta², L. Laurenti¹

¹Fondazione Policlinico Universitario Agostino Gemelli IRCCS; ²Dipartimento di Biomedicina e Prevenzione Università degli studi di Roma "Tor Vergata"; ³Department of Cellular Biotechnologies and Hematology, Sapienza University; ⁴Ospedale Belcolle; ⁵Policlinico Universitario Sant'Andrea; ⁶Ospedale di Latina; ⁷IRCCS Istituto Nazionale dei Tumori Regina Elena; ⁸Ospedale F. Spaziani, Italy

Introduction: Elderly patients with chronic lymphocytic leukemia (CLL) are often treated with chemoimmunotherapeutic regimens. Because of ineligibility for Fludarabine-based protocol, Bendamustine plus Rituximab (B-R) or Chlorambucil plus Rituximab (Chl-R) are the alternative schemes that are usually chosen, in fit and unfit elderly patients, which can be applied with expectation of a good response and less toxicity. In literature overall response rates (ORR) between 66% to 84% have been reported Chl-R; for B-R, the ORR ranged between 88% and 98%.

Aim and Methods: We compared these two regimens in a multicentre group to understand why and how physicians could choose from among these schemes and to establish the advantages and disadvantages of each one in terms of safety and efficacy. To this end, we performed a subgroup analysis: high-risk group (HR) included patients with 17p or 11q and/or unmutated IGHV, standard-risk group (SR) patients without 11q or 17p but with mutated IGHV. We conducted a retrospective analysis on the experience, in clinical practice, of B-R and Chl-R as frontline treatment for elderly (\geq 65 years) CLL patients treated at 8 haematological centres in the Lazio region.

Results: We enrolled 192 untreated CLL patients, 111 treated with B-R and 81 with Chl-R; a median dose of 300 mg for B and 90 mg for Chl was used for each cycle. Patients' characteristics are summarized in Table 1. The ORR (93.6% in B-R and 86.5% in Chl-R) was not statistically different between the two groups, such as the time-dependent outcomes PFS, TTR and OS. B-R group showed a higher haematological (p=0.007) and extra-haematological (p=0.008) toxicity compared to Chl-R arm. When comparing toxicities according to the age, we noted that extra-haematological toxicity was higher in very elderly patients (> 75 years) treated with B-R than in elderly patients treated with Chl-R (p=0.03). The biological characteristics, excluded del17p, seem not to have any impact in the treatment choice in this group of elderly untreated CLL patients.

Conclusions: This retrospective study confirms the feasibility of B-R and Chl-R in elderly untreated CLL patients. In real life, very elderly (>75 years) and unfit patients were more frequently treated with Chl-R. This scheme allows to achieve the same ORR, PFS, TTR and OS when compared with B-R because of haematological and extra-haema-

toxicities due to B, in which a greater dose reduction has been shown in comparison to Chl.

Table 1. Patients' clinical and biological characteristics.

Patients' characteristic	B-R	Chl-R
	111 patients	81 patients
Median age at treatment	69 years (range 65-81)	75 years (range 65-85)
Age 65-75	90 (81.0%)	46 (56.8%)
Age >75	21 (19.0%)	35 (43.2%)
Male/Female	65/46	50/31
CIRS score >6	9 patients (8.1%)	20 patients (24.7%)
ECOG score 0	81 (73.0%)	51 (63.0%)
ECOG score ≥1	30 (27.0%)	30 (37.0%)
Lymphocytes count (range)	59.5x10 ⁹ /L (3.0-240.0)	64.0 x10 ⁹ /L (3.0-240.0)
Haemoglobin levels (range)	12.8 g/L (5.8-16.6)	12 g/L (7.7-16.6)
Platelet count (range)	164 x10 ⁹ /L (23-472)	142 x10 ⁹ /L (41-362)
Binet A	33 (29.7%)	42 (51.8%)
Binet B	67 (60.4%)	33 (40.7%)
Binet C	11 (9.9%)	6 (7.4%)
FISH analysis	88/111 patients	75/81 patients
Normal karyotype	35 (39.8%)	20 (26.7%)
13q deletion	25 (28.4%)	33 (44.0%)
+12	15 (17.0%)	15 (20.0%)
11q deletion	9 (10.2%)	7 (9.3%)
17p deletion	4 (4.5%)	-
IGHV	61/111	51/81
Unmutated	42 (68.9%)	29 (56.9%)
Mutated	19 (31.1%)	22 (43.1%)
Subgroup	55/111	52/81
Low-risk group	18 (32.7%)	20 (38.5%)
Intermediate-risk group	37 (67.3%)	32 (61.5%)
B and Chl dose/patient (range)	1680 mg (200-2700)	600 mg (210-980)
Median dose for cycle (range)	300 mg (120-450)	90 mg (60-130)
R dose/patient (range)	4600 mg (1270-7750)	3900 mg (600-7350)
Median dose for cycle (range)	775 mg (600-1000)	666 mg (350-1000)

P113

MOLECULAR ANALYSIS AND PROGNOSTIC IMPLICATIONS IN PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA

F. Culurgioni, R. Murru, S. Uda, C. Musiu, G. La Nasa

Hematology and Stem Cell Transplantation Unit, Ospedale Oncologico "A. Businco", Azienda Ospedaliera Brotzu, Cagliari, Italy

Chronic lymphocytic leukemia (CLL) is a genetically heterogeneous disease characterised by genomic alterations and gene mutation that may portend worse survival or resistance to treatments. We analyzed a monocentric real life cohort of 211 unselected CLL patients with a long term follow up (median: 10 yrs; range: 1-29). All patients, have been observed in our institution from 01/1988 to 07/2018; 136 were male and 75 female; median age at diagnosis was 63 (37-88). In this work we tried to correlate known biological factors (FISH, LDH) with other innovative ones (NOTCH1). Despite the small numbers and the retrospective characteristics of data collection we are able to make some interesting observations. In particular, also in our series the tris12 is significantly correlated with the increase of LDH values (diagnosis 612 U/L range 193-1188 pre-treatment 850 U/L range 220-1759), distinguishing itself from the other FISH anomalies. NOTCH1 alleles were investigated in 146 patients, with NOTCH1 mutation in 22 patients (15%). Furthermore, approximately 50% of patients with tris12 are also

characterized by a NOTCH1 mutation. A further field of investigation was to verify the incidence of NOTCH1 mut in the different FISH sub-categories identified, in order to verify the known correlation between NOTCH1 and tris12 mutations and any other possible associations. 46% of patients with tris12 also had a NOTCH1 mutation, in line with what was reported in the most recently published literature. In the other sub-categories the percentage association was the following: FISH neg: 25.7%, TP53 +: 25%, Del11: 20%, Complex mutations: 12.5%, Del13: 10.5%, Del17: absent. Then we confirm the prognostically unfavorable value of the NOTCH1 mutation, with worsening of the overall survival outcome. This unfavorable prognostic impact is further evident in the IGVH unmutated / NOTCH1 mut subgroup. In conclusion the desirable future is represented by the introduction and validation of new prognostic markers - among these NOTCH1 and others currently under study - which will constitute a valid instrument of evaluation and prognostic stratification together with what has already been used in clinical practice. The recent implementation of new biological diagnostic investigations and their evolution in the field of oncohematological pathologies are able to ensure increasingly innovative investigation tools.

P114

EFFICACY OF BCL2 INHIBITOR IN CLL PATIENTS WHO PROGRESSED AFTER B-CELL RECEPTOR INHIBITORS: A RETROSPECTIVE MULTICENTER ITALIAN EXPERIENCE

I. Innocenti¹, F. Morelli², F. Autore³, A. Piciocchi⁴, A. Frustaci⁵, F.R. Mauro⁶, L. Schiattone⁷, L. Trentin⁸, G. Del Poeta⁹, G. Reda¹⁰, G.M. Rigolin¹¹, A. Ibatucci¹², S. Ciolli¹³, M. Coscia¹⁴, P. Sportoletti¹⁵, R. Murru¹⁶, L. Levato¹⁷, M. Gentile¹⁸, G. D'Arena¹⁹, M.R. Villa²⁰, R. Fontana²¹, A. Tedeschi²², L. Scarfò²³, A. Cuneo²⁴, R. Foà²⁵, L. Laurenti²⁶

¹Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; ²Institute of Hematology, Università Cattolica del Sacro Cuore, Rome, Italy; ³Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; ⁴Italian Group for Adult Hematologic Diseases (GIMEMA), Data Center, Rome, Italy; ⁵Hematology, Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; ⁶Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy; ⁷Division of Experimental Oncology, IRCCS Istituto Scientifico San Raffaele and Università Vita-Salute San Raffaele, Milan, Italy; ⁸Hematology and Clinical Immunology, Department of Medicine, Padua, Italy; ⁹Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy; ¹⁰IRCCS Ca' Granda - Università degli Studi, Milan, Italy; ¹¹Hematology section - University of Ferrara, Italy; ¹²Division of Hematology and Bone Marrow Transplant, IRCCS San Martino-IST, Genova, Italy; ¹³Hematology Unit, Careggi Hospital, Firenze, Italy; ¹⁴Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino and Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy; ¹⁵Hematology and Clinical Immunology, University of Perugia, Perugia, Italy; ¹⁶Hematology and Stem Cell Transplantation Unit, Ospedale Oncologico A. Businco, AO Brotzu, Cagliari, Italy; ¹⁷Hematology Unit, A. Pugliese Hospital, Azienda Ospedaliera Pugliese Ciaccio, Catanzaro, Italy; ¹⁸Hematology Section, Cosenza Hospital, Cosenza, Italy; ¹⁹Hematology and Stem cell Transplantation Unit, IRCCS CROB, Referral Cancer Center of Basilicata, Rionero in Vulture, Italy; ²⁰UOC Ematologia, PO Santa Maria di Loreto Nuovo, Naples, Italy; ²¹AOU San Giovanni di Dio e Ruggi d'Aragona, Ematologia - Salerno, Italy; ²²Hematology, Niguarda Cancer Center, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy; ²³Division of Experimental Oncology, IRCCS Istituto Scientifico San Raffaele and Università Vita-Salute San Raffaele, Milan, Italy; ²⁴Hematology section - University of Ferrara, Italy; ²⁵Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy; ²⁶Institute of Hematology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; Institute of Hematology, Università Cattolica del Sacro Cuore, Rome, Italy

B-cell receptor signaling inhibitors (BCRi), as Ibrutinib (Ibr) and Idelalisib (Idela), have changed the management of chronic lymphocytic leukemia (CLL), and the BCL2 inhibitor Venetoclax (Ven) has shown efficacy in relapsed/refractory patients (pts). The exact sequencing of new inhibitors outside clinical trials is not well established. In this multicenter retrospective real life study we evaluated the overall response rate (ORR), progression-free survival (PFS) and overall survival (OS) of CLL pts treated with Ven after one or two BCRi and the reasons for

BCRi discontinuation, adverse events (AE) vs progressive disease (PD). We report an analysis of 76 CLL pts from 18 Italian centers treated with Ibr and/or Idela prior to Ven. Twenty-four pts were treated with one BCRi, 52 with both. Median age was 63 years (26-87); 78% of pts had unmutated IGHV and 32% had TP53 disruption. The sequence of drugs' administration was: Ibr, Idela, Ven in 16 pts; Idela, Ibr, Ven in 8 pts; Ibr, Ven in 37 pts; Idela, Ven in 15 pts. No statistically significant difference was found in terms of ORR to Ven in pts who received one or two BCRi, 74% or 50% respectively ($p=0.114$). Pts who received a single BCRi prior to Ven showed a better PFS and OS at 12 months if compared to double BCRi treatment: 76% vs 35% ($p=0.011$) and 88% vs 57% ($p=0.015$), respectively. BCRi treatment was stopped in 70% of pts due to PD and in 30% due to AE. When we considered the impact of the first BCRi discontinuation on Ven response, the ORR was better in pts who stopped due to AE vs PD: 91% vs 49% ($p=0.03$). PFS and OS at 12 months were also better in pts who stopped treatment due to AE vs PD, with a PFS of 84% vs 45% ($p=0.003$) and an OS of 93% vs 62% ($p=0.028$). In conclusion, the number of BCRi received before the BCL2i does not affect ORR to Ven. A single prior BCRi is better than both in terms of PFS and OS following treatment with Ven, regardless of the type of BCRi used. Discontinuation of the first BCRi for AE is associated with a better PFS and OS on Ven compared to pts who experienced PD. The discontinuation of both BCRi for AE did not impact on PFS to Ven. On the other hand, pts who stopped both BCRi for PD showed a trend to a worse PFS on Ven, without reaching statistical significance. Finally, these preliminary data support the use of double BCRi before Ven for pts who discontinued due to AE; on the contrary, we suggest the use of Ven after single BCRi when the reason of discontinuation is PD.

P115

HYPOGAMMAGLOBULINEMIA AND MONOCLONAL GAMMOPATHY AS INDEPENDENT PROGNOSTIC FACTORS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A RETROSPECTIVE MULTICENTRIC EXPERIENCE

A. Corbingi¹, I. Innocenti², A. Tomasso¹, R. Pasquale³, A. Visentin⁴, M. Varettoni⁵, F. Autore², F. Morelli¹, L. Trentin⁴, G. Reda³, L. Laurenti^{1,2}

¹Hematology, Università Cattolica del Sacro Cuore, Roma; ²Hematology, Fondazione Policlinico Universitario "A. Gemelli", Roma; ³Hematology, Fondazione IRCCS Ca'Granda, Ospedale Maggiore Policlinico, Milano; ⁴Hematology, Università degli Studi di Padova; ⁵Hematology, Fondazione IRCCS, Policlinico San Matteo, Pavia, Italy

Background: Chronic Lymphocytic Leukemia (CLL) is a B-cell lymphoproliferative disorder. Several prognostic factors regarding CLL B-cell biology have been detected so far stratifying the prognosis and outcome in CLL patients (pts). However all data above reported are not available to all medical centers while data concerning the presence of hypogammaglobulinemia (HYPO), IgM or IgG monoclonal gammopathy (MG) are easily achievable.

Aim: The aim of the study is to evaluate the prevalence and the outcome of monoclonal IgM/CLL, IgG/CLL and HYPO compared with CLL pts with normal immunoglobulin (Ig) levels.

Methods: We collected from four different Italian centers 1505 pts diagnosed with CLL from 1987 to 2017 with a baseline assessment of serum Ig, immunofixation, chromosomal aberrations and clinical features evaluating their impact on progression free survival (PFS), time to treatment (TTT) and overall survival (OS).

Results: Once assessment was made, 1505 pts met eligibility criteria and were included in our study. Median age of 65.5 was similar in all the groups and data gathered from the four centers showed an overlapping rate about prevalence and time-dependent-parameters in all CLL subclasses. The overall prevalence of MG is 14.8% of whom 149pts (9.9%) with IgG/CLL, 73pts (4.9%) with IgM/CLL. HYPO was detected in 200 pts (13.2%) while 1083pts (72%) had no evidence of paraprotein. Median PFS ($p<.0001$) was reached in IgM/CLL group at 33 months compared to HYPO/CLL (54 months), IgG/CLL (62 months) and normal/CLL (96 months). A projection at 150-months showed an estimated OS of 62% in IgM/CLL group versus 68.3% in HYPO/CLL, 80.6% in IgG/CLL and 85.8% in normal/CLL ($p<.0001$). The worst OS in HYPO group was associated with a higher incidence of infections rather than progressive diseases, or CLL-related death causes. In our study, the worst outcome is

identified among IgM/CLL because of a more advanced stage at diagnosis according to Binet staging ($p<0.002$) and a major frequency of del17p ($p<.022$). No differences were noticed regarding IgHV mutation status ($p=0.237$) or other chromosomal abnormalities detected by FISH ($p=0.29$ for del13, $p=0.43$ for del11). Summary. Our study provided data about prevalence and prognosis of MG and HYPO in CLL patients. Serum Ig paraprotein was present in 14.8% of all CLL pts with a worst outcome in IgM/CLL. Thus, making of serum Ig, a simple data to obtain, useful to assess CLL prognosis.

P116

PROSPECTIVE EVALUATION OF CD160 AND CD200 IN B-LLC AND MBL: DIAGNOSTIC AND PROGNOSTIC INDICES

L. Albertazzi, P. Zaldini, R. Longo, O. Bonanno, F. Ilariucci, A. Ferrari, L. Coppolecchia

Laboratory of Chemical Chemistry; Hematology. Azienda Unità Sanitaria Locale IRCCS di Reggio Emilia, Italy

Introduction: CLL is characterized by clonal proliferation and accumulation of CD5 and CD23 positive mature B cells. Are considered atypical CLLs cases: CD5 or CD23-, FMC7 or CD11c or CD79b +, or with high-intensity fluorescence Ig. In cases with atypical data seem useful a cytofluorimetric evaluation of the CD160 and CD200. CD160 is expressed in CLL and in HCL; CD200 is up-regulated in CLL and HCL, it is absent in MCL. The combined use of CD160 and CD200 facilitates the differential diagnosis in B-LLC with atypical phenotype. Antigens could contribute to the diagnostic definition in 20-30% of the cases.

Aim: This paper presents a prospective observational study with the aim of: 1. to assess how much the CD160-CD200 antigens contribute to the diagnostic definition of cases (diagnostic confirmation) in particular in the differential diagnosis in chronic lymphoproliferative diseases and in atypical CLL cases. 2. evaluate the CD160-CD200 antigens during the disease both as a percentage of positive cells and using the MFI (Mean Fluorescence Intensity) as a surrogate measure of the cellular "concentration" of the antigens to (prognostic exploratory).

Material and Methods: The duration of the study is 5 years (3 for enrollment; 2 for follow-up) starting from February 2017. Until April 2019 we enrolled all patients from the Hematology Unit with suspected CLL/MBL/B Lymphoproliferative Disorders, with no immediate drug treatment requirements and never treated before. In total 51 patients of which 27 with at least one follow-up check. Positivity for CD160/CD200 was defined with a 20% cut-off of positive cells. The MFI of CD200 and CD160 is evaluated on the CD5+ /CD19+ /CD2- population. For the cytofluorimetric measurement we use a cytofluorimeter NAVIOS 10 Beckman-Coulter.

Results: From the confirmatory-diagnostic point: all patients, but 1, are CD5+; this patient is CD200+ and CD160-; all patients, but 1, are CD200+; 3 patients, whose diagnosis was not confirmed, show: CD160- and CD200+ From an exploratory prognostic point: in 9 patients the percentage of CD160 positive cells increased significantly (Wilcoxon test; $p<0.01$) in the one-year follow-up; CD160 and CD200 MFI evaluation: significant reduction (t-test; $p<0.01$) in the 1-year follow-up compared to the diagnosis.

Conclusions: It is necessary to evaluate in the follow-up of the enrolled patients in the new cases if data will be confirmed, also in association with other biochemical parameters of clinical significance (eg: B2 microglobulin).

P117**A RARE CASE OF CERVICAL LYMPHADENOPATHY**S. Pascale¹, A. Sau¹, D. Onofrillo¹, M. Fusillo², A. Spadano³¹Unità Operativa Semplice Oncoematologia Pediatrica, Ospedale Civile di Pescara; ²Unità Operativa Complessa di Chirurgia pediatrica, Ospedale Civile di Pescara; ³Unità Operativa Complessa di Ematologia Clinica, Ospedale Civile di Pescara, Italy

Kikuchi-Fujimoto disease (KFD) is a benign and self-limiting disease that mainly affects young women. Recognition of this condition is critical as it can mimic tuberculosis, lymphoma, or even adenocarcinoma. Awareness of this entity helps to prevent misdiagnosis and inappropriate treatment. We have reported a case of a 15-year old South American woman presented to our department with complaints of multiple right-sided neck swellings. She reported a 2-week history of fever following which she noticed a right cervical and supraclavicular mass that progressively increased in size. She also complained of fatigue and intermittent chills. She reported taking low-dose prednisone and antibiotics for 1 week at her house. On examination she was lethargic but otherwise looked well. Significant findings were palpable right cervical and supraclavicular lymphadenopathy. The lymph nodes were firm and non-tender. There was no hepatosplenomegaly or clinically appreciable lymphadenopathy elsewhere. Blood tests revealed neutropenia (ANC (1100/ L), raised LDH (936 U/l), mild increase in CRP (2 mg/dl). Viral markers showed a past CMV and Toxoplasma gondii infection while markers for EBV, Adenovirus and Parvovirus were negative. ECG and chest X-rays were normal. US showed the presence of hypochoic and slightly inhomogeneous lymph node packages, with rich vascularized hilum and no evidence of colliquative phenomena. Given the association of fever, cervical lymphadenopathies and mildly elevated inflammatory index, oral antibiotic therapy with amoxicillin clavulanic acid and analgesic therapy with paracetamol were started. Due to persistence of fever, lymphadenopathy, worsening of neutropenia (ANC 800/ L), raised LDH (2400 U/l) and the appearance of a erythematous rash on the face, an excisional lymph node biopsy and bone marrow aspiration were performed. The cytometric investigation showed non-malignant features. The histologic exam revealed histiocytic necrotizing lymphadenitis, which was diagnostic for KFD. She was treated with prednisone 50 mg daily for 1 week which was subsequently tapered. She responded well to therapy with fevers subsiding, lymph nodes reducing in size and facial rash completely resolved. This case report highlights the importance of including KFD disease as a differential for lymphadenopathy. KFD, although rare, should be part of the differential diagnosis in patients presenting with fever and cervical lymphadenopathy.

Cytogenetics, Molecular Genetics and Myelodysplastic Syndromes**P118****DIGITAL PCR IS A PROMISING METHOD FOR SENSITIVE DETECTION OF THE KIT D816V MUTATION IN PATIENTS WITH SUSPECTED SYSTEMIC MASTOCYTOSIS**

M. Martelli, L. Bavaro, G. De Matteis, M. Rondoni, R. Zanotti, M. Bonifacio, C. Papayannidis, A. Curti, C. Monaldi, S. De Santis, M. Mancini, M. Cavo, S. Soverini

Department of Experimental Diagnostic and Specialty Medicine - DIMES, Institute of Hematology "L. e A. Seràgnoli", University of Bologna, Azienda Ospedaliera di Verona, Azienda USL della Romagna, Italy

Introduction: Detection of the D816V KIT mutation in the bone marrow (BM) is one of the minor criteria for the diagnosis of systemic mastocytosis (SM). Since in patients (pts) with indolent SM the level of BM infiltration by tumor mast cells (MCs) is very low, the European Competence Network on Mastocytosis recommends routine use of assays like ASO-qPCR, conjugating high sensitivity with the possibility to quantitate the allele burden - which may provide prognostic information. A highly sensitive assay would also be useful for the screening of peripheral blood (PB) in cases of suspected SM with no skin lesions and/or normal tryptase levels. Digital PCR (dPCR) is a promising alternative to ASO-qPCR which allows absolute quantification of somatic mutations. We evaluated a commercial dPCR assay for the detection of KIT D816V in SM.

Methods: PB samples from 13 healthy donors (HDs) were used as negative controls, to calculate the limit of blank; the D816V-positive HMC 1.2 cell line was used as positive control. 35 BM samples from pts with suspected SM, either with neoplastic MC infiltration <0.01% by flow cytometry and low level positivity by ASO-qPCR or negative both by flow cytometry and ASO-qPCR were used for dPCR validation. 10 matched PB or BM samples from pts with suspected SM were prospectively analyzed. Droplet dPCR was performed on a BioRad QX200 instrument using the ddPCR™ Mutation Assay: KIT p.D816V, Human (Bio-Rad). The allele burden (VAF) of KIT D816V was calculated by dividing the number of mutated copies by the total number of KIT copies.

Results: Merged analysis of multiple wells of dPCR reaction for each sample ranged from 30'000 to 40'000 and increased assay sensitivity. In HMC 1.2 cells, dPCR measured a VAF of ±50%, consistent with the heterozygous mutation status. No KIT D816V-positive events were detected in any of the HD samples. Comparison between dPCR and ASO-qPCR revealed high concordance in mutation detection and quantitation, even at very low VAF levels. dPCR identified the D816V in 5/10 pts prospectively evaluated for diagnosis of SM. All pts positive for the D816V in BM also tested positive in the PB by dPCR.

Conclusions: dPCR and ASO-qPCR yielded highly concordant results, also in terms of VAF measurement. dPCR could easily detect the D816V mutation in PB. We thus propose dPCR as a valid alternative to ASO-qPCR. Multicenter standardization projects are the next crucial step towards the implementation of dPCR in clinical practice.

P119**NANOPORE TARGET SEQUENCING FOR RAPID GENE MUTATIONS DETECTION IN ACUTE MYELOID LEUKEMIA**C. Cumbo¹, C.F. Minervini¹, P. Orsini¹, L. Anelli¹, A. Zagaria¹, A. Minervini¹, N. Coccaro¹, L. Impera¹, G. Totà¹, E. Parciante¹, M.R. Conserva¹, M. Delia¹, O. Spinelli², A. Rambaldi², G. Specchia¹, F. Albano¹¹Department of Emergency and Organ Transplantation (D.E.T.O.) - Hematology Section - University of Bari; ²Hematology and Bone Marrow Transplant Unit, University of Milan, Italy

Acute myeloid leukemia (AML) clinical settings cannot do without molecular testing to confirm or rule out predictive biomarkers for prognostic stratification, in order to initiate or withhold targeted therapy. Next generation sequencing offers the advantage of simultaneous investigation of numerous genes, but these methods remain expensive and time consuming. In this context, we present a nanopore-based

assay for rapid (24 hours) sequencing of six genes (NPM1, FLT3, CEBPA, TP53, IDH1, and IDH2) that are recurrently mutated in AML. The study included 22 AML patients at diagnosis subdivided into two groups for the purposes of the barcoding process. For each case, three multiplex long-PCRs were performed on genomic DNA extracted from bone marrow mononuclear cells. Amplicons were purified, barcoded and prepared for MinION sequencing (MS). All data were compared with the results of S5 sequencing (S5S) and discordant variants were validated by Sanger sequencing (SS). Variant calling, filtering and annotation, produced a total of 34 variants, excluding FLT3 internal tandem duplications (ITDs). Among them, ten were discordant and needed validation. After SS analysis, three discordant variants resulted false negatives (one from MS and two from S5S analysis, respectively) and seven variants were false positives (two from MS and five from S5S analysis, respectively). Moreover, nanopore ability to generate long reads allowed a more accurate detection of longer FLT3 ITDs and phasing double CEBPA mutations. Furthermore, nanopore approach showed substantial advantages in terms of speed and low cost. In conclusion, we propose a cheap, rapid workflow that can potentially enable all basic molecular biology laboratories to perform detailed, targeted gene sequencing analysis in AML patients, in order to define the prognosis and the treatment.

P120

LA DIGITAL DROPLET PCR "DROP OFF" COME NUOVA TECNICA PER LA DIAGNOSI ED IL MONITORAGGIO DELLE MUTAZIONI DI IDH2 NEI PAZIENTI AFFETTI DA LEUCEMIA MIELOIDE ACUTA

S. Galimberti¹, S. Grassi¹, F. Guerrini¹, E. Ciabatti¹, S. Salehzadeh¹, E. Benedetti², F. Caracciolo², S. Balducci¹, P. Rossi¹, F. Martini¹, E. Mazzantini¹, F. Ricci¹, R. Tavarozzi¹, M.R. Metelli², M. Petrini¹

¹Dipartimento di Medicina Clinica e Sperimentale, Sezione Ematologia, Università di Pisa; ²UO Ematologia, AOUP, Italy

Background: After availability of the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) inhibitors, the screening of these mutations became fundamental. IDH2 mutations (R140 or R172) are found in 20% of patients with acute myeloid leukemia (AML). Prognostic assessment of IDH2 mutations is still controversial, but the introduction of enadenisib opened the possibility of using IDH2 as a marker for minimal residual disease. Sanger sequencing is the gold standard method for detecting IDH2 mutations, but it is not a real quantitative tool and has limited sensitivity. Various allele-specific PCR methods have been thus developed, based on the amplification refractory mutation system (ARMS PCR), but they are not very accurate.

Methods: The "drop-off" ddPCR FAM/HEX Assay (Biorad®) was set; it requires a single pair of probes to detect and quantify different mutations in a single reaction: the FAM-labeled probe binds a reference sequence distant from the target but within the same amplicon, while the HEX probe binds the wild-type sequence in the target site. Thus, wild-type samples present signals from both FAM and HEX probes, while the mutated ones display only the FAM signal.

Results: We enrolled in this study 60 AML consecutive patients; at diagnosis, we identified 11/60 (18.3%) IDH2-mutated cases; the median mutational burden was 13.7% (range 0.4%-44%); it did not correlate with blasts percentage or histotype. Sanger was not able to identify mutations in 5 of the 11 cases positive by ddPCR, probably because of their low mutational burden (0.4-12%) (our method reaches a sensitivity of 2×10^{-3}). The IDH2 mutational status did not significantly impact on survival, neither on the quality of the clinical response to treatment. Then, ddPCR was made in follow-up samples in 4 IDH2 mutated patients (2 CRMRD-, 1 CRMRD+ and 1 resistant). In the CRMRD- patients, the IDH2 allele burden was reduced and became negative, such as NPM1 and WT1 markers; in the resistant one, the IDH2 status correlated with WT1 and FLT3 persistence; in the CRMRD+ case, the IDH2 mutation reduced to 0%, while the MRD positivity was still detected by using NPM1 and WT1; this patient later relapsed.

Conclusions: our study clearly showed that ddPCR is useful for identifying AML cases carrying IDH2 mutations and following them during treatment. ddPCR is simple to be performed, with acceptable costs, rapid and allows to give an accurate quantitation of the mutational burden, with a satisfying sensitivity.

P121

DIAGNOSTIC WORK-UP OF NEUTROPENIA: A SINGLE CENTER "REAL LIFE" EXPERIENCE

F. Tarantini, I. Attolico, A. Ricco, M.C. Longo, M.L. Di Noi, E. Arcuti, S. Zucano, M.A. Urbano, A. Piccolomo, P. Nierychlewska, F. Albano, G. Specchia

Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, University of Bari, Italy

Background: Neutropenia in adult patients (pts) is a frequent cause of access to Haematology Units. Even if an absolute neutrophil count (ANC) <1500 cells/ml is the criterion, many pts with milder neutropenia are referred for investigation. This finding involves a broad spectrum of diseases, and it's a true diagnostic challenge.

Aim: We collected data on pts referred to our center for neutropenia to analyze the "real-life" diagnostic approach.

Results: From 2010 to 2018, 85 pts (median age: 47,7 yrs, range 18-65,79 F; 6 M) with newly diagnosed neutropenia referred to our clinic. The median ANC was $1351,3 \times 10^9/\text{mcl}$ (range 371-2050). RBC, HB and PLT counts were normal. Peripheral blood (PB) smear and CFM analysis for EPN clones were performed. No dysplastic features were found; no EPN clones were found. Bone marrow (BM) aspirate and cytogenetics were performed in 18 pts (21,8%) with ANC <500 cells/ml. Median BM blasts (BM smears) was 1% (range 0-3%). All pts had a normal karyotype. Dosages of IgG, IgA and IgM, LDH, urate, RDW, B12 and folate were normal in all pts; ESR was elevated in 59 (69,4%) pts. Screening for ANA was positive in 49 (57,6%) pts. Abdominal ETG was done in 83 (97,6%) pts; 2 (2,3%) pts had splenomegaly (LD: 16 and 17 cm). 53 pts (63,2%) had a diagnosis of autoimmune disease (mostly Hashimoto Thyroiditis and Connectivitis). The remaining 32 pts (36,8%) had a idiopathic neutropenia. None of the pts received neutropenia inducing drugs. Gaucher's disease was excluded in 2 pts presenting with also mild splenomegaly.

Discussion: This is an homogeneous series of pts with neutropenia. Most of them are young women, with an underlying immune disease and without other clinical or laboratory signs of haemopathy. We didn't investigate for CHIP but RDW, often altered in CHIP, was normal in all our pts. With a median follow-up of 5 years, we didn't observe evolutions to any primary hematological disease. Based on our experience, we suggest a baseline performance of PB smear and EPN CFM analysis, serum chemistry (including LDH, urate, dosage of IgG, IgA, IgM, ESR, B12 and folate) and abdominal ETG. BM aspirate/biopsy and cytogenetics do not always seem to be mandatory. Pts not presenting with the previously indicated features should be assessed with a case-oriented approach.

P122

ABSTRACT WITHDRAWN

P123

DRIED BLOOD SPOTS (DBS) FOR -GLUCOSIDASI ENZYME ACTIVITY IN PATIENTS WITH HAEMATOLOGICAL DISEASES

N. Sgherza, A. Quarta, A. Pasanisi, M.P. Solfrizzi, M.R. Coppi, A. Spina, F. Merchionne, M.S. De Candia, A. Romano, D. Pastore

U.O.C. di Ematologia – Centro Trapianti di Midollo Osseo – Centro della Microcitemia, P.O. "A. Perrino"- Brindisi, Dipartimento di Scienze Mediche Chirurgiche e Sperimentali - Università di Sassari, Italy

Gaucher Disease (GD) is a rare autosomal recessive disease caused by a deficiency in the lysosomal enzyme -glucocerebrosidasi (GBA) due to mutations in the GBA1 gene. As for many other lysosomal enzymes, also for GBA, screening methods using Dried Blood Spots (DBS) have been developed. Here we report our single-centre experience of 3 cases of concomitant haematological disease and GBA1 mutations. Case1. In August 2004, a 27-year-old woman, was admitted to our institution for lymphadenopathies and mediastinal mass; after a lymph node biopsy a diagnosis of Hodgkin Lymphoma (HL) was made. At bone marrow examination presence of Gaucher's cells was revealed; consequently activity of GBA and measurement of plasma chitotriosidase, on peripheral blood, were performed, resulting decreased and increased respectively. At last, PCR analysis revealed a pathological mutation of GBA1 (N370S/N370S) and a definitive diagnosis of GD type 1 was made.

Case2. In February 2016, DBS for GBA was performed in a 24-year-old man affected by major thalassemia and with not justifiable splenomegaly and grade of anemia, despite the good compliance to on-going treatment. DBS showed: GBA activity < 1 mol/l/h (n.v. > 4,6 mol/l/h), Lyso-Gb1: 5,6 ng/ml (n.v. < 4,8 ng/ml). Even if a not pathogenic mutation of GBA1 was found, a heterozygous variant in exon 8 of the gene [c.1223C>T p.(Thr408Met)] was detected. GBA activity was measured also in peripheral blood leucocytes and was normal. Case3. In October 2018, a 56-year-old woman was admitted to our institution for pancytopenia and splenomegaly. Among first level exams, for increased LDH, hyperferritinemia and splenomegaly also mutational analysis for JAK2V617F and DBS for GBA were performed; then a total body computed tomography showed different lymphadenopathies and after lymph node biopsy a diagnosis of HL was made. JAK2V617F was wild type. DBS showed: GBA activity: 1,4 mol/l/h (n.v. > 4,1 mol/l/h), Lyso-Gb1: 1,5 ng/ml (n.v. < 10 ng/ml) and a heterozygous variant in exon 9 of the gene [c.1226A>G p.(Asn409Ser)]. GBA activity was measured also in peripheral blood leucocytes and was normal. In conclusion, since that coexistence of GBA1 mutations and other haematological diseases is possible, our single centre experience suggests that probably, a simple diagnostic method, such as DBS, could be added to other laboratory tests, to identify GD among adult subjects primarily suspected or affected by other haematological diseases.

P124

EFFICACY OF HIGH-DOSES OF ALPHA-ERYTHROPOIETIN IN PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES: A RETROSPECTIVE SINGLE CENTER ANALYSIS

S. Rosati, M. Ansuinelli, I. Carosino, E. Scalzulli, S. Mohamed, M. Porrazzo, A. Bruzzese, M. Molica, L. Rizzo, E. Mariggio, D. De Benedittis, M. Mancini, M. Breccia, R. Foà, R. Latagliata

Dipartimento di Medicina Traslazionale e di Precisione sezione di Ematologia Università "Sapienza", Italy

Myelodysplastic syndromes (MDS) are an heterogeneous group of myeloid neoplasms, characterized by ineffective hematopoiesis, anemia in >70% of patients and a propensity to leukemic transformation. Standard doses of erythropoiesis-stimulating agents (ESAs) are recommended by the current guidelines for the treatment of anemia in lower-risk MDS; however, the best initial dose is still uncertain. We conducted a retrospective analysis on 193 MDS patients (M/F 94/99) with a median age at diagnosis of 74.9 years (interquartile range IQR 68.4-81) treated at our Center between March 2001 and February 2019 with an initial high dose of erythropoietin (EPO)-alpha (80,000-UI/week), to evaluate the efficacy of such approach in the real-life clinical practice. Clinical main features at the start of EPO are shown in Table 1. Median time from diagnosis to EPO start was 7.1 months (IQR 2.3-18.9). According to the International Working Group (IWG) 2006 criteria, erythroid response was achieved in 103 patients (53.3%), after a median time from EPO start of 1.8 months (IQR 1.1-2.6): among these responding patients, 49 (47.6%) relapsed after a median of 12.8 months (IQR 4.9-30.5): the median response duration was 27.2 months (95%CI 13.6-40.9). Different features at baseline - Hb >8 g/dl (p=0.001), no previous transfusion requirement (p<0.001), endogenous EPO levels <50 mUI/ml (p=0.001), age >65 years (p=0.029), IPSS low (p=0.022), r-IPSS very low (p=0.016) and ferritin levels <250 ng/ml (p=0.049) - had a positive predictive role for erythroid response at univariate analysis. At multivariate analysis, only endogenous EPO levels <50 mUI/ml (HR 3.7-95%CI 1.6-8.6, p=0.002) and absence of previous transfusion requirement (HR 5.5-95%CI 2.2-13.1, p<0.001) were independent predictors of response. Evolution into acute myeloid leukemia occurred in 23 patients (11.9%) after a median time from EPO start of 13.3 months (IQR 5.1-46.3). At the last follow-up, 71 patients (36.7%) had died, 51 (26.4%) were lost to follow-up, 19 (9.8%) were alive after EPO discontinuation and 41(21.2%) were alive in response and still on EPO treatment. The 5-year overall survival (OS) of the entire cohort was 55.2% (95%CI 45.8-64.6); the 5-year event-free survival (EFS) was 20.9% (95%CI 14.1-27.7), considering as events: resistance to EPO, relapse and death for any reason. In conclusion, high doses of EPO seem effective in more than 50% of anemic lower risk MDS patients, with a median response duration >2 years.

Table 1. Clinical characteristics at the start of EPO treatment.

Males/Females, n° (%)	94/99 (48.7/51.3)
Median age, years (IR)	74.9 (68.4 – 81)
WHO classification, n° (%)	
MDS-Unilineage Dysplasia	30 (15.5)
MDS-UD-with Syderoblasts	5 (2.6)
MDS-Multilineage Dysplasia	71 (36.8)
MDS-MD-with Syderoblasts	19 (9.8)
MDS-Excess Blasts 1	25 (12.9)
MDS-Excess Blasts 2	15 (7.8)
5q Syndrome	24 (12.4)
Not evaluable	4 (2)
IPSS, n° (%)	
Low	42 (21.8)
Int-1	91 (47.1)
Int-2	15 (7.8)
High	2 (1)
r-IPSS, n° (%)	
Very low	23 (12)
Low	79 (41)
Int	26 (13.5)
High	18 (9.3)
Very high	4 (2)
Median interval from diagnosis, months (IR)	7.1 (2.3 – 18.9)
Median Hb, g/dl (IR)	8.8 (8.1 – 9.5)
Median ferritin level, ng/ml (IR)	260 (114 – 455)
Median EPO level, mUI/ml (IR)	77 (39 – 168)
Transfusion requirement, n° (%)	
Yes	74 (38.3)
No	119 (61.7)

P125

HIGHER-RISK MDS PATIENTS NOT ELIGIBLE FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT): REAL-LIFE EXPERIENCE IN A LONG-TERM SURVIVAL COHORT

A. Ricco, P. Carluccio, I. Attolico, M. Delia, A.V. Russo Rossi, E. Arcuti, M.L. Di Noi, G. Specchia, F. Albano

A.O.U. Policlinico Consorziale di Bari, Italy

Introduction: Evidence from clinical trials has demonstrated clear improvements in delaying progression to AML and in survival for patients (pts) with higher-risk MDS who are not eligible for HSCT treated with azacytidine (AZA). However, many questions remain with regard to treatment duration and the identification of pts who may benefit from treatment. Much of the evidence is focused on treatment responsiveness rather than survival outcomes

Aim: We evaluated the outcome of long-term survivor high-risk MDS pts who were treated at our institution with AZA between 2010 and 2019, to determine the characteristics of this subset. AZA was administered subcutaneously in an outpatient basis at a dose 75 mg/m² s.c. with a schedule of 5 + 2 + 2 or of 7 consecutive days every 28, until disease progression or unacceptable toxicity

Results: Out of a cohort of 102 higher risk MDS pts treated with AZA in our institution, we identified 35 long survivors, with a median follow up of 36 months (range 12-103) after the start of treatment. Median number of AZA cycles received was 34 (range 12-103). Median age at diagnosis was 72 years (range 51-85). Diagnosis according to the WHO 2016 Classification was MDS-MLD in 2 cases, MDS-EB-1 in 14, MDS-EB-2 in 20. IPSS Cytogenetic risk was good in 29 cases (80.6%), intermediate in 3 (8.3%) and not evaluable in 4 cases; no poor cytogenetic risk was found in this subgroup. According to IWG 2006 criteria, best response achieved was: Complete in 5 pts (13.9%), Partial in 10 (27.8%) and Stable Disease in 21 (58.3%). After a median of 43 cycles of therapy (range 22-103), 15pts (41.7%) are still alive and continuing to receive AZA, 13 died due to disease progression to AML, 21 due to MDS related causes, among which only one patient died of infection disease

(pneumonia). Peripheral cytopenias were the most common advent events

Discussion: There are conflicting data concerning the efficacy and the goals of prolonged administration of AZA. Our experience shows that patients achieving at least SD should continue AZA treatment until disease progression, since SD is actually associated with a significantly reduced risk of death and improvement in Quality of Life. Our cohort shows a follow up clearly longer than that expected on the basis of IPSS prognosis. Moreover cytogenetic risk confirms to have a significant prognostic weight among this group of higher risk, not HSCT-eligible MDS patients.

P126

APPLICATION OF AN NGS-BASED 14 GENE PANEL AND A CE-IVD ANALYSIS SOFTWARE IN MYELOPROLIFERATIVE NEOPLASMS

F. Orsini¹, F. Pallotti², C. Lapucci¹, F. Crò¹, C. Businaro¹, L. Germagnoli¹

¹Synlab Italia srl; ²Università degli Studi dell'Insubria, Italy

Molecular routine diagnostics for BCR ABL1 negative myeloproliferative neoplasms (MPN) in many realities is currently focused on classical mutations in JAK2, CALR and MPL. It was recently demonstrated the need to extend the genes mutational analysis, looking for the presence of another clonal marker, as recommended by WHO 2017. We set up and validated a Next Generation Sequencing-based 14 gene panel that could help the clinicians to better characterize MPNs. In this panel we included genes involved in classical MPN - CALR, JAK2 and MPL for canonical and non-canonical mutations, DNA methylation - DNMT3A, IDH1, IDH2, TET2, chromatin modification - ASXL1, EZH2, cell signaling - CBL, SH2B3, RNA splicing - SF3B1, SRSF2 and TP53 as suggested in recent (2017) recommendations. We adopted a custom kit, based on hybrid capture technology on a NextSeq platform. For NGS data analysis, after evaluation between a totally in house bioinformatic solution and at least two commercial software, we choose a CE-IVD software for alignment, variant calling, variants prioritization and interpretation; this choice allowed us to apply custom panels to a CE-IVD totally customized workflow and gave us the possibility to manage FastQ, BAM and VCF files without the support of an external provider for data analysis. In the first phase of workflow validation, we processed 50 DNA samples. Data analysis showed no regions of low coverage in the target genes. A second phase of validation was performed on 18 clinical samples from patients with suspect of PV, ET, MF. The target region was analyzed with a coverage over 500X (more than 80% had coverage of 1000X) allowing us to detect somatic mutations even at low allelic frequency. The NGS analysis on these 18 samples showed the following

Results:

- 3 samples were positive for JAK2 V617F
- 2 samples were positive for JAK2 V617F and DNMT3A (1 for p.Phe752Leu and 1 for p.Arg882His)
- 1 sample was positive for JAK2 V617F and SH2B3 p.Glu208Gln
- 1 sample was positive for DNMT3A c.89A>G p.Glu30Ala
- 1 sample was mutated in CALR p.Lys385Asnfs*47
- 1 sample was mutated in MPL W515L

The mutational status was confirmed using Real Time PCR, Sanger Sequencing and Fragment Analysis. In the next future this NGS workflow will be applied in a large number of samples to prove the benefit of multigenic approach in clinical practice, for a deeper molecular characterization of MPN's triple negative and a better understanding of the evolution of the disease.

P127

PERLS' STAIN GRADE IN THE BONE MARROW ASPIRATE CORRELATES WITH OVERALL SURVIVAL IN LOW RISK MYELODYSPLASTIC PATIENTS

F. Pilo, G. Caocci, G. Mele, V. Serreli, E. Brundu, G. La Nasa

Hematology and Bone Marrow Transplant Unit, A. Businco Hospital, Cagliari; Department of Medical Sciences, University of Cagliari, Cagliari, Italy

Myelodysplastic syndromes (MDS) are heterogeneous group of acquired clonal hematopoietic stem cell disorders characterized by atypical stem cells maturation and genetic instability leading to an enhanced risk of progression to acute myeloid leukemia. These patient present at

diagnosis different grade of cytopenia, among whom, anaemia is the most common, therefore are incline to long term accumulation of iron in the organs due mostly to red blood cell transfusion (RBC) but iron overload may also occurs in patients who do not receive RBC transfusions due to the ineffective erythropoiesis. It is well known the effect of oxidant-mediated tissue's injury through the formation of free reactive iron species (ROS) in the liver and heart site but also in the bone marrow nice. We aimed to investigate, for the first time, the predictive value of iron bone marrow accumulation, as demonstrated by Perls' staining, on long-term outcome in such patients. We retrospectively analyzed 114 consecutive low risk-intermediate-I MDS patients who had diagnosed in our institution in the last 20 years. Patients had undergone bone marrow aspiration as part of the diagnostic work up for their MDS. Two or three different experienced hematologist analyzed samples. Perls' Prussian blue stain was used to stain bone marrow, iron was assessed by modified Gale's grading (Table 1) and then correlated with outcome. Twenty-seven patients had grade 1 (+), 31 grade 2 (++) and 56 grade 3 (+++). None of these patient had received iron chelation before marrow examination. Probability of overall survival (OS) was estimated by the Kaplan-Meier method and the significance was assessed by the log-rank test. Results showed that 20-year OS was significantly lower in patients with higher Perls' score (median = 80 ±7 months in grade 3; median = 70 ±17 months in grade 2; median = 144 ±18 months in grade 1, P=0.011) Figure 1. Multivariate analysis showed that higher bone marrow's iron overload (p=0.003; HR=) and transfusion dependency (0.001; HR=2.6) negatively impacts on survival. Although Perls' grading is a qualitative method, it is still the gold standard to detect iron storage in the bone marrow. We conclude that higher grade of iron storage at diagnosis can impact on outcome in low risk MDS patients. Perls' stain, together with Ferritin and blood transfusional burden could be another marker of iron-related tissue toxicity at diagnosis that predict overall survival.

Table 1. Iron stain score system.

GRADE	GALE'S GRADE	Bone marrow iron store
1 (+)	0-1-2	None, small or slight iron particles visible under high and low power magnification
2 (++)	3-4	Numerous small iron particles present in reticulum cells throughout the marrow fragment and/or tendency to aggregate into clumps
3 (+++)	5-6	Heavy and Very Heavy clumps of iron throughout the fragment both intra- and extra-cellular, obscuring cellular detail in the fragments.

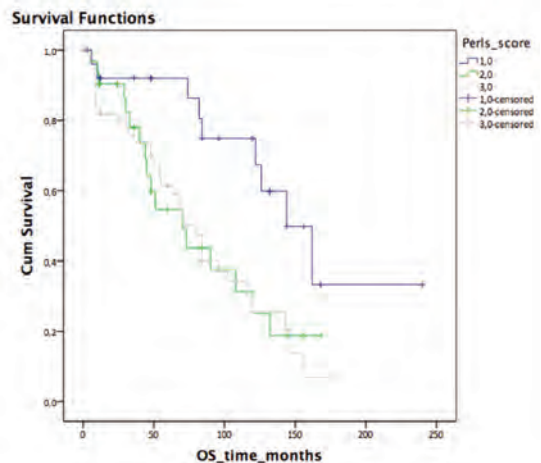


Figure 1. 20 year OS in MDS patients according to Perls' score.

P128

EFFICACY OF DPS30 AND IL2 FOR THE DEFINITION OF COMPLEX KARYOTYPE IN CLL PATIENTS: MONOCENTRIC EXPERIENCE

I. Caliendo¹, C. Fabbriatore¹, M. Ergoli¹, L. Strino¹, T. Palma¹, C. Califano¹

¹U.O.C. Ematologia P.O. A. Tortora Pagani (SA), Italy

Introduction: Chronic Lymphocytic Leukaemia (CLL) is the most prevalent leukaemia in the western Hemisphere. Cytogenetic examination and the detection of chromosomal aberrations in CLL is an integral part of prognostic stratification and treatment decisions. Improvements in cytogenetic methods, notably the introduction of the cell stimulation method, have led to the detection of chromosomal aberrations in CLL cases. A comprehensive analysis of cytogenetic aberrations recently showed that complex karyotypes (CKs) defined as the detection of either three or more or five or more chromosomal changes in a karyotype have a poor prognostic impact. Here we present data on two mitogens: CpG-oligonucleotide DPS30/Interleukin-2 (IL-2) and 12-O-tetradecanoylphorbol-13-acetate (TPA).

Methods: We analysed 40 cases of CLL with cytogenetics from January 2018 to April 2019. Three cultures were set-up: unstimulated, DPS30/IL-2 and TPA-stimulated, according to laboratory's standard protocols. Chromosome analyses were performed on G-banded metaphase cells of bone marrow aspirate and peripheral blood specimen. The culture period ranged from 3 to 5 days. A minimum of 20 metaphase cells were examined and the karyotypes were classified according to the International System For Human Cytogenetic Nomenclature (ISCN) 2016

Results: CK was detected more often in cases analysed with DPS30/IL-2 in 15/40 (40%) CLL patients, compared to the TPA protocol 8/40 (20%). This difference may be attributed to higher effectiveness of DPS30/IL-2 stimulation protocol.

Conclusions: DPS30/IL-2 offers a high abnormality detection rate for CLL in routine diagnostic cytogenetics with a single stimulated culture and may improve rate of finding new novel and potentially clinically significant abnormalities. So the use of DPS30/IL-2 is recommended in many B-Cell malignancies as an effective mitogen in culture of the malignant clone.

P129

WT1 AS PROGNOSTIC MARKER IN MIELODYSPLASTIC SYNDROMES

S.P. Pascale, R. Nuccorini, S. Coluzzi, A. Tramutoli, G. Gioia, A. Amendola, M. Cimminiello, N. Filardi, A. Maturro, D. Vertone, M. Pizzuti

UOC Ematologia AOR SAN CARLO, Potenza, Italy

Background: Myelodysplastic syndromes (MDS) are a group of hematological neoplasms associated with ineffective hematopoiesis and that transform to acute myeloid leukemia (AML). The International Prognostic Scoring System (IPSS) has been successfully used for accurate diagnoses and treatment, with a recent revision of the IPSS (IPSS-R) further improving prognostic risk stratification and optimal treatment selection. WT1 gene is overexpressed in different types of solid tumors and AML and it is a useful marker to monitor minimal residual disease.

Aim of Study: We evaluated the usefulness of WT1 levels at diagnosis for predicting the prognosis of MDS.

Patients and Methods: We analyzed 55 patients (pts) with MDS, 19 M e 36 F, with a median age of 67 (27-86) and with a median follow up of 12 months (1-103). We evaluated WT1 expression by Real Time PCR. WT1 expression was normalized by evaluating the expression of the gene Abl, using the calculation: WT1/ABLx104. A cut-off of 80 was utilized to discriminate pts with higher expression of gene than lower one.

Results: Pts were stratified in terms of the IPSS-R risk category, 7 were classified as Very Low, 17 as Low, 11 as Intermediate, 14 as High and 6 as Very High. The median value of WT1 was 32,85 (0.4- 4296.6), 20 pts showed a higher expression of WT1 (median: 545, range 87,4 – 4296,6). The level of WT1 was not related to the percentage of blasts CD34+ in BM, to the Karyotype groups and to the parameters of peripheral blood. Interestingly, considering WT1 as a single molecular marker in MDS at diagnosis our results showed a reduction in both overall survival (OS) and leukemia-free survival (LFS) with increasing WT1 levels as showed in Figure 1. Further, WT1 level was significantly related to the prognos-

tic risk categories of MDS by IPSS-R. Among IPSS categories, Very Low + Low + Int (lower risk group) had significantly lower WT1 level than High + Very High (higher risk group) (p=0.006). We assessed incidence of progression in AML, significant differences were observed between pts with low- and over-expression of WT1 (p:0.035) and when we added WT1 to IPSS-R risk categories we were able to better stratify MDS pts both in terms of OS and LFS.

Conclusions: Although the limited number of cases, we assumed that WT1 expression was related to disease characteristics and could be at diagnosis a useful prognostic marker to add to the prognostic scoring systems, in use today, in order to better characterize MDS pts and to target them to optimal treatment.

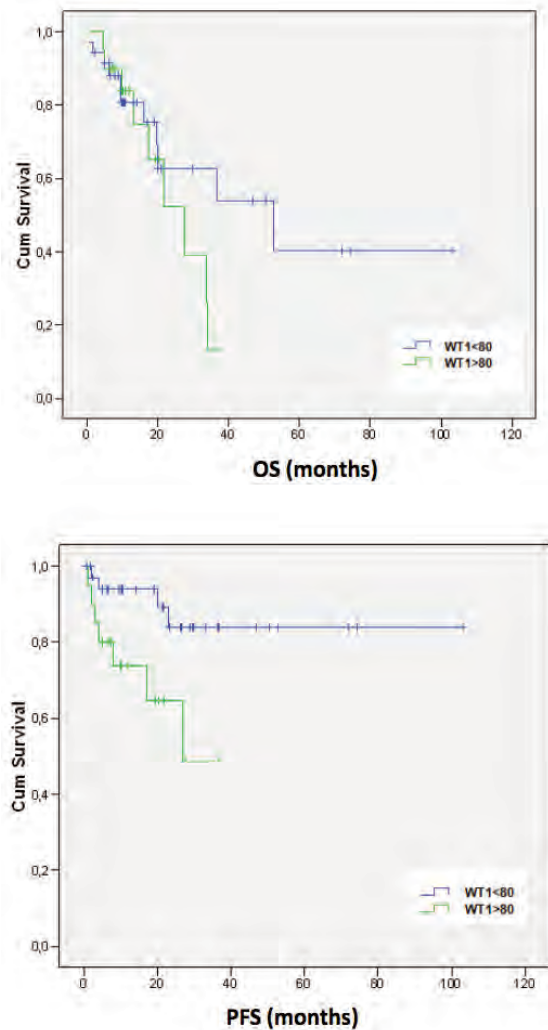


Figure 1. 20 year OS in MDS patients according to Perls' score.

Non Hodgkin Lymphoma 2

P130

PRE-TREATMENT WITH BENDAMUSTINE DOES NOT AFFECT STEM CELL MOBILIZATION IN PATIENTS WITH INDOLENT LYMPHOMAS: A MULTICENTRIC STUDY IN 45 PATIENTS

S. Luminari, L. Farina, F. Cocito, I. Defrancesco, G. Gini, A. Arcari, G. Scapinello, M. Gentile, M. Goldaniga, G. Loseto, E. Cencini, A. Greco, A. Ferrario, F. Rossi, S. Bolis, F. Passamonti, L. Arcaini, M. Merli

Ematologia Ospedale di Circolo e Fondazione Macchi ASST Settelaghi, Ematologia Arcispedale S. Maria Nuova IRCCS, Ematologia Fondazione IRCCS Istituto Nazionale dei Tumori, Dipartimento di Ematologia Ospedale Universitario S. Gerardo, Divisione di Ematologia Fondazione IRCCS Policlinico San Matteo, Dipartimento di Ematologia Ospedali Riuniti, Ematologia Azienda AUSL, Ematologia Dipartimento di Medicina Università di Padova, Oncoematologia Azienda Ospedaliera di Cosenza, Ematologia Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Ematologia IRCCS Ospedale Oncologico Giovanni Paolo II, Ematologia Azienda ospedaliera Senese Università di Siena, Ematologia Ospedale G. Panico, Ematologia Ospedale di Circolo e Fondazione Macchi ASST Settelaghi, Ematologia Fondazione IRCCS Ca'Granda Ospedale maggiore Policlinico, Dipartimento di Ematologia Ospedale Universitario S. Gerardo, Ematologia Ospedale di Circolo e Fondazione Macchi ASST Settelaghi, Dipartimento di Medicina Molecolare Università di Pavia, Ematologia Ospedale di Circolo e Fondazione Macchi ASST Settelaghi

Bendamustine plus rituximab (BR) is now the most popular standard treatment of patients (pts) with indolent non Hodgkin lymphomas (iNHL). Autologous stem-cell transplant (ASCT) is the standard option in young fit pts with R/R iNHL and improved survival of follicular lymphoma (FL) pts with early treatment failure (ETF). The effectiveness of CD34+ cells collection in pts pre-treated with B, which has a structure similare to fludarabine, a well-known stem cell poison, is essentially still unknown and based on few small series. We retrospectively analyzed rates of CD34+ cells collection in 45 consecutive pts with iNHL undergoing PBSC mobilization attempt, who had previously received treatment with B in 12 centers of the Fondazione Italiana Linfomi. 43 pts experienced disease relapse and initiated 2nd line treatment, while 2 where mobilized during 1st line therapy. The majority of pts had FL (n=39), 13 of whom (33%) relapsed within 24 months (ETF). Median lines of therapy at PBSC mobilization were 2 (1- 4). Eleven pts (24%) received B in 1st line, 29 as part of 2nd line, and 5 in further lines of therapy. The most frequent mobilization strategy was CT plus G-CSF. At 1st mobilization attempt, 38 out of 45 pts (84%) collected $\geq 2 \times 10^6$ CD34+/Kg. 5 of 7 pts who failed first mobilization underwent a second attempt collecting all $\geq 2 \times 10^6$ CD34+/Kg. Considering all mobilization attempts, 43/45 pts (96%) reached successful harvest of PBSC and 38/45 (84%) obtained an optimal PBSC collection ($\geq 5 \times 10^6$ /Kg). Median number of PBSC collected was 6.46×10^6 /kg (3-18). Pre-mobilization bone marrow involvement and response at mobilization significantly affected PBSC mobilization ($p < 0.05$). Overall, 30 out of 43 pts who efficiently mobilized PBSC (70%) finally underwent ASCT, with complete and fast engraftment. At a median follow-up of 6.3 yrs, only 3 pts died (2 for progression and 1 for viral encephalitis), with 8-yr OS of 89.6% and a median PFS from ASCT of 8.1 yrs. Notably, FL pts experiencing ETF did not exhibit worse OS (85.7 vs 87.1% at 8-yr, $p = 0.7$). High rate of success in PBSC collection reported in this large real-world study supports the evidence that pre-treatment with B does not affect PBSC mobilization in iNHL pts, although we confirmed that B does not display per se a mobilizing capacity. Salvage strategy including ASCT resulted in overall favorable outcome in young pts with R/R iNHL and seemed able to overcome the negative prognostic impact of ETF in FL.

Table 1. Patients' characteristics, treatments and pre-mobilization parameters.

Parameter	N (%)	p (on PBSC mobilization)
Age, median (range)	52 (35-66)	0.16
Gender, M/F (%)	26/19 (58/42)	0.72
Histology		0.85
FL	39 (87)	
grade 1-2	25	
grade 3A	7	
grade unknown	7	
MZL	3 (6.5)	
NMZL	1	
MALT MZL	2	
LPL/WM	3 (6.5)	
Stage III-IV, N (%)	38 (84)	0.9
BM involvement at baseline, N (%)	20 (44)	0.73
First line of therapy		0.11
R-CHOP	27 (60)	
BR	11 (24)	
Other	7 (16)	
Second line of therapy		0.37
BR	30 (67)	
R-CHOP	7 (16)	
Other	8 (17)	
Lines of therapy before mobilization, median (range)	2 (1-4)	0.9
Response at mobilization		0.02
CR	26 (58)	
PR	15 (33)	
SD/PD	4 (9)	
BM involvement before mobilization, N (%)	4 (9)	0.03
First mobilization regimen		0.039
CT + G-CSF	40 (89)	
(+ Plerixafor)	4	
G-CSF only	2 (4)	
(+ Plerixafor)	1	
bendamustine + G-CSF	3 (7)	
(+ Plerixafor)	1	
Cumulative dose of bendamustine before mobilization		0.9
>720 mg/m ² BSA	21 (47)	
≤ 720 mg/m ² BSA	24 (53)	
Interval between last bendamustine dose and mobilization		0.34
>3 months	37 (82)	
≤3 months	8 (18)	
Prior use of:		0.90
radiotherapy	9 (20)	
radioimmunotherapy	1 (2)	/
fludarabine	1 (2)	/

Table 2. Mobilization outcome, characteristics of ASCT and engraftment.

Parameter	N (%)
Peak of peripheral blood CD34+ at mobilization, median (range)	53.5/ μ l (4-143)
Collected CD34+ cells $\times 10^6$ /Kg at first collection, median (range)	6.43 (1.4-10.2)
$\geq 2 \times 10^6$ CD34+/Kg at first collection (%)	38 (84)
$\geq 5 \times 10^6$ CD34+/Kg at first collection (%)	27 (60)
Median days of leucoapheresis (range)	1 (1-4)
Second harvest procedure (%)	5 (11)
Successful second harvest procedure ($\geq 2 \times 10^6$ CD34+/Kg) (%)	5 (100)
Overall collected CD34+ cells, median (range)	6.46 (3-18)
Overall collection $\geq 2 \times 10^6$ CD34+/Kg (%) [primary endpoint]	43 (96)
Overall collection $\geq 5 \times 10^6$ CD34+/Kg (%)	38 (84)
Patient proceeding to ASCT (%)	30 (70)
Reasons for which pts did not proceed to ASCT	
ASCT planned but still not performed at data cut-off	3
Alternative consolidation (radioimmunotherapy)	5
Toxicity	2
Progression	1
Patients' refusal	2
Reinfused CD34+ cells $\times 10^6$ /Kg, median (range)	4.97 (3-12)
Days to ANC recovery $>600/\mu$ l, median (range)	11 (9-19)
Days to PLT recovery $>20000/\mu$ l, median (range)	13 (8-35)

P131**IMMUNOHISTOCHEMISTRY EXPRESSION OF SOX11, KI67 AND P53 AS A PROGNOSTIC FACTOR IN MANTLE CELL LYMPHOMA: A MONOCENTRIC RETROSPECTIVE STUDY**

B. Puccini¹, L. Mannelli², F. Vergoni³, R. Santi³, I. Naldi⁴, F. Pancani⁵, L. Signori⁵, S. Kovalchuk¹, V. Carrai¹, B. Scappini¹, L. Rigacci⁶, A. Bosi¹

¹SOD Ematologia AOU Careggi; ²CRIMM centro di ricerca e innovazione per le malattie mieloproliferative AOU Careggi; ³Dipartimento di Medicina Sperimentale e Clinica Università degli studi di Firenze; ⁴SOD Istologia Patologica e Diagnostica Molecolare AOU Careggi Firenze; ⁵SOD Oncologia Medica AOU Careggi; ⁶Dipartimento di Medicina Sperimentale e Clinica unità funzionale di Ematologia Università degli studi di Firenze; ⁶SOD Ematologia Azienda San Camillo Forlanini, Italy

Background: Mantle cell lymphoma (MCL) is a rare type of non-Hodgkin lymphoma and associated with a dismal outcome with a median overall survival (OS) of only 3 to 5 years. To date, MIPI and c-MIPI score stratify patients in 3 risk class. To evaluate the prognostic impact of the immunohistochemistry expression of SOX11, ki67 and p53 on overall survival and progression free survival (PFS)

Methods: We retrospectively collected 172 consecutive patients (pts) affected by MCL diagnosed in AOU Careggi, Florence from 1999 to 2017. 141 pts were analyzed for this study, 31 pts were excluded for unavailability of clinical data. Based on literature and pathological guidelines, pts were classified as: ki67 low and high for ki67 expression <30% or ≥30% respectively; SOX11 positive for diffuse staining or strong staining in <30% of neoplastic cells; p53 positive for strong staining in ≥30% of neoplastic cells. 105 samples were evaluable for ki67 staining (28<39%;77≥30%); 91 samples were evaluated for SOX11 staining (14 negative; 77 positive); 83 samples were evaluated for p53 expression (71 negative; 12 positive).

Results: In our cohort, the median overall survival and progression free survival was 86 months and 42 months. Based on MIPI score we stratified pts as low risk (25%), intermediate (29%) and high risk (46%), with a median overall survival not reached, 156 e 45 at 120 months respectively. Ki67 had an impact on OS (median OS not reached at 120 vs 48 months; p<0.001) and PFS (median PFS 49 vs 33 months; p=0.004). SOX11 positivity affected OS (median OS not reached at 120 months vs 75 months; p=0.026) but not on PFS. P53 staining affected the OS (median OS 86 vs 33 months) but not PFS. On multivariate analysis high risk MIPI, high ki67 and p53 staining maintained a significant impact on OS and PFS, except for p53 staining. Based on ki67 and p53 evaluation, we distinguished 2 groups of pts, ki67<30%-p53- and ki67≥30%-p53+ pts with different OS (OS median 97 vs 27 months; p<0.001) and PFS (median PFS 42 vs 9 months; p<0.001). Furthermore, the p53 positivity on low and intermediate MIPI risk class identified pts with significant inferior OS (p=0.04 and p=0.002 respectively).

Conclusions: Based on our study and literature data we recommend to perform ki67, SOX11 and p53 immunohistochemistry staining at diagnosis in order to identify high risk pts that could be classified as low or intermediate risk by MIPI score and in view of the new therapies pts could be undertreated.

P132**THE ROLE OF FLOW CYTOMETRY IN THE PREDICTION OF CENTRAL NERVOUS SYSTEM INVOLVEMENT IN DIFFUSE LARGE B CELL LYMPHOMA**

E. Maiolo, S. Bellesi, P.L. Puggioni, E. Alma, F. D'Alò, A. Cuccaro, D. Resta, R. Malafrente, V. De Stefano, S. Hohaus

Fondazione Policlinico A. Gemelli IRCCS Università Cattolica del Sacro Cuore, Italy

Diffuse Large B Cell Lymphoma (DLBCL) patients (pts) have a 5% risk of central nervous system (CNS) events that mainly occur within one year after diagnosis and carry a dismal prognosis. This pattern of early relapse suggests that occult malignant cells can be present in the CNS at diagnosis. Early identification of CNS localization is critical for improved treatment and prognosis. Several studies show that cerebrospinal fluid (CSF) flow cytometry (FC) detects occult disease with high sensitivity and specificity. We retrospectively collected data from CSF FC analysis of DLBCL pts considered at high risk of CNS localization diagnosed between December 2015 and December 2018. Standard prophylaxis consisted in intrathecal administration of methotrexate 12

mg with the first 4 cycles of chemotherapy. We analyzed fresh samples with a 8-color tube as follows: Kappa-V450/CD45-V500/CD20-FITC/CD79b-PE/CD5-PerCy5.5/CD19-PECy7/CD10-APC/Lambda-APC-H7. Data were acquired by BD FACS Canto II and analyzed by Diva Software (Becton Dickinson). Clonal neoplastic cells were detected by the presence of light chain restriction. We considered a cluster of clonal restricted events as representative if at least 10 events were registered. 73 pts and 262 PL were considered in our analysis. Only in 5 pts and in 6/262 samples (2.3%) we detected a clonal B cell population, despite a normal cell count in standard liquor analysis. In 4 of 5 pts the positivity was documented only in the first exam, in one patient (pt) also the second exam was positive. In another one pt the positivity was documented only in the second exam performed. The result of FC analysis had no impact on treatment. With a median follow up of 27 months from diagnosis (range 15-41) no pts with FC positivity had a CNS event. 6 pts developed CNS events (4 leptomeningeal, 2 parenchymal; 2 with concomitant systemic progression) despite intrathecal prophylaxis. FC analysis of the 24 samples of these pts resulted all negative. FC is a useful method to detect occult CSF disease with a sensitivity level higher than standard liquor analysis. Our data suggest that detection of a clonal B cell population using FC is rare when intrathecal chemotherapy is administered for prophylaxis without clinical suspicion. In this setting CNS prophylaxis appears sufficient to prevent leptomeningeal progression. On the other hand, FC failed to identify pts who will develop CNS events despite intrathecal prophylaxis.

P133**LONG-TERM EFFICACY AND SAFETY OF LENALIDOMIDE MAINTENANCE IN PATIENTS WITH RELAPSED DIFFUSE LARGE B-CELL LYMPHOMA WHO ARE NOT ELIGIBLE FOR AUTOLOGOUS TRANSPLANTATION (ASCT)**

P. Angelillo, M. Sassone, F. Zaja, A. Re, M. Spina, A. Di Rocco, A. Fabbri, C. Stelitano, M. Frezzato, C. Rusconi, R. Zambello, A. Arcari, G. Bertoldo, D. De Lorenzo, S. Volpetti, T. Calimeri, S. Perrone, C. Cecchetti, F. Ciceri, M. Ponzoni, A.J.M. Ferreri

Lymphoma Unit Department of Onco-Hematology IRCCS San Raffaele Scientific Institute Milan Italy; SC Ematologia Azienda Sanitaria Universitaria Integrata Trieste Italy; Division of Hematology Spedali Civili Brescia Italy; Division of Medical Oncology National Cancer Institute Aviano Italy, Division of Hematology University La Sapienza Rome Italy; Division of Hematology Azienda Ospedaliera Università Senese Siena Italy; Division of Hematology Azienda Ospedaliera Bianchi-Melacrino-Morelli Reggio Calabria Italy; Division of Hematology San Bortolo Hospital Vicenza Italy; Division of Hematology Niguarda Hospital Milan Italy; Division of Hematology Azienda Ospedaliera di Padua University of Padua Italy; Department of Oncology and Hematology G. da Saliceto Hospital Piacenza Italy; U.O. di Oncologia ed Ematologia Oncologica Ospedale di Mirano Mirano Italy; Clinica Ematologia Azienda Sanitaria Universitaria Integrata DAME Udine Italy; Hematology and Bone Marrow Transplantation Unit Department of Onco-Hematology IRCCS San Raffaele Scientific Institute Milan Italy; Ateneo Vita-Salute IRCCS San Raffaele Scientific Institute Milan Italy

Background: A multicentre phase II trial showed that lenalidomide maintenance (mLENA) was safe and effective in pts with chemosensitive relapse of DLBCL not eligible for or relapsed after ASCT. However, late toxicity and events remained undefined as median follow-up was 25 months and LENA was ongoing in 41% of pts at time of report. Herein, we report results of the trial after mLENA completion in all pts and a median follow-up of 5 ys.

Methods: HIV-neg pts with *de novo* or transformed DLBCL responsive to salvage therapy were treated with LENA 25 mg/day for 21/28 days, for a minimum duration of 2 ys, or until progression or unacceptable toxicity. Primary endpoint was 1-yr PFS (Simon's optimal design). To demonstrate a 1-yr PFS improvement from 30% to 50%, 47 pts were needed. Maintenance would be considered effective if ≥19 pts were progression-free at 1 yr.

Results: 48 pts were recruited (2009-2015); 46 were assessable (median age 72 ys; range 34-86); all pts were previously treated with R-CHOP or similar, plus ASCT in 6 pts. Most pts had unfavourable features, with an IPI ≥2 in 83% of pts. Salvage therapy before LENA contained HD-cytarabine or HD-ifosfamide in 66% of pts; response at registration was complete in 26 pts and partial in 20. LENA was well tolerated after an average of 16 courses/pt (range 3-82); 16 pts received LENA for ≥2 ys.

With the exception of neutropenia, g4 toxicities occurred in <1% of courses. Infections were rare and well controlled with oral antibiotics. 3 pts died of toxicity during LENA (intestinal infarction, meningitis, sudden death) and 2 died due to myelodysplastic syndrome at 31 and 62 months. 25 pts required dose reduction (transient in 21). After 1 year from registration, 31 pts were progression free, which was significantly higher than the pre-determined efficacy threshold ($n \geq 19$). At a median observation period from LENA completion of 35 months, 22 pts remain relapse-free, with a 1- & 5-yr PFS of $67 \pm 7\%$ & $48 \pm 7\%$, respectively. The duration of response to LENA was longer than response to prior treatment in 30 (65%) pts, and benefit was observed both in de novo and transformed DLBCL, and in GCB and nonGCB subtypes (NanoString Technology & Hans algorithm). 27 (59%) pts are alive, with a 5-yr OS of $58 \pm 7\%$.

Conclusions: These long-term results soundly promote the use of mLENA in pts with relapsed DLBCL not eligible for or failed after ASCT. Further studies on immunomodulators maintenance in this poor-prognosis pts are warranted.

P134

PROGNOSTIC SIGNIFICANCE OF GENOMIC ALTERATIONS IN MANTLE CELL LYMPHOMA (MCL). A MONOCENTRIC RETROSPECTIVE STUDY.

L. Signori¹, F. Pancani¹, L. Mannelli², A. Di Rocco⁴, F. Vergoni³, C. Celino¹, R. Santi³, C. Lucarelli³, C. Mannarelli², A. Farcomeni⁴, B. Puccini⁵, B. Scappini⁵, A. Bosi⁵

¹Dipartimento di Medicina Sperimentale e Clinica unità funzionale di Ematologia Università degli studi di Firenze; ²CRIMM centro di ricerca e innovazione per le malattie mieloproliferative AOU Careggi Dipartimento di Medicina Sperimentale e Clinica Università degli studi di Firenze; ³SOD Istologia Patologica e Diagnostica Molecolare AOU Careggi Firenze; ⁴Dipartimento di Sanità Pubblica e Malattie Infettive Sapienza Università di Roma; ⁵SOD Ematologia AOU Careggi Firenze, Italy

Background: The clinical course of MCL is variable and involves both indolent and highly aggressive forms. Mutations in TP53, biologic MIPI high risk and blastoid or pleomorphic cytomorphology are associated with aggressive disease course and inferior outcome. Advances in molecular techniques have led to identification of novel genomic alterations in MCL with different prognosis.

Aims: Using a next-generation sequencing (NGS) platform we analyzed in FFPE tissues samples collected at the diagnosis a panel of 11 genes frequently mutated in MCL (ATM, TP53, BIRC3, KMT2D, NOTCH1, NOTCH2, UBR5, WHSC1, CCND1, MEF2B, TLR2), trying to define a new predictive score on the basis of the identified mutations and assess their impact on progression-free survival (PFS) and overall survival (OS).

Results: In this study we enrolled 54 patients MCL referred to the Institute of Hematology AOU Careggi from 1999 to 2017; 81% were male (44) 19% female (10). Overall, we identified 1880 point mutations and 14 CNV. Conventionally we considered only alterations occurred in at least 5 samples (368 SNPs). Within them, two mutations resulted to impair PFS: NOTCH2 D1306N (median PFS 0,8 years, versus 3,5 years for the reference allele); KMT2D P523 (1 years versus 4 years). Six mutations were significant for OS: 3 were missense mutations (NOTCH1 G919R, KMT2D R2801Q, NOTCH2 D1306N) reducing OS from 8.3 years to 2.5, 2.5 and 1.3 years respectively; 3 were silent mutations (UBR5 P833, KMT2D P523 and KMT2D P916 pathogenic variant in neuroblastoma) impairing OS from 8.3 to 1.9, 1.1 and 2.5 years respectively. A score based on 3 mutations on 3 genes (NOTCH1, NOTCH2, and KMT2D) resulted to identify 4 classes of patients (score 0, 1, 2, >2) with significantly different PFS (median PFS group 0 was 3.9 years versus 2.7, 1.3, 0.5 and 0.2 years for groups 1, 2, 3 and 4 respectively); while a score taking in account of 5 mutations on 3 different genes (2 on NOTCH1, 2 on ATM and 1 on KMT2D) selected 3 categories (score -3 to 0 group 0, -1 to -2 group 1, >2 group 2) of cases with distinct OS (median OS group 1 was 4 years, group 2 1.2 years).

Conclusions: Our data seem to suggest that mutational status of MCL patients at the diagnosis may allow to identify cases with particular poor prognosis, independently from other conventional risk factors. They might be used to select high risk patients for novel therapy. Further studies possibly in a prospective setting are warranted.

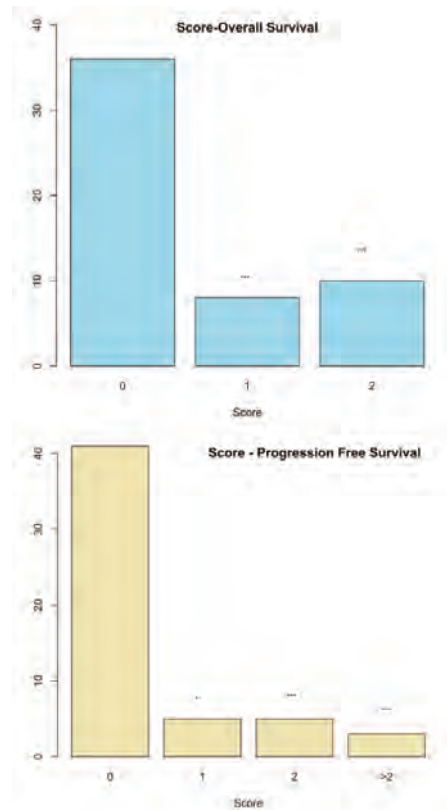


Figure 1.

P135

A 50-GENE SIGNATURE ASSOCIATED TO CHOLESTEROL METABOLISM IDENTIFIES M1-LIKE TUMOR-INFILTRATING MACROPHAGES AND PREDICTS SURVIVAL IN DIFFUSE LARGE B CELL LYMPHOMA

M.C. Vegliante¹, S. Ciavarella¹, S. De Summa², S. Mazzara³, M. Fabbri³, G. Opinto¹, F. Melle³, G. Motta³, A. Gulino⁴, G. Loseto¹, C. Minioia¹, S. Tommasi², A. Scattone⁵, A. F. Zito⁵, C. Tripodo⁴, A. Moschetta⁶, S.A. Pileri³, A. Guarini¹

¹Hematology and Cell Therapy Unit- IRCCS-Istituto Tumori 'Giovanni Paolo II'; ²Molecular Diagnostics and Pharmacogenetics Unit- IRCCS-Istituto Tumori 'Giovanni Paolo II'; ³Unit of Diagnostic Hematopathology-European Institute of Oncology; ⁴Tumor Immunology Unit- Dipartimento per la Promozione della Salute e Materno Infantile "G. D'Alessandro"-University of Palermo; ⁵Pathology Department- IRCCS-Istituto Tumori 'Giovanni Paolo II'; ⁶Department of Interdisciplinary Medicine-Aldo Moro University of Bari, Italy

Diffuse large B cell lymphoma (DLBCL) is a heterogeneous disease with high variability in clinical outcome, genetic features and cells of origin (COO). Gene expression profiling (GEP) studies have identified stromal signatures reflecting extracellular matrix deposition and macrophage infiltration as predictive of favorable outcome to standard immune-chemotherapy. However, while a reliable gene panel has been recently developed to detect stromal compartment, no reproducible macrophage biomarkers applicable to clinical practice are currently available. Tumor-associated macrophages (TAM) can display a M1-like phenotype with an antitumor activity, or act as M2-like subpopulation promoting tumor progression. As described for many immune cells, environmental and metabolic signals including oxygen, cytokine and nutrient gradients induce profound reprogramming also in macrophages and their precursors. In particular, the cholesterol metabolism, mainly regulated by the nuclear liver X receptors (LXR and LXR β), is known to control functional polarization, inflammatory response and apoptosis processes. We investigated whether LXRs may play a role in DLBCL biology and modulate the function of macrophages in tumor microenvironment, ultimately affecting prognosis. By combining GSEA, CIBERSORT deconvolution, in situ and in vitro approaches, we found that

LXR but not LXR was significantly up-regulated in DLBCL non-malignant cells, showing a strong association with M1-like macrophages. We also identified a panel of 50 genes closely related to LXR by using the machine learning approach Random Forest, and explored their prognostic potential in a validation cohort of 203 fresh frozen DLBCLs. A hierarchical clustering analysis identified three subgroups with different clinical outcome. In particular, cases with low expression of the signature showed a significant shorter overall survival compared to those with intermediate and high expression. Moreover, this difference was independent from COO, and identified a group of GCB-DLBCLs with unexpected less favorable outcome. In conclusion, we demonstrated that the metabolic regulator LXR may represent a reliable functional biomarker of M1-like macrophages in DLBCL. Furthermore, we identified a signature related to cholesterol metabolism that recognizes distinct prognostic subgroups of DLBCLs independently from their COO, providing the rationale to design clinical trials exploring new immunomodulatory drugs.

P136

LYMPHOCYTE TO MONOCYTE RATIO (LMR) PREDICTS PROGRESSION-FREE SURVIVAL INDEPENDENTLY FROM FLIPI AND IS USEFUL IN IDENTIFYING PATIENTS WITH EARLY DISEASE PROGRESSION

G. Gritti¹, C. Pavoni¹, S. Ferrari¹, P. Stefanoni¹, A. Rossi¹, F. Delaini¹, A.M. Barbui¹, A. Rambaldi^{1,2}

¹Hematology and BMT Unit, Ospedale Papa Giovanni XXIII; ²Department of Oncology and Hemato-oncology, University of Milan, Italy

Background: Despite the overall good prognosis of follicular lymphoma (FL), 20-30% of the cases experience early progression after first line therapy and suffer adverse survival (Casulo *et al.*, 2015; Shi *et al.*, 2017). Lymphocyte to monocyte ratio (LMR) has been previously identified as a useful prognostic markers in diffuse large B-cell lymphoma treated with Rituximab-based chemotherapy (Rambaldi *et al.*, 2013). In this study we aimed to evaluate the potential role of LMR in identifying patients with FL experiencing early progression after first line therapy.

Methods: 831 patients with a diagnosis of grade 1-3a FL were identified in the Bergamo Lymphoid Cancer Registry (NCT03131531) between 1983 to 2017. Patients with stage II-IV and receiving first line chemotherapy at diagnosis were included (N=448). The majority of the patients were treated with R-based therapy (72%), chemotherapy regimens included CHOP (67%), CVP (17%) or others (16%). Patients with early progression were defined as those with a PFS event before 30 months from first-line treatment start (PFS30).

with patients with LMR <2 or >5.2 (N=176) being at higher risk for a PFS event compared to those with LMR >2 and <5.2 (N=272) (Figure 1B). In multivariate analysis for PFS, LMR retained its significance independently from FLIPI (p=0.0001). The simple addition of LMR to standard FLIPI permitted to refine patient prognosis in terms of PFS (Figure 1C) and OS (Figure 1D), with three groups identified i.e. low risk (FLIPI low and favorable LMR), intermediate risk (FLIPI low and unfavorable LMR; FLIPI int/high and favorable LMR) and high risk (FLIPI int/high and unfavorable LMR). LMR was significantly associated with OS in univariate analysis (P=0.047), but not in multivariate analysis with FLIPI (p=0.11). The combined LMR and FLIPI index predicted survival with a 30-months OS of 96%, 94% and 85% in the low, intermediate and high risk category (p=0.0032).

Conclusions: LMR is a significant prognostic factor for PFS in FL, independent from FLIPI and closely related to the use of Rituximab. The combination of LMR with currently available prognostic markers can be useful in identifying high patient before the start of first line treatment.

P137

PROGNOSTIC CORRELATION OF CELL-OF-ORIGIN AND MYC, BCL-2 AND BCL-6 STATUS IN HIV-ASSOCIATED DIFFUSE LARGE B-CELL LYMPHOMAS

C. Rusconi¹, L. Crucitti¹, A. Re², L. Bandiera³, M. Spina⁴, G. Gini⁵, M. Paulli⁶, M. Lucioni⁶, F. Facchetti⁷, G. Goteri⁸, V. Canzonieri⁹, L. Lorenzi⁷, P. Balzarini⁷, S. Fisogni⁷, R. Riboni⁶, A. Malfitano¹⁰, M. Bonfichi¹¹, M.C. Moiola¹², M. Nichelatti¹³, F. Melle¹⁴, G. Motta¹⁴, S.A. Pileri¹⁴, R. Cairoli¹

¹Division of Hematology ASST Grande Ospedale Metropolitano Niguarda; ²Division of Hematology ASST Spedali Civili di Brescia; ³Division of Pathology ASST Grande Ospedale Metropolitano Niguarda; ⁴Division of Medical Oncology National Cancer Institute; ⁵Division of Hematology Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona; ⁶Anatomic Pathology Department of Molecular Medicine University of Pavia Fondazione IRCCS Policlinico San Matteo; ⁷Department of Molecular and Translational Medicine ASST Spedali Civili di Brescia University of Brescia; ⁸Department of Biomedical Sciences and Public Health Section of Pathologic Anatomy Polytechnic University of the Marche Region United Hospitals; ⁹Division of Pathology National Cancer Institute and Department of Medical Surgical and Health Sciences University of Trieste; ¹⁰ Division of Infectious Disease IRCCS Policlinico San Matteo; ¹¹Division of Hematology IRCCS Policlinico San Matteo; ¹²Division of Infectious Disease ASST Grande Ospedale Metropolitano Niguarda; ¹³Servizio di Statistica Fondazione Malattie del Sangue ASST Grande Ospedale Metropolitano Niguarda; ¹⁴Unit of Hematopathology European Institute of Oncology

Background: Diffuse Large B-cell lymphoma (DLBCL) outcome in HIV negative population differs according to the Cell-Of-Origin (COO) subtypes classified by gene expression profiling, being the prognosis of the germinal center B-cell type (GCB) superior to that of the activated B-cell type (ABC) and of the unclassifiable type (UC). Gene rearrangements of MYC plus BCL-2 and/or BCL-6 are further adverse prognostic factors. In HIV positive DLBCL patients (pts), limited data are available on prognostic impact of COO subtypes and genes rearrangements.

Aims: To evaluate in HIV associated DLBCL: the proportion of COO subtypes defined by immuno-histochemistry (IHC) using Hans algorithm and NanoString (NS), and the concordance of these methods; the prognostic impact of COO and of MYC, BCL-2 and BCL-6 protein expression and gene rearrangements detected by fluorescence in situ hybridization (FISH).

Methods: We retrospectively analyzed 66 HIV positive pts with newly diagnosed DLBCL treated with rituximab-CHOP; histological samples have been centrally reviewed for diagnosis confirmation, Hans COO assignment and MYC, BCL-2 and BCL-6 protein expression. NS Lymph2Cx assay for COO identification and FISH using break-a-part probes for MYC, BCL-2 and BCL-6 gene analysis were applied.

Results: By Hans COO assigned was GCB in 31 (47%) and non-GCB in 35 (53%) cases. NS was performed in 60 pts: 34 pts were GCB (57%), 11 ABC (18%) and 15 UC (25%). Hans algorithm and NS assay concordantly assigned COO subtypes in 80% of cases (Cohen's kappa=0.604; p<0.0005). After a median follow-up of 50 months, 2-yr progression-free survival (PFS) and overall survival (OS) were 56% and 61%. No significant survival difference was found according to IHC

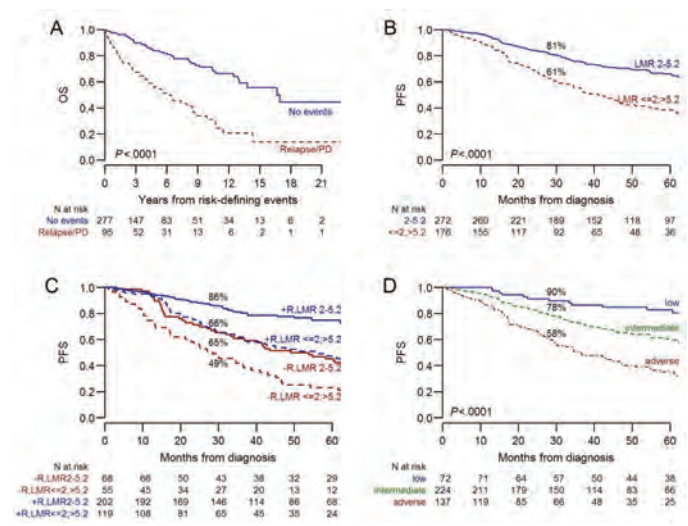


Figure 1.

Results: In the study population, 26% of the patients experienced early progression and were characterized by a shorter median OS compared to the other group (6.3 vs 17 years, p<0.0001, Figure 1A). The optimal cut-off of LMR to predict PFS30 was identified by ROC analysis,

and NS COO assignment, although a trend for a superior OS in GCB subtype defined by NS was observed (2-yrs OS: 71% vs 53%, $p=0.078$). MYC, BCL-2, BCL-6 proteins expression and gene rearrangements are summarized in Table 1. MYC protein overexpression and MYC, BCL-2, BCL-6 gene rearrangements did not impact survival, while BCL-2 overexpression (22 pts, 34%) was associated with an inferior OS (2-yrs OS 48% vs 67%, $p=0.047$).

Conclusions: In HIV associated DLBCL we found an high concordance between Hans algorithm and NS in the COO subtypes allocation, with subgroups distribution by NS similar to what described in the HIV negative population. The trend for superior outcome in GCB type by NS and prognostic role of MYC, BCL-2, BCL-6 status need to be confirmed.

Table 1.

MYC, BCL-2, BCL-6 status according to COO by NS	GCB n=34	ABC n=11	UC n=15
MYC overexpression, N (%)	13 (38%)	5 (45%)	6 (40%)
BCL-2 overexpression, N (%)	6 (18%)	7 (64%)	8 (53%)
Double Expressor (MYC and BCL-2 overexpressed), N (%)	3 (9%)	3 (27%)	3 (20%)
MYC rearranged, N (%)	5 (15%)	2 (18%)	4 (27%)
BCL-2 rearranged, N (%)	1 (3%)	0 (0%)	0 (0%)
BCL-6 rearranged, N (%)	8 (23%)	4 (36%)	5 (33%)
Double Hit (MYC and BCL-6 rearranged), N (%)	0 (0%)	1 (9%)	2 (13%)
Triple Hit (MYC and BCL-6 and BCL-2 rearranged), N (%)	0 (0%)	0 (0%)	0 (0%)

P138

THE ROLE OF CENTRAL NERVOUS SYSTEM PROPHYLAXIS AND CONTRALATERAL TESTIS RADIOTHERAPY IN PRIMARY TESTICULAR LYMPHOMA: LONG TERM ANALYSIS OF THE PHASE II IELSG10 STUDY AND PRELIMINARY RESULTS OF THE IELSG30 STUDY OF INTERNATIONAL EXTRANODAL LYMPHOMA STUDY GROUP AND FONDAZIONE ITALIANA LINFOMI

A. Chiappella¹, E. Zucca², A.J.M. Ferreri³, G. Ciccone⁴, A. Vanazzi⁵, M. Martelli⁶, A. Tucci⁷, M. Balzarotti⁸, F. Merli⁹, M.G. Cabras¹⁰, L. Nassi¹¹, V. Pavone¹², S. Storti¹³, M. Tani¹⁴, A.L. Molinari¹⁵, A. Conconi¹⁶, H. Gomez¹⁷, A. Lopez-Guillermo¹⁸, A.H. Sarris¹⁹, U. Ricardi²⁰, M.K. Gospodarowicz²¹, F. Cavalli²², U. Vitolo²³

¹Ematologia, AOU Città della Salute e della Scienza di Torino; ²Università della Svizzera italiana, Institute of Oncology Research; ³Istituto Scientifico San Raffaele; ⁴SSD Epidemiologia Clinica e Valutativa, Clinical Trial Center, AOU Città della Salute e della Scienza di Torino e CPO – Piemonte; ⁵Ematologia, Istituto Europeo Oncologico IRCCS; ⁶Ematologia, Dipartimento Cellulare di biotecnologie ed Ematologia, Università La Sapienza; ⁷Ematologia, ASTT Spedali Civili di Brescia; ⁸Ematologia, Istituto Clinico Humanitas; ⁹Ematologia, Azienda Ospedaliera Arcispedale Santa Maria Nuova – IRCCS; ¹⁰Ematologia, Ospedale Oncologico Armando Businco; ¹¹Ematologia, AOU Maggiore della Carità di Novara; ¹²Unità di Ematologia e Trapianto, Università di Bari; ¹³Ematologia, Università Cattolica del Sacro Cuore; ¹⁴Ematologia, Ospedale Santa Maria delle Croci; ¹⁵Ematologia, Ospedale degli Infermi di Rimini; ¹⁶Ematologia, Ospedale degli Infermi di Biella; ¹⁷Instituto Nacional de Enfermedades Neoplásicas; ¹⁸Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer; ¹⁹Hygeia Hospital Marousi; ²⁰Dipartimento di Oncologia, Radioterapia Oncologica, Università degli Studi di Torino; ²¹Princess Margaret Hospital; ²²Università della Svizzera italiana, Institute of Oncology Research; ²³Ematologia, AOU Città della Salute e della Scienza di Torino

The high risk of central nervous system (CNS) and contralateral testis recurrences are frequent in primary testicular lymphomas (PTL), Zucca et al, JCO 2003. The IELSG and FIL designed a phase II trial, IELSG10, to address the activity of Rituximab-CHOP21 (R-CHOP21) associated with CNS intrathecal prophylaxis (4 doses of 12 mg intrathecal methotrexate, IT-MTX) and contralateral testis radiotherapy (IF-RT, 25-30 Gy). Results of IELSG10 were previously reported by Vitolo et al, JCO 2011 on 53 patients (median age 64 years, range 22-79). In order to evaluate a potential increase in the incidence of CNS and contralateral testis relapses in this series of patients, a long-term analysis was

conducted. At a median follow-up of 12 years, 10-years progression free survival (PFS) was 65% (95%CI: 50-76%). Relapses involved lymph node alone in 3 patients, extranodal organs (pleura, skin, CNS) with or without lymph nodes in 7 patients. No contralateral testis relapses were observed. The 10-years overall survival (OS) was 71% (95%CI: 56-81%). Four patients experienced CNS relapses: two of them had isolated relapses (one meningeal and one brain parenchymal), one had a concurrent meningeal and lymph nodal relapse and the fourth one had a parenchymal CNS relapse while in second remission after nodal relapse. The 10-years cumulative incidence of CNS relapse, considering the competitive risk of death, was the same reported at 5-years, 6% (95%CI, 0-12%). Despite of CNS prophylaxis, the risk of CNS relapse was still present; based on these results, FIL and IELSG designed the international phase II trial, IELSG30, with 6 courses of R-CHOP21 associated to an intensified CNS prophylaxis, including 4 doses of 50 mg intrathecal liposomal cytarabine (from cycle 2 to 5) and a consolidation, at the end of induction, with two courses every 14 days of systemic methotrexate at intermediate dose (1.5 g/sqm). After chemotherapy, patients underwent IF-RT to the contralateral testis. From September 2009 to July 2017, 54 patients were enrolled. Median age was 64 years (range 37-80), stage II in 22 (41%), bilateral testis involvement in 2 (4%) and no CNS involvement at diagnosis. All the patients completed the treatment and no toxic deaths were reported. In conclusion, the introduction of CNS prophylaxis and contralateral testis radiotherapy was safe in this prevalently elderly populations and was able to significantly reduce the risk of CNS recurrence and contralateral testis relapses in PTL.

Myeloma and Monoclonal Gammopathies 3

P139

CLINICAL PROFILE OF MULTIPLE MYELOMA PATIENTS WITH LONG TERM SURVIVAL

M. Raimondo¹, M. Viscovo¹, C. Cimmino¹, G. Delle Cave¹, M. Calabrò¹, V. Caprioli¹, A. Giordano¹, G. Piccolo¹, R. Della Pepa¹, N. Pugliese¹, M. Picardi¹, S. Capalbo², L. Catalano¹, F. Pane¹

¹Department of Hematology University Federico II Naples; ²Department of Hematology Hospital University Riuniti Foggia, Italy

Background: Although cure is still considered unattainable in multiple myeloma (MM), a small number of patients has a remarkably long survival. With "long term survivors", we usually refer to patients surviving more than 10 years after the diagnosis of myeloma. It represents a small percentage among MM patients. Studies on this subgroup are few and, to our knowledge, there are not Italian studies.

Aims: Identifying clinical profile of patients affected by multiple myeloma who survived more than 10 years after the first line of treatment.

Patients and Methods: Patients treated in the Department of Hematology, University Federico II with survival longer than 10 years after the diagnosis of symptomatic disease, were preliminary analysed. The diagnosis of solitary plasmacytoma was an exclusion criteria. Main features at baseline are summarized in the Table 1.

Table 1.

Clinical characteristics of patients at diagnosis	n=25	
Sex		
Female	18	(72%)
Male	7	(28%)
Age, years		
Median (range)	62,5	(38-70)
Survival, years		
Median (range)	13	(10-20)
Heavy chains		
IgG	20	(80%)
IgA	2	(8%)
IgD	1	(4%)
Micromolecular	1	(4%)
Not-secretory	1	(4%)
Light chains		
kappa	17	(68)
lambda	7	(28)
DSS		
I A	1	(4%)
II A	5	(20%)
III A	18	(72%)
I B	0	-
II B	0	-
III B	1	(4%)
ISS		
I	1	(4%)
II	19	(76%)
III	5	(20%)
Creatinine		
>2 mg/dl	1	(4%)
<2 mg/dl	24	(96%)
Beta-2 microglobulin		
<3,5 mg/L	1	(4%)
3,5- 5,5 mg/L	19	(76%)
>5,5 mg/L	5	(20%)
Hemoglobin		
<10 mg/dl	19	(76%)
>10 mg/dl	6	(24%)

Results: HDM-ASCT was performed in 84 % of patients. About 76% of the patients were from the pre-proteasome inhibitor era, and thalidomide was part of their upfront therapy (Protocol Bologna 2002). Among 21 patients undergoing HDM-ASCT, 4 (19%) received tandem autolo-

gous transplant treatment. Maintenance after HDM-ASCT was performed in 85% of patients; alpha interferon was administered in 52% of patients. Relapsed patients were 18 (72%) and in 84 % was administered lenalidomide plus dexamethasone. The median PFS of multiple myeloma long survival was 108 months. We also determined PFS2 (time from the beginning of the first line to the second recurrence) and was found to be 216 months. In the group of patients who underwent HDM-ASCT, we observed that PFS was 108 months for single-ASCT (HR 0.812, IC 95% 0.22-3.02) and 132 for tandem-ASCT (HR 1.22, IC 95% 0.33-4.5). However the difference between the two groups does not appear to be statistically significant.

Conclusion: The clinical profile of patients surviving longer than 10 years, that emerge from our analysis, is characterized by a younger median age (54 years), a greater incidence in the female sex (70%), a previous history of MGUS, isotype IgG-k, low ISS stage at diagnosis and the median therapeutic lines performed is 2. The achievement of continuous complete remission occurs in 28% of patients. PFS could be used as a predictor of long survival, because the patient who has a prolonged progression free survival has a greater probability of a long survival compared to those who go in rapid progression.

P140

POMALIDOMIDE-DEXAMETHASONE IN THE MANAGEMENT OF HEAVILY PRETREATED MULTIPLE MYELOMA

C. Cerchione¹, G. Martinelli¹, D. Nappi², A.E. Pareto², M. Di Perna², K. Ferrara², S. Ronconi¹, D. Cangini¹, V. Di Battista¹, M. Ceccolini¹, M. Altini¹, F. Pane², L. Catalano²

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; ²Hematology, AOU Federico II, Italy

Pomalidomide is a new generation IMiD, with a very good compliance, thanks to oral administration, which can be used also in heavily pretreated patients, in a domestic setting. In this retrospective observational trial, it has been evaluated efficacy and tolerance of pomalidomide plus dexamethasone (PD) as salvage regimen in heavily pretreated patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. 33 patients (19 M/14 F), with rrMM, median age at diagnosis 69 years (r. 52-84), and median age at start of treatment 76 years (r. 56-89) treated with several lines of treatments (median 7, r. 2-11), every refractory to all the drugs previously received (also Bortezomib, Thalidomide and Lenalidomide), received Pomalidomide-Dexamethasone (Pomalidomide 4 mg for 21 days, Dexamethasone 40 mg days 1,8,15,22, pegfilgrastim day +8) every 28 days, until progression. ISS was equally distributed, and cytogenetic at relapse was evaluable in 14 patients. All the patients had previously been treated with schedule containing bortezomib and IMiDs. 60% (20/33) of them had undergone at least to a single ASCT. All patients were relapsed and refractory to last therapies received before PD. Pomalidomide was well tolerated, with grade 3 anemia in 51% (17/33) of patients, 36.3% (12/33) grade 3 neutropenia (pegfilgrastim in primary prophylaxis was given, no hospitalization was required, no septic shocks were observed), 30.3% (10/33) grade 3-4 thrombocytopenia without hemorrhagic events and transfusion-dependence. No severe extra-hematologic toxicity was observed. According to IMWG, ORR1 (\geq PR) was 45.4% (15/33: 4 CR, 5 VGPR, 6 PR), but, considering that we are evaluating a cohort of heavily pretreated patients, with poor prognosis, another parameter should be considered, ORR2 (\geq SD), considering stable disease as a successful result in progressive MM. ORR2 was 78.7% (26/33: 4 CR, 5 VGPR, 6 PR, 11 SD). These can be considered as impressive result in this subset of patients. Oral treatment gives a really good compliance, in frail and unfit patients, and response, when present, is always really fast (median time to response: 2 months (r.1-6)), median OS from diagnosis was 92 months (range 21-234), median OS from start of pomalidomide was 9 months (range 1-25). Pomalidomide-dexamethasone has shown significant efficacy and a very good compliance, thanks to oral administration, in a particularly severe setting of heavily pretreated patients, relapsed and refractory to all available therapeutic resources.

P141**IMMUNOTHERAPY IN MULTIPLE MYELOMA: EXPERIENCE OF THE MULTIPLE MYELOMA GIMEMA LAZIO GROUP**

F. Vozella, A. Siniscalchi, M. Rizzo, T. Za, G. Antolino, U. Coppetelli, A. Piciocchi, A. Andriani, O. Annibaldi, L. De Rosa, G. Cimino, G. La Verde, V. De Stefano, M. Cantonetti, T. Caravita di Toritto, R. Foà, M.T. Petrucci

Sapienza Università di Roma, Ospedale S.Eugenio, Università Tor Vergata, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Ospedale S.Andrea, Ospedale S. Maria Goretti, GIMEMA Data Centre, Ospedale F. Spaziani, Università Campus Bio-Medico, Ospedale San Camillo Forlanini, Italy

Treatment of multiple myeloma (MM) patients (pts) has radically changed over the last years following the introduction of next generation proteasome inhibitors (PI) and immunomodulatory derivative (IMiDs). Almost all pts eventually relapse despite their responses to PI, IMiDs or both. Recently, one further therapeutic option for MM patients is represented by daratumumab, an anti-CD38 monoclonal antibody. We report the experience of the Multiple Myeloma GIMEMA Lazio Group in 50 relapsed/refractory MM pts treated with daratumumab as monotherapy. Twenty-nine pts (58%) were men and 21 (32%) women. Median age was 62.3 years (range, 43.1 – 85.7); 32 pts (64%) were refractory to the last line of therapy; 26 (52%) had previously received a stem cell transplant. After a median follow-up from diagnosis of 54.5 months (range 1.0 – 203.0) and a median of 3 previous lines of therapy (range 2 – 8), pts received a median of 3 cycles (range 1 – 23) of daratumumab. Forty-seven pts (94%) performed at least one cycle and were evaluable for response. The overall response rate was 74%; in particular, 2 pts obtained a CR (4.2%), 3 pts a VGPR (6.3%), 17 pts a PR (36.2%) and 15 pts a SD (32%), while 10 pts (21.3%) presented a PD. After a median follow-up of 5.3 months (range 1 – 31), 24 pts (65%) were still in response and alive, one pt (5.8%) died in PR due to post-allograft GVHD and 12 (32%) experienced a PD (1 CR, 1 VGPR, 6 PR and 4 SD). Seven (19%) pts died and 30 (81%) are still alive. With regard to the 3 pts not evaluable for response, 2 died early and 1 has not yet completed the first cycle. The median time to response, duration of response, progression-free survival and overall survival were 1.5 months (range 1.0 – 6.0), 6.7 months (95% CI, 4.14 – 14.21), 5.7 months (95% CI, 3.26 – 13.75) and 22.5 months (95% CI, 11.6 – 36.1), respectively. Daratumumab was well tolerated; the most common adverse events, of any grade, were infections in 20 pts (42.0%) and anaemia in 21 pts (44.0%), which did not lead to treatment discontinuation. Infusion-related reactions were observed in 7 pts (14.8%), grade I-II (4 pts), grade III (3 pts). Daratumumab monotherapy is an effective strategy for heavily pre-treated and refractory pts with multiple myeloma, with a favorable safety profile. This treatment option needs to be considered for pts not eligible for combination therapy of daratumumab with bortezomib or lenalidomide, recently approved also in our country.

P142**SUBCUTANEOUS BORTEZOMIB CONTAINING REGIMENS AS UPFRONT TREATMENT OF NEWLY DIAGNOSED TRANSPLANT ELIGIBLE MULTIPLE MYELOMA PATIENTS**

I. Rizzello, E. Zamagni, L. Dozza, M. Quaresima, M.T. Petrucci, V. De Stefano, E. Antonioli, P. Tosi, M. D'Agostino, M. Offidani, A. Gozzetti, G. Rivoli, N. Testoni, M. Martello, P. Tacchetti, L. Pantani, K. Mancuso, S. Rocchi, I. Caratozzolo, M. Cavo

Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Arcispedale Santa Maria Nuova IRCCS Centro Oncologico ed ematologico di Reggio Emilia, Dipartimento di biotecnologia cellulare ed Ematologia Università La Sapienza di Roma, Istituto di Ematologia Università Cattolica Fondazione Policlinico Universitario Gemelli di Roma, Azienda Ospedaliero Universitaria Careggi di Firenze, Dipartimento Oncoematologico Ospedale di Rimini, Dipartimento di Ematologia Università di Torino AOU Città della Salute e della Scienza di Torino, Clinica di Ematologia AOU Ospedali Riuniti di Ancona, Unità di Ematologia Azienda Ospedaliero Universitaria Senese Università di Siena, Clinica Ematologica Ospedale San Martino di Genova, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto

di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna, Istituto di Ematologia Seràgnoli Università di Bologna

Bortezomib (B)-based regimens currently represent the standard of care as induction therapy prior to autologous stem cell transplantation (ASCT) in newly diagnosed multiple myeloma patients (ND-MM pts). Data from prospective published studies are based on the intravenous (IV) administration of B, while, after the demonstration of similar efficacy with reduced neuro-toxicity in relapsed/refractory patients (Moreau *et al.*, Lancet Oncol 2011), the subcutaneous (sc) way of administration is currently used in daily clinical practice. We conducted a retrospective observational study aimed at defining the outcomes of pts treated with sc B-based induction regimens prior to ASCT. One hundred and thirty-one pts, enrolled in 10 haematologic institutions, were included in the present analysis. The median age was 61 yo (27% were between 65 and 72). ISS and R-ISS stage 2 and 3 were the following: 34% and 20%, 46% and 6%, respectively. B and dexamethasone were administered as induction therapy combined with thalidomide (VTD) in 86%, cyclophosphamide (VCD) in 5% and alone (VD) in 9% of the pts, respectively, for a median of 4 cycles. 52% of the pts received a single and 48% a double ASCT. 37% of the pts received in addition a consolidation therapy after ASCT, 89% with VTD and 11% with VD, for a median of 2 cycles. B dose-reduction, due to toxicity, was needed in 16% and 8% of the pts during induction and consolidation, respectively. 46% of the pts showed \geq grade 2 adverse event (AE). Most common toxicities were gastrointestinal disorders (27%) and peripheral neuropathy (PN) (25%). Grade \geq 2 PN rate during induction was 23%, 16% grade 2, 7% grade 3. Haematological AEs presented in 7% of the pts. The overall response rate (ORR) was 93.1% (70.2% \geq VGPR), 97.7% (83.2% \geq VGPR) and 100% (93.8% \geq VGPR), after induction, ASCT and consolidation, respectively. With a median follow-up of 45.3 months, median PFS and PFS2 were 55.8 months and 72 months, respectively, and median OS was not reached. Our data, derived from a real-life population of pts up to the age of 72 yo, compare well with the results coming from randomized clinical trials of IV B-based combinations (Cavo *et al.*, Lancet 2010; Moreau *et al.*, Blood 2011; Rosinol *et al.*, Blood 2012), with a lower rate of PN, and similar efficacy. In conclusion, B-based regimens, administered sc, as induction therapy before, and consolidation after ASCT, are safe and not inferior to published IV B results.

P143**CHARACTERIZATION OF BONE MARROW CD8+ TISSUE-RESIDENT MEMORY T CELLS (TRM) IN MULTIPLE MYELOMA**

M. Carlisi, S. Mancuso, V. Orlando, M.P. La Manna, G. Bertuglia, S. Raso, M. Santoro, N. Caccamo, S. Siragusa

Department of Surgical Oncological and Stomatological Disciplines, Biomedical Department of Internal Medicine and Specialties (DiBiMIS), Central Laboratory for Advanced Diagnosis and Biomedical Research (CLADIBIOR)- and Department Bi.N.D.

Background: CD8 T cell responses are an essential component of the adaptive immune system. After resolution of infection a small population of memory cells is formed. In relation to circulatory patterns, different subsets of memory CD8+ T cells can be identified: the central memory (TCM) and the effector memory T cells (TEM). Also, it has been described a subset of resident memory T cells (TRM) permanently living in peripheral tissues, including the bone marrow (BM). It is conceivable that these cells can contribute to the defence toward haematological tumours infiltrating the BM. Therefore, we have evaluated the frequency and the phenotype of BM CD8 TRM in multiple myeloma (MM) patients.

Patients and Methods: We prospectively analysed 15 consecutive patients with a new diagnosis of IgA, IgG and light chain MM. Clinical and laboratory data about the patients and disease were collected. At the time of the BM assessment, we took a sample to perform phenotypical and functional analysis and *in vitro* cell culture. Moreover, to evaluate the role of the microenvironment on maintenance of these cells, we performed *in vitro* experiments of BM-derived PBMC of MM patients cultured in the presence of homeostatic cytokines in maintain-

ing these cells for a long time.

Results: The *ex vivo* average frequency of CD8+ TRM in MM patients was of 0.49% and the phenotype was represented mainly by T (EM) (78%) followed by T (EMRA) (12.2%) and (5,19%) of naïve cells and (4,53%) of T (CM). BM derived mononuclear cells from MM patients were then cultured in vitro for 4 days in the presence of IL15, IL7+IL15, IL7+IL15+TGF β , and medium alone. After in vitro culture, we observed an increased frequency of CD8 TRM, moreover the analysis of the phenotype distribution showed an increase of the CM subset.

Conclusions: The increase of CD8 TRM cell with a CM phenotype could have an anti-tumor role in the control of MM. Further studies are needed to investigate the biological role of these cells in the perspective of their use in therapeutic programs.

P144

BENDAMUSTINE-BORTEZOMIB-DEXAMETHASONE (BVD) IN HEAVILY PRETREATED MULTIPLE MYELOMA: OLD/NEW IN NOVEL AGENTS' ERA

C. Cerchione¹, L. Catalano², S. Rocco³, S. Palmieri³, D. Nappi², A.E. Pareto², S. Ronconi¹, M. Ceccolini¹, F. Pane², G. Martinelli¹, F. Ferrara³

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola; ²Hematology, AOU Federico II; ³Hematology, A.O.R.N. Cardarelli, Italy

Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM). In this retrospective study, it has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed. 56 patients (31 M/25 F), with rrMM, median age at diagnosis 57.3 years (r. 36-82), median age at start of treatment 61.8 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (B 90 mg/sqm days 1,2; V 1.3 mg/sqm days 1,4,8,11, D 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. All patients had previously been treated with schedule containing bortezomib and IMiDs, and 30% had also received radiotherapy. 67% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD. Bendamustine was well tolerated, with grade 3 transfusion-dependent anemia in 41% of patients, and 37% grade 3 neutropenia (no hospitalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, after a median follow-up of 14 months (r.2-36), ORR was 64% (36/56: 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second auSCT, and for two patients a bridge to alloSCT. Three patients have shown a notable PR after failure of novel agents (i.e. Carfilzomib and Pomalidomide). Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (r.6-151), median OS from start of Bendamustine was 9.8 months (r.2-36). The triplet Bendamustine-Bortezomib-Dexamethasone has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogeneic SCT.

P145

CARFILZOMIB, LENALIDOMIDE AND DEXAMETHASONE (KRD) IN RELAPSE/REFRACTORY MULTIPLE MYELOMA (MM): REAL LIFE EXPERIENCE AND NEW PERSPECTIVES ON DOSE-DENSE CHEMOTHERAPY

A. Giordano, C. Giordano, N. Pugliese, M. Raimondo, G. Piccolo, I. Zacheo, L. Catalano, F. Pane
Università Federico II, Napoli, Italy

Background: The natural history of (MM) is characterized by improved survival rates but relapses are still common, thus new therapeutic

approaches are still needed. The aim of this study is to describe the real life of our patients treated with KRD and to propose a new concept of therapy.

Material and Methods: Data from patients with relapse/refractory MM treated with KRD in the Federico II University of Naples, were retrospectively analyzed. We analyzed the time to next treatment (TTNT) of the entire population. Patients were divided into three cohorts as per chemotherapy dose-density: in the first group patients received $\leq 50\%$ of the expected cycles; in the second group patients received a dose comprised between 50% and 75% of the expected cycles; in the third group $\geq 75\%$ of the expected cycles was employed. The Overall Response Rate (ORR) of the entire population was evaluated. Disease progression and treatment response were determined in accordance with IMWG Uniform Response Criteria. Disease assessments were performed on day 1 of each cycle. In patients that had obtained a PR, VGPR, CR in at least 3 consecutive measurements carried out at monthly intervals during treatment it was decided to discontinue the therapy until subsequent reassessment to 30 days. Treatment was reintroduced when disease progressed according to clinical and/or biochemical parameters.

Results: From Jan 2016 to Jan 2019 the forty-two patients with a diagnosis of MM treated with KRD, relapsed after a previous therapy (1-7) including bortezomib and Immunomodulatory Drugs (IMiDs). The ORR of the entire cohort was 95% TTNT was 13 months for the entire cohort while the median TTNT for cohort treated with $< 50\%$ dose was not reached; the cohort treated with 50-75% and the cohort treated $> 75\%$ dose had 13 and 5 months of TTNT respectively.

Discussion: This subgroup analysis indicates that KRD improved outcomes in patients in their first relapse, with ≥ 2 previous lines of therapy, with or without previous exposure to bortezomib and/or IMiDs. Although the therapies for MM continue until progression, our data suggest that less dose-density appears to have the same efficacy of full dose but with a great economic saving and toxicity. The rationale behind this conclusion is that these patients harbor a minimum residual disease, which is refractory to the agent combination administered. By using a less aggressive therapeutic regimen, the selection for these refractory cells will take longer.

Survival proportions: Survival of Three groups

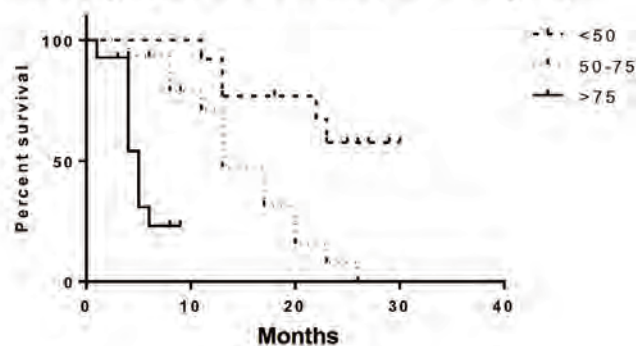


Figure 1.

P146

STUDY OF MULTIPLE MYELOMA CHROMOSOMAL ABNORMALITIES BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION: CORRELATIONS WITH FLUORESCENCE IN SITU HYBRIDIZATION

F. Carbone, F. Nozza, G. Vona, C. Mannarella, A. Dell'Acqua, O. Scariolla, A. Ciancio, B. Daraia, C. Bitetti, G. Pietrantuono, R. Nuccorini, S. Pascale, M. Pizzuti, P. Musto, A. Fragasso

Laboratory "Muscas" - Hematology Unit - Madonna delle Grazie Hospital, "Carla Alagia" Laboratory of Clinical Research and Advanced Diagnostics - IRCCS-CROB, Hematology Unit - Madonna delle Grazie Hospital, Genetics - Madonna delle Grazie Hospital, Department of Onco-Hematology - IRCCS-CROB, Hematology Unit - San Carlo Hospital, Hematology Department of Basilicata Region, MATERAIL

Chromosomal abnormalities play an important role in prognostic stratification of Multiple Myeloma (MM). Standard current technology used to evaluate such genetic abnormalities is Fluorescence In Situ Hybridization (FISH). Nevertheless, it is difficult for FISH to screen all lesions simultaneously, due to high costs and technique limitations. Multiplex Ligation-dependent Probe Amplification (MLPA) is a polymerase chain reaction that permits the amplification of multiple targets by a single reaction. This technique is useful to detect Copy Number Variations (CNVs); however, it is unable to find out balanced translocations and detects abnormalities only if they are present in 30-40% of pathological cells. In this study we evaluated the MLPA technique in the identification of mutations occurring in MM patients, comparing the results with those obtained by FISH. Cohort study included 44 newly diagnosed MM patients (27 M, 17 F, range 53-87 y). After evaluating the presence and percentage of plasma cells, a sample part of bone marrow (BM) was used for the FISH assay. MLPA tests were conducted on DNA extracted from marrow aspirates. In 27 out of 44 samples PCs were purified from MM samples using CD138 microbeads and magnet-assisted cell sorting. Excluding translocations involving IgH genes (not evaluable by MLPA), FISH analysis detected genetic alterations in 31 of 44 tested patients (del13q: N=18; del17p: N= 5; amp1q: N= 5; del1p: N=1). We could identify genetic alteration in 22/44 patients by using MLPA (del13q: N=15; amp1q: N=10; del1p: N=4; del17p: N=3). Additional lesions found by MLPA included: amp5q (N=10), amp15q (N=7), del12p (N=4), amp9 (N=8), del16q (N=1), delChrY (N=1). Overall, concordant results were detected in 37/44 cases and discordant in 7/44. Out of these 7 cases, MLPA tests were made on DNA isolated from whole BM mononuclear cells in 4 patients. Interestingly, MLPA identified del1p in 1 patient, not showed by FISH, because the probes do not cover the region interested by the deletion (1p21-1p12). Overall, in this study there was a consistent concordance between FISH and MLPA for detection of CNVs. However, some discrepancies emerged, which were probably attributable to the non-isolation of CD138 cells in some samples. Notably, we could identify by MLPA some additional cytogenetic abnormalities. Taken together, MLPA and FISH represent complementary techniques, that could be both useful in MM patients. The study is still recruiting new patients.

P147

SIRT2 AND SIRT3 EXPRESSION CORRELATES WITH ADVANCED DISEASE AND BONE LESIONS IN MULTIPLE MYELOMA PATIENTS

V. Innao¹, A. Allegra¹, M. Aguenouz², A. Alibrandi³, R. Oteri², A.G. Allegra¹, F. Polito², M. Bruzzese¹, R.M. Di Giorgio², C. Musolino¹

¹Division of Hematology – Department of Human Pathology in Adulthood and Childhood “Gaetano Barresi” – University of Messina; ²Department of Neurosciences – University of Messina; ³Department of Economics – Unit of Statistical and Mathematical Sciences – University of Messina, Italy

Mammalian sirtuins (SIRT) deacetylases comprise seven family elements (SIRT1-7) that have been demonstrated to be essential controllers of cell signaling pathways. SIRT2 is a class III NAD⁺ dependent deacetylase, which regulates a large variety of biological actions, comprising cancer inhibition. Instead, SIRT3 has probably a dual role in tumorigenesis, but data are controversial. The aim of the present study is to evaluate the expression of SIRT2 and SIRT3 in 17 patients with multiple myeloma (MM) at the onset, and 10 healthy controls, trying to correlate these values with the variables generally used to evaluate the disease status, its extent, and the presence of damage of organ secondary to myeloma. Moreover, we perform NAD⁺/NADH determination and the analysis of glutathione peroxidase (GPx), and hydrogen peroxide (HP) in mononuclear cells from MM patients and controls. In fact, NAD⁺/NADH ratio have long been known to regulate the activity of numerous oxidoreductase enzymes, including the SIRT family of NAD⁺-dependent protein deacetylases. SIRT2 and SIRT3 expressions are reduced in MM patients with respect to controls ($p < 0.001$ and 0.0001 respectively). Moreover, SIRT2 reduction is associated with advanced clinical stage ($p = 0.035$). Finally, SIRT2 and SIRT3 expressions are associated with more advanced bone lesions ($p = 0.007$ and 0.02). Mononuclear cells of MM patients have low NAD⁺ values compared to controls, and lower levels of GPx ($p < 0.0001$). Moreover, mononuclear cells of MM patients with bone lesions have lower levels of NAD⁺ and

GPx than patients without signs of bone disease ($p < 0.0001$). Finally, mononuclear cells of MM patients have higher levels of HP than controls ($p < 0.0001$). The in vitro results confirm the close correlation that exists between NAD⁺ and sirtuins. The results also confirm the existence of a profoundly altered oxidative stress in MM patients. Our preliminary data suggest that reduction of the SIRT gene expression in MM subjects could have a potential role in the onset and progression of this pathology.

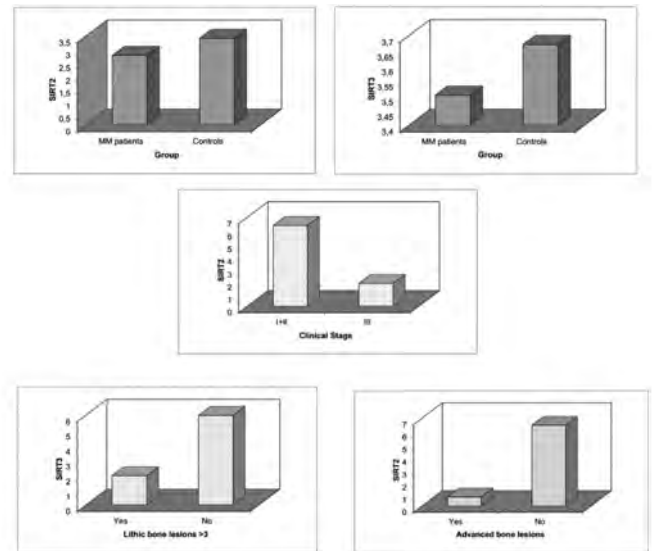


Figure 1.

P148

EXPLORING GRANULOCYTES PROFILE AND FUNCTION IN THE TUMOUR IMMUNE MICROENVIRONMENT DOES NOT DISCLOSE ANY ASSOCIATION WITH CLINICAL OUTCOME OF PATIENTS AFFECTED BY WALDENSTROM DISEASE

N.L. Parrinello, D. Cambria, S. Frazzetto, F. Puglisi, C. Bellofiore, C. Giallongo, D. Tibullo, G. Schinà, V. Leotta, C. Conticello, V. Del Fabro, F. Di Raimondo, A. Romano

AOU Policlinico Vittorio Emanuele di Catania - Divisione di Ematologia, Italy

Background: In the tumor immune microenvironment (TIME) of Waldenström Disease (WD), the role of granulocytes in promoting tumor growth and inducing immune suppression is not well-established. A recent study (Condamine, 2018) indicated that Lectin-type oxidized LDL receptor-1 (LOX-1) was a distinct surface marker for human granulocytic myeloid-derived suppressor cells (PMN-MDSC). Recent reports identify NLR, the ratio between absolute neutrophils count (ANC) and absolute lymphocyte count (ALC), as a predictor of clinical outcome in cancer.

Methods: We evaluated by multidimensional flow cytometry the pre-established phenotype of granulocytes in bone marrow (BM) and peripheral blood (PB) samples from WD (n=8, 5/8 Myd88L265Pmut), IgM-MGUS (n=8, 3/8 Myd88L265Pmut) and healthy subjects (n=5). We then used in vitro experiments to determine their immune suppressive potential. NLR was tested in a cohort of 28 newly diagnosed WD and 30 IgM-MGUS;

Results: As previously shown in MGUS and MM patients, PMN-MDSCs have been defined as a unique cluster displaying a CD11b-, CD14-, CD15+, CD33+ and HLADR- phenotype in both BM and PB. The percentage of cells with a CD14-CD15+CD33+HLADR- phenotype was higher in BM than in PB ($p = 0.01$), but similar in the BM of IgM-MGUS and WD patients (median 50 (IQ 49-63)% of white blood cells). Thus, we next used MFC to identify three additional subsets of neutrophils in whole PB from IgM-MGUS and WD patients, based on homogeneous CD14-CD15+CD33+HLADR- expression but differential reactivity against CD11b, Lox-1 and the immunosuppressive marker ARG-1. Both CD11b+ CD14-CD15+CD33+HLADR- and Lox-1+ PMN-MDSC were higher in WD than IgM-MGUS ($p = 0.01$), without

significant differences in the expression of ARG-1. Conversely, there were no significant differences in the amount of ARG-1 in sera in WD, MGUS and healthy patients. We could not identify any correlation between the amount of PMN-MDSC and clinical variables at diagnosis, including mutational status of Myd88. According to functional studies on granulocytes, NLR was weakly higher in WD than IgM-MGUS (median 1.88, IQR 0.5-5.8, $p=0.07$). In 12/28 WD patients we found $NLR \geq 2$, with an enrichment of Myd88L265Pmut cases ($p=0.03$), not associated to clinical outcome.

Conclusions: Lox-1+PMN-MDSC are increased in WD and IgM-MGUS patients; however, NLR is not predictor of clinical outcome, differently from what previously reported for other plasma cells dyscrasias.

Myeloproliferative Neoplasms 2

P149

IN VIVO STUDY (MYCEC0617) OF CD34+ HSC AND CEC MUTATIONAL PROFILE FOR INVESTIGATING CELL ORIGIN AND PATHOGENESIS OF PRIMARY MYELOFIBROSIS (PMF)

M. Farina^{1,2,3}, S. Bernardi^{1,4}, M. D'Adda², C. Foroni^{1,4}, C. Zanaglio^{1,4}, A. Dunbar³, F. Re^{1,4}, F. Cattina¹, B. Rambaldi¹, T. Zollner¹, A. Turra¹, E. Morello¹, M. Malagola¹, R.L. Levine³, G. Rossi², N. Polverelli¹, D. Russo¹

¹Chair of Hematology, Dept of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili di Brescia; ²Division of Hematology, ASST-Spedali Civili di Brescia; ³Center for Hematologic Malignancies, Memorial Sloan Kettering Cancer Center; ⁴CREA Laboratory (Centro di Ricerca Emato-Onco-logica AIL), ASST-Spedali Civili di Brescia, Italy

Background: Primary myelofibrosis (PMF) is characterized by increased bone marrow vascularity and vascular complications. Furthermore, *in vitro* studies show that endothelial cells (EC) may bring the JAK2 mutation. These data suggest that PMF could harbor from a precursor common to CD34+ hematopoietic stem cells (HSC) and EC. To investigate this biological issue the mutational profile of the HSC and of circulating endothelial cells (CEC) was analyzed.

Aims: To analyze the mutational profile of cell-sorted HSC and CEC in PMF patients (pts).

Methods: 14 PMF pts untreated with Jak2 inhibitors (median age: 66 ys) and 1 healthy control were enrolled in the MyCEC0617 study, approved by Ethical Committee. HSC were selected through CD34+ immunomagnetic positive selection, while the CEC were detected by the CellSearch system. CEC (CD146+, CD105-PE+, DAPI+ and CD45-APC-) were sorted with DEPArray system, based on a combination of dielectrophoresis technology with high quality image-based cell selection. DNA has been analyzed by MiSeq Illumina NGS platform, using a 54 genes custom panel.

Table 1. Subjects clinical characteristics and molecular analysis. In bold the mutations in common between CEC and HSC. In red the CEC mutations in MF driver genes.

ID	Sex	Age at diagnosis (years)	Months from diagnosis to analysis	Diagnosis	Grade of Marrow Fibrosis	PMN-MDSC (per 1000)	WBC (per 1000)	Hb (g/L)	Hct (%)	PLT (per 1000)	CEC detected	Total number of CEC	Mutations on HSC (n=4)	Mutations on CEC (n=4)
MYCEC0617	M	71	4	PMF	2	none	142	89%	101	Yes	Yes	9	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%, TET2 (Y105C) 12%, IDH1 (R132G) 12%, IDH2 (R140Q) 12%, IDH3 (R132G) 12%	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%, TET2 (Y105C) 12%, IDH1 (R132G) 12%, IDH2 (R140Q) 12%, IDH3 (R132G) 12%
MYCEC0617	M	78	2	PMF	1	NA	142	89%	101	Yes	Yes	3	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%
MYCEC0617	M	57	6	PMF	NA	NA	142	89%	101	Yes	Yes	2	JAK2 (G2019S) 47%, MPL (W515L) 26%	JAK2 (G2019S) 47%, MPL (W515L) 26%
MYCEC0617	M	72	121	PMF	1	NA	142	89%	101	Yes	Yes	3	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%
MYCEC0617	F	88	18	PMF	2	NA	142	89%	101	Yes	Yes	1	MPL (W515L) 26%	MPL (W515L) 26%
MYCEC0617	F	64	8	PMF	2	none	142	79%	101	Yes	Yes	9	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%
MYCEC0617	M	66	15	PMF	1	none	142	89%	101	Yes	Yes	9	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%	JAK2 (G2019S) 47%, MPL (W515L) 26%, NPM1 (Y2539C) 15%, SRSF2 (R228Q) 20%, KIT (D816V) 19%, DNMT3A (G84E) 12%, ASXL1 (I105V) 12%
MYCEC0617	M	68	NA	Control	NA	NA	NA	NA	NA	NA	NA	NA	none	none

Results: CEC were successfully collected in 12 (80%) of 15 samples (including normal control). A median of 26 (1-122) CEC in 4 ml of peripheral blood was recovered per pts. Genetic analysis is ongoing in 4 cases, thus this preliminary analysis refers to 8 cases (7 pts and 1 control). The characteristics and molecular analysis are summarized in Table 1. All the 7 pts had mutations on both HSC and CEC, while none was found in the control. JAK2V617F mutation was confirmed on HSC in 5 of 6 pts and 5 of the 7 pts shared one or more mutations both on HSC and CEC. In particular, 2 pts shared JAK2V617F together with ASXL1 and IDH1, TET2, ABL1, respectively. The other 3 pts shared SRSF2, KIT and WT1, respectively. 1 pts had a MPL-W515L mutation on CEC, without sharing any other mutations. Therefore, all the 7 pts had mutations on both HSC and CEC and all of them are related with PMF. The presence of common mutations on HSC and CEC (in 1 case for 4

genes; in 1 case for 2 genes) clearly support a common clonal cell origin. One MPL gene mutation was found in CEC but not in HSC, while no CARL gene mutation was found.

Conclusions: HSC and EC in PMF share one or more mutations of genes, which are known to be correlated with the onset and pathogenesis of PMF. These first *in vivo* findings support the theory that PMF may harbor from a common HSC/EC precursor. Further data are needed to validate these findings.

P150

CYTOGENETIC ABNORMALITIES IN PRIMARY MYELOFIBROSIS: CLINICAL ASSOCIATION AND OUTCOME IN AN ITALIAN MULTICENTER SERIES

A. Iurlo, D. Cattaneo, F. Palandri, E.M. Elli, B. Martino, C. Bucelli, M. Sciumè, D. Vincelli, G. Auteri, F. Brioschi, G.A. Croci, I. Cortinovis, A. Bossi, V. Rosti, L. Baldini, U. Gianelli

Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan; Institute of Hematology "L. and A. Seràgnoli", S. Orsola - Malpighi Hospital; Hematology Division, San Gerardo Hospital, ASST Monza; Division of Hematology, Azienda Ospedaliera "Bianchi Melacchino Morelli"; Division of Pathology, Department of Pathophysiology and Transplantation, University of Milan, and Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico; Laboratory G. A. Maccacaro, Department of Clinical Sciences and Community Health, University of Milan; Center for the Study of Myelofibrosis, Laboratory of Biochemistry, Biotechnology, and Advanced Diagnosis, IRCCS Policlinico San Matteo Foundation

Cytogenetic abnormalities have been reported in 30-70% of primary myelofibrosis (PMF) patients (pts) and specific alterations have been associated with worse outcome. We analyzed cytogenetic data at diagnosis in 395 out of 480 PMF pts to evaluate any possible association between karyotype and clinical phenotype and its impact on prognosis. All the cases were diagnosed at five Italian Hematological Centers between November 1983 and December 2016. G-banding technique were used and at least 20 metaphases analyzed. Bone marrow (BM) biopsies were reviewed to adhere to WHO 2016 criteria. An abnormal karyotype (AK) was found in 69 (17.5%) pts: 56 (81.2%) were sole abnormality, 10 (14.5%) double abnormalities, and three (4.3%) complex karyotype (of which one was monosomal karyotype).

Table 1. Presenting clinical and laboratory features of the 395 PMF patients according to karyotype.

	Total N=395	Abnormal N = 69	Normal N = 326	P value
Male gender, n (%)	204 (51.6)	49 (73.1)	155 (47.6)	<0.001
Age ≥ 65 years, n (%)	201 (50.9)	42 (60.9)	159 (48.8)	0.007
Pre-fibrotic PMF, n (%)	280 (70.9)	38 (55.1)	242 (74.2)	0.002
Overt fibrotic PMF, n (%)	115 (29.1)	31 (44.9)	84 (25.8)	
Hb < 10 g/dl, n (%)	53 (13.4)	20 (29.0)	33 (10.1)	<0.001
WBC ≥ 25 x 10 ⁹ /L, n (%)	13 (3.3)	1 (1.45)	12 (3.68)	0.345
PLT < 100 x 10 ⁹ /L, n (%)	12 (3.0)	7 (10.1)	5 (1.5)	0.001
Blasts > 1%, n (%)	28 (9.5)	13 (18.8)	15 (4.6)	<0.001
Splenomegaly, n (%)	178 (45.1)	41 (59.4)	137 (42.0)	0.008
Constitutional symptoms, n (%)	45 (11.4)	11 (16.2)	34 (10.4)	0.175
Driver mutation type:				0.955
CALR type 1, n (%)	50 (12.7)	8 (11.6)	42 (12.9)	
CALR type 2 or other / JAK2V617F / MPL, n (%)	312 (79.0)	55 (79.7)	257 (78.8)	
Triple-negative, n (%)	33 (8.4)	6 (8.7)	27 (8.3)	
IPSS risk category:				<0.001
Low, n (%)	141 (35.7)	15 (21.7)	126 (38.7)	
Intermediate-1, n (%)	186 (47.1)	31 (44.9)	155 (47.6)	
Intermediate-2 / High, n (%)	68 (17.2)	23 (33.3)	45 (13.8)	

Abbreviations: PMF: primary myelofibrosis; Hb: hemoglobin; WBC: white blood cells; PLT: platelets; IPSS: international prognostic scoring system.

Table 1 reports clinical and laboratory features of PMF pts according to AK and normal karyotype (NK) status. AK clustered differently according to BM fibrosis grade as it was found in 31 (27.0%) cases with overt fibrotic and 38 (13.6%) with pre-fibrotic PMF (p=0.001). Male sex, older (>65 years) age, anemia (Hb <10 g/dl), thrombocytopenia (PLTs <100x10⁹/L), circulating blasts >1%, splenomegaly and higher

LDH were significantly associated with AK. A significant association was also found between higher IPSS and AK (p<0.001). Concerning driver mutations, chromosomal abnormalities were described in eight (16.0%) out of 50 type 1 CALR-mutated, in 55 (17.6%) out of 312 non-type 1 CALR / JAK2 or MPL-mutated, and in six (18.2%) out of 33 triple-negative pts. After a follow-up of >6 years, 101 deaths (25.6%) were recorded. Survival was different between AK and NK patients with an estimated median OS of 8.9 and 25.7 years, respectively (p=0.015). Blast phase (BP) transformation occurred in 20 (5.1%) pts and 82 (20.8%) suffered from thrombosis. Any relationship between karyotype and BP, nor between karyotype and thrombotic events was observed. In conclusion, around 20% of pts showed an AK, with the latter clustering more frequently in pts with an advanced BM fibrosis grade and clinical-laboratory features indicative of a more aggressive disease. As in secondary myelofibrosis (MYSEC project), any significant difference in AK distribution according to driver mutations was found. This present study showed that an AK confers a more severe clinical phenotype and significantly influenced OS, thus representing an additional tool to be considered in the evaluation of PMF prognosis.

P151

DO DIRECT ORAL ANTICOAGULANTS PROVIDE A LIMITED PROTECTION AGAINST RECURRENCES IN MPN PATIENTS WITH VENOUS THROMBOEMBOLISM?

V. De Stefano¹, S. Betti¹, T. Za¹, A. Ciminello¹, F. Bartolomei¹, F. Di Landro¹, P. Chiusolo¹, E. Rossi¹, T. Barbui²

¹Istituto di Ematologia, Università Cattolica, Fondazione Policlinico A. Gemelli IRCCS; ²Fondazione FROM, Ospedale Papa Giovanni XXIII

In patients with Ph-negative myeloproliferative neoplasms (MPN) and venous thromboembolism (VTE) long-term treatment with vitamin K-antagonists (VKA) is suggested. However, there is concern about the hemorrhagic risk, being the MPN pts. more prone to bleeding. In non-cancer and non-MPN pts. with VTE direct oral anticoagulants (DOACs) are suggested over VKA, because of a non-inferior efficacy and a lower hemorrhagic risk. Efficacy and safety of DOACs in the MPN pts. are unknown. We analysed the medical records of 13 pts. (M/F 8/5, median age at diagnosis 66 yrs, range 37-82) with MPN (polycythemia vera=7, essential thrombocythemia=4, myelofibrosis=2) receiving DOACs after VTE. Thrombosis involved leg veins (n=6), splanchnic veins (n=3), cerebral veins (n=2), and pulmonary arteries (n=2). Eleven pts. took rivaroxaban, and 2 apixaban; circulating levels were checked once in all pts. and were found at therapeutic levels. Five pts. had been shifted from VKA, and 8 received DOACs as first therapy. The median time on DOACs was 18 months (range 1-39), for overall 19.8 pt-yrs, and 14.5 months (range 1-39) in the first-users. Eleven pts. took hydroxyurea. Three recurrent thromboses were recorded in first-users of DOACs: 1 pulmonary embolism 1 month after leg deep vein thrombosis (DVT) (progression), 1 cerebral vein thrombosis (CVT) 21 months after leg DVT, and 1 TIA 18 months after splanchnic vein thrombosis (SVT). A fourth patient with previous CVT was shifted to DOACs after 18 years on VKA because of intracranial hemorrhage and developed a SVT 27 months later. No major bleeding was recorded on DOACs; a clinically relevant non-major bleeding (epistaxis) was recorded in the only patient receiving also aspirin. The incidence of thrombosis on DOACs resulted 20 per 100 pt-yrs (95%CI 5.4-51.2), with a cumulative probability at 2 years of 36.7% (95%CI 1.5-71.8). This compares unfavourably with the 10.7% cumulative probability of thrombosis at 2 years recorded on VKA in pts. with MPN and VTE (Leukemia 2016;30:2032). Obvious limitations of this study are the small number of pts., the short duration of exposure to DOACs, and the retrospective design, and no firm conclusion can be reached. However our data induce some caution in prescribing DOACs after VTE in MPN pts., particularly after CVT and SVT. Those findings urgently call for a multicenter study aimed to assess efficacy and safety of DOACs in this setting on a statistically powered patient sample size.

P152

ARTERIAL THROMBOSIS IN YOUNG PATIENTS WITH POLYCYTHEMIA VERA

G. Bogoni, I. Bertozzi, G. Biagetti, F. Fabris, M.L. Randi

Dept of Medicine, DIMED, University of Padua, Italy

Polycythemia vera (PV) is a myeloproliferative neoplasm occurring in median advanced age but rarely in young patients. PV natural history is marked by vascular complications, but little is known about thrombotic events in young patients. This study evaluates arterial thrombotic complications in this setting. Out of 204 consecutive patients (105 males, 99 females) diagnosed with PV in our Department in agreement with WHO 2016 criteria, 38 patients (18.6%, median FU 12.42 y, 02-43.5) were younger and 166 (81.4%, median FU 5.95 y, 0.02-30.2) were older than 45 y at diagnosis [≤ 45 grp; >45 grp], respectively]. In all patients the presence of cardiovascular risk factors (CVRF) and thrombotic events were recorded. They received low dose aspirin, phlebotomies to maintain hematocrit below 45% and cytoreduction when indicated. The Fisher's exact test or the χ^2 test were used to compare categorical variables among the different categories. Comparison of incidence rate (IR) of thrombosis among categories have been performed with incidence rate ratio (IRR). A two-tailed P value of less than 0.05 was considered significant. CVRF were present in 145 patients (76% of >45 grp and 50% of ≤ 45 grp, $p < 0.01$). Arterial thrombosis occurred in 6 (15.8%) of ≤ 45 grp and in 33 (19.9%) of >45 grp ($p = NS$), among them CVRF were present in 4 (66.6%) and 29 (87%) respectively ($p = NS$). Arterial thrombotic incidence rate was 2,13% pt-ys in ≤ 45 grp with CVRF and 1,72% pt-ys in ≤ 45 grp with no CVRF, with an IRR of 1,2. In >45 grp we found IR=3,49% pt-ys and IR=2,21% pt-ys in those with and without CVRF respectively, with an IRR of 1,6. Comparing patients with CVRF, age appeared as an independent arterial thrombosis risk factor (IRR=2,8). CVRF are more common in older patients and seem to increase the arterial thrombotic risk in this group. In contrast, the presence of CVRF seems to have a small significance in younger patients, representing PV a risk factor per se for arterial thrombosis. The retrospective design of the study and the small number of young patients require larger studies to confirm our findings.

P153

UNDERSTANDING THE ROLE OF INFLAMMATION IN MYELOFIBROSIS AND THE RESPONSE TO RUXOLITINIB: A SINGLE CENTRE EXPERIENCE

S. Tomassetti, F. Rotondo, A. Merli, G. Tolomelli, A.M. Mianulli, M. Imola, A.L. Molinari, P. Tosi

U.O. Ematologia Ospedale Infermi, Italy

Background/Aim: Chronic myeloproliferative neoplasms are characterized by clonal hematopoiesis and persistent inflammatory reaction. This report aims to investigate clinical associations of inflammatory biomarkers in patients with myelofibrosis (MF) treated with Ruxolitinib (RUX).

Patients and Methods: We retrospectively analyzed a cohort of 23 myelofibrosis patients treated with RUX and we compared responsive patients to resistant ones in terms of Neutrophil-to-lymphocyte-ratio (NLR), Platelet-to-lymphocyte-ratio (PLR) and C-reactive protein (CRP) in addition to other clinical and disease specific parameters.

Results: Median age at diagnosis was 72 years (range 53-82) and male/female ratio was 8/15. Median follow-up from MF diagnosis was 23 months (range 1-167) and median RUX exposure was 9 months (range 1-28). At 6 months from RUX start, 9 patients achieved a spleen response (39%) according to the 2013 IWG-MRT criteria. At diagnosis NLR, PLR and CRP mean values in responsive group were not statistically significantly different from the group of resistant patients. After 6 months of treatment NLR mean value showed a significant decrease exclusively among responders: 10.41 (SD 10.98) at diagnosis versus 3.8 (SD 1.90) after 6 months ($p = 0.05$). Moreover at 6 months resistant patients had a significantly higher increase in CRP mean level: 5.26 (SD 6.32) at diagnosis versus 13.81 (SD 15.52) after 6 months ($p = 0.026$). No significant differences have been found in PLR levels between the two groups in any time points of RUX therapy. The 23 patients have also been evaluated for other clinical and laboratory features: IPSS/DIPSS, molecular status, allele burden, unfavorable karyotype, transfusion dependence, circulating blasts, grade of bone marrow fibrosis, BMI, constitutional symptoms, median time from diagnosis to RUX start,

survival, thrombotic and bleeding events: no correlations have been found between these variables and the above inflammation parameters.

Conclusions: despite the retrospective nature of our investigation, the small number and the high heterogeneity of observed cases, we can confirm the important role of chronic inflammation in MF and we can suggest a possible predictive value of inflammatory parameters in RUX responders. Larger prospective cohorts of patients are needed in order to validate these findings.

P154

COEXISTENCE OF CHRONIC MYELOID LEUKEMIA (CML) AND CALR-MUTATED CHRONIC MYELOPROLIFERATIVE NEOPLASM (MPN): A CASE REPORT AND LITERATURE REVIEW

F. Guidotti, V. Saccà, A. Gardellini, M. Zancanella, F. Alberio, M. Feltri, L. Ambrosiani, M. Turrini

Division of Hematology, Valduce Hospital, Como, Italy, Department of Pathology, Valduce Hospital, Como, Italy

Background: Cases of Ph+ and JAK2 MPN have been described in literature and a study confirmed the coexistence of JAK2 V617F mutation and BCR-ABL fusion gene in about 0.2-0.5% of patients, mainly in 2 different clones. Reports of MPNs with concurrent BCR-ABL translocation and CALR mutation are few and we have summarized them in Table 1. Case Report: A 80-year old man presented with leukocytosis (WBC $65.8 \times 10^9/L$) and thrombocytosis ($468 \times 10^9/L$): BCR-ABL fusion gene and Ph chromosome were demonstrated in peripheral blood. A diagnosis of CML, intermediate Sokal risk, was made and imatinib 400 mg/d started. After 3 months of therapy, despite a normal WBC count, platelets were $1563 \times 10^9/L$. Hydroxyurea was started and bone marrow biopsy performed: marrow cellularity was 80% with markedly increased myeloid to erythroid ratio and some myeloid shift; megakaryocytes in occasional loose clusters were increased with some atypical large forms with hyperlobated nuclei, some small forms with hypolobated nuclei and some micromegakaryocytes; reticular stain showed 1+ fibrosis. He had obtained a partial cytogenetic response but BCR-ABL transcript level was 22.31%IS. Considered the apparent hematological failure and molecular warning, BCR-ABL mutational status was performed revealing the presence of 2 aminoacidic variants (E499E, M388I) and the patient was switched to second line therapy with Bosutinib. At the same time, he was further investigated: while JAK2 V617F mutation was negative, CALR resulted mutated (type 1) demonstrating the coexistence of CML and a Ph-MPN. The patient rapidly obtained molecular response and continued HU in order to maintain platelets $< 450 \times 10^9/L$. Discussion: The coexistence of different molecular defects may change the clinical and laboratory manifestation of MPN and may result in an inappropriate interpretation of TKI response in CML patients: CHR still represents a milestone in confirming optimal response, and this case highlights the need to pay particular attention to avoid an early switch. Here, together with a strange timing of thrombocytosis, bone marrow morphology and in particular the presence of large atypical megakaryocytes with fibrosis, was an initial clue warranting additional investigations. To date, there are no data concerning impact on survival but the coexistence of a concurrent JAK2/MPL/CALR clone may have implications on response to a TKI in the treatment of CML and may contribute to worse disease outcome.

Table 1.

	First diagnosis	Second diagnosis	CALR mutation	Time to second diagnosis
Bonzheim et al, 2015	ET	CML	Type 2	3 years
Cabagnols et al, 2015	CML	PMF	Type 1	6 months
Dogliotti et al, 2017	CML	ET/pre-fibrotic PMF	Type 1	14 years
Loghavi et al, 2015	CML	PMF	Type 1	7 months
Diamond et al, 2016	PMF	CML	Type 1	4 years
Seghatoleslami et al, 2016	MPN + CML blast crisis		Type 1	0
Lawandowski et al, 2017	CML	MPN	Type 1	10 years
Boddu et al, 2018	PMF	CML	Type 2	2.5 years
Boddu et al, 2018	CML + PMF		Type 1	0
Xie et al, 2018	ET	CML	Type 1	4 years

P155

CLINICAL AND PROGNOSTIC FEATURES OF ESSENTIAL THROMBOCYTHEMIA: COMPARISON OF WHO 2001 VERSUS WHO 2008/2016 CRITERIA IN A LARGE SINGLE CENTER COHORT

S. Chiatamone Ranieri, M.A. Arleo, S. Trasarti, L. Bizzoni, I. Carosino, M.L. De Luca, S. Mohamed, E. Mariggio, E. Scalzulli, D. De Benedittis, G. Colafigli, M. Molica, L. Rizzo, A. Ferretti, E. Baldacci, M. Mancini, C. Santoro, M. Breccia, M. Vignetti, R. Latagliata

Department of Translational and Precision Medicine, "Sapienza" University of Rome, Policlinico Umberto I, Rome, Italy

According to the World Health Organization (WHO) 2008/2016 classification, a platelet (PLT) count $\geq 450 \times 10^9/l$, thus reduced from the previous WHO 2001 level $\geq 600 \times 10^9/l$, was considered the new PLT threshold for the diagnosis of Essential Thrombocythemia (ET). To validate in a setting of current clinical practice this important diagnostic change, clinical and hematological features at diagnosis and during follow-up of 219 patients with ET according to WHO 2008/2016 criteria, diagnosed in our center from 1/2008 to 12/2017, were retrospectively analyzed. Patients were divided into Group A (G-A) (PLT $\geq 600 \times 10^9/l$ at diagnosis) (167 patients - 76.3%) and Group B (G-B) (PLT $\geq 450 \times 10^9/l$ < $600 \times 10^9/l$ at diagnosis) (52 patients - 23.7%) and compared for clinical features at onset, and during the clinical course. Main clinical features at diagnosis of the entire cohort as well as of G-A and G-B are reported in the Table 1.

Table 1. Clinical features at presentation in the whole cohort and according to PLT number at diagnosis.

	All Patients	Group A (PLTs > 600 x 10 ⁹ /l)	Group B (450 X 10 ⁹ /l PLTs < 600 x 10 ⁹ /l)	P
Patients n° (%)	219	167 (76.3%)	52(23.7%)	
M/F, n° (%)	81/138 (37/63)	66/101 (39.5/60.5)	15/37 (28.8/71.2)	0,164
Median Age, years (RIQ)	63.3 (48.1-70.4)	63.9 (48.0-70.8)	61.5 (50.1-69.3)	0,449
JAK-2 V617F mut(%)	130(59.4)	96(57.5)	34(65.4)	0,421
WHO Histology TE (%)	162 (74)	124(74.3)	38(73.1)	0,866
Early-PMF(%)	57(26)	43(25.7)	14(26.9)	
Median Hb, g/dl (RIQ)	14.1 (13.1-15.0)	14.1 (13.1-15.0)	14.0 (13.5-14.6)	0,975
Median Hct, % (RIQ)	42.0 (39.7-45.0)	41.9 (39.5-45.2)	42.0 (40.3-44.0)	0,807
Median WBC, x 10 ⁹ /l (RIQ)	8.83 (7.34-10.3)	9.07 (7.53-10.4)	7.53 (6.1-9.88)	0,01
Median Cholesterol, mg/dl (RIQ)	191 (168-220)	189 (164-217)	202 (179-234)	0,260
Median triglycerids, mg/dl (RIQ)	102 (75-150)	103 (75-151)	97 (67-149)	0,8760
Ipertension, Yes/No (%)	104/115 (47.5/52.5)	80/87 (47.9/52.1)	24/28 (46.2/53.8)	0,825
Diabetes, Yes/No/Na (%)	5/193/21 (2.3/88.1/9.6)	4/143/20 (2.4/85.6/12)	1/50/1 (1.9/96.2/1.9)	0.114
Smoking history Yes/no/Na (%)	79/107/33 (36.1/48.8/15.1)	64/82/21 (38.4/49.0/12.6)	15/25/12 (28.9/48.1/23.0)	0,262
History of thrombosis yes/no (%)	37/182 (16.9/83.1)	28/139 (16.8/83.2)	9/43 (17.3/82.7)	0,928

Clinical and Prognostic Features of Essential Thrombocythemia: Comparison of Who 2001 Versus Who 2008/2016 Criteria in a Large Single Center Cohort

Among clinical features, only median value of leukocytes was significantly higher in G-A [$9.07 \times 10^9/l$, interquartile range (IQR) 7.53-10.4 vs $7.53 \times 10^9/l$, IQR 6.1-9.88; $p=0.01$]. Cytostatic treatment was administered in 148 patients (67.6 %) of entire cohort at different intervals from diagnosis, with a significantly higher rate in patients of G-A (74.3% versus 46.2%, $p<0.001$). After a median follow-up of 37.5 months (IQR 19.8 - 60.7), 10 thrombotic events (4.6%) were recorded in the entire cohort, without difference between G-A and G-B [3.6%] in the G-A and 4 (7.7%) in the G-B, $p=0.216$]. No patient evolved in myelofibrotic phase, 1 patients evolved in blastic phase (BP) after 42 months in the G-B. At the last follow-up, 4 patients (1.8%) died (1 from BP, 1 from cerebral hemorrhage, 2 from unavailable cause), 10 (4.5%) were lost to follow-up and 205 (93.7%) are still alive and currently followed at our Institute. The 5-year Overall Survival (OS) of the entire cohort was 96.2% (95%CI 92.3 - 100), without differences between the two groups [96.3% (95%CI 92.0 - 100) in the G-A versus 96.7% (95%CI 87.3 - 100)

in the G-B, $p=0.819$]. Our data indicate a substantial homogeneity in terms of clinical presentation, risk of thrombosis and survival among ET patients regardless of the PLT number at diagnosis, thus confirming the usefulness of 2008/2016 WHO diagnostic criteria. However, it can not be excluded that the most aggressive treatment in patients with higher PLT count at diagnosis may have reduced any differences between the two groups.

P156

SPLANCHNIC VEIN THROMBOSIS (SVT) IN MYELOPROLIFERATIVE NEOPLASMS.

V. Accurso¹, M. Santoro¹, A.D. Contrino¹, M. Sardo¹, S. Raso¹, F. Di Piazza², A. Perez², A. Russo², S. Siragusa¹

¹Ematologia A.O.U.P Paolo Giaccone, ²Oncologia Medica A.O.U.P Paolo Giaccone, Italy

Myeloproliferative neoplasms (MPNs) are the most common cause of splanchnic vein thrombosis (SVT). In this analysis we determined the prevalence and the features of splanchnic vein thrombosis (SVT) in MPNs. From 2010 to 2018 our center has collected 514 cases of Philadelphia-negative Myeloproliferative Neoplasms (MPNs). 278 are females and 236 males. The median age is 65.71 years (14.5 - 93.7). 196 ET, 143 PV, 141 MF (31 PREMF) have been identified. There were 129/514 cases of thrombosis or cardiovascular events at diagnosis or during follow up (25,10%), 20/129 (15,5%) cases are defined as splanchnic venous thrombosis (SVT). 13 are portal thrombosis and splenic thrombosis. 4 mesenteric thrombosis and portal vein, 2 are thrombosis of the isolated mesenteric vein, finally 1 suprahepatic vein thrombosis (Budd-Chiari Syndrome). There were no significant differences in the mutational state between the SVT and the remaining thrombosis. 9/20 SVT occurred in patients with polycythemia vera. It is interesting to note in our experience that the median age of SVT patients is significantly lower than in patients with other thromboses: 55.33 years versus 65.92 ($p=0.003$). **Conclusions:** MPN is the most important risk factor for SVT, In our experience the presence of SVT correlates with the young age.

P157

MYELOPROLIFERATIVE NEOPLASMS IN THE YOUNG: OUR EXPERIENCE WITH 108 PATIENTS AGE < 51 YEARS .

V. Accurso¹, M. Santoro¹, A.D. Contrino¹, M. Sardo¹, S. Raso¹, F. Di Piazza², A. Perez², A. Russo², S. Siragusa¹

¹Ematologia A.O.U.P Paolo Giaccone, ²Oncologia Medica A.O.U.P Paolo Giaccone Palermo, Italy

From 2010 to 2019 our center has collected 515 cases of Philadelphia-negative Myeloproliferative Neoplasms (MPNs). Data from these patients were collected in a specific database and analysed with simple statistic tools. 108 patients with myeloproliferative neoplasms (MPN), age <51 years, were seen, constituting 20,97% of all MPN patients seen during the same time period disease-specific incidences were 27,77% in polycythemia vera (PV; n = 30), 38,88% in essential thrombocythemia (ET; n = 42) and 13,88% in primary myelofibrosis (PMF; n = 15). 8,33% in pre-fibrotic/early PMF (pre-MF; n=9). Compared to patients ≥ 51 years, younger patients were more likely to present with low risk disease ($p<0.001$) and display female preponderance in ET ($p=0.05$). Younger patients were also more likely to express CALR mutations, (11,21% in younger and 7,16% in older patients $p=0.04$). No difference has been noted in the frequency of cardiovascular events in the two groups of patients, but in our experience, younger patients display higher incidence of splanchnic veins thrombosis (SVT) (34,61% versus 10,68% in older patients $p=0.005$). Median survival in our younger patients is significantly higher and perhaps for this reason there is a greater incidence of evolution in myelofibrosis and leukemic transformation. Young MPN patients comprise a unique disease subset defined by mutational backdrop and conspicuously longer survival compared to their older counterparts

P158

YOUNG MPNS PATIENTS: CLINICAL CHARACTERISTIC AND OUTCOMES

N. Pugliese, A. Leone, S. Vitiello, I. Cappuccio, C. Cimmino, G. Delle Cave, F. Fanelli, A. Giordano, V. Marafioti, G. Piccolo, V. Martinelli, F. Pane

Università degli Studi Di Napoli Federico II, Dipartimento di Medicina Clinica e Chirurgia, Italy

Introduction: Natural history of patients with Ph-negative MPNs (i.e. Polycythemia Vera (PV), Essential Thrombocythosis (ET), Primary Myelofibrosis (PMF) and Prefibrotic Myelofibrosis (pre-MF) is characterized by an increased risk for long-term complications including thrombotic events, overt myelofibrosis and leukemic transformation. The median age at diagnosis ranges between 56 to 70 years. Younger patients with MPNs show different clinical features and CV risk factors. However, optimal treatment, disease course and clinical characteristics of these patients are still not well defined. In this study we describe the clinical features and outcome of MPN patients younger than 45 years.

Methods: A monocentric retrospective study, including patients younger than 45 years, diagnosed with MPNs between 1985-2018.

Results: 92 young MPN patients were included with a median age was 34.7 years (range 5-44), 48 (52.2%) were females. Among entire study population, 20 were PV (21.7%), 70 were ET (76.1%) and 2 were pre-PMF (2.2%), and 72 patients had received cytoreductive therapy. In 9 patients (9.7%) thrombosis preceded MPN diagnosis and unusually, in all of them thrombotic events were arteriosus. After a median follow-up of 8.7 years (range 1-27.8), 6 patients (6.5%) had at least one thrombotic event: 2 of them were treated with hydroxyurea, 4 with interferon as cytoreductive therapy and all patients had received antiplatelet therapy at the time of thrombosis. Thrombotic events were equally distributed between venous and arteriosus. In a univariate analysis, MPN related features or thrombotic risk factors were unable to predict thrombosis. Two patients (2.2%) developed post ET-MF and none experienced evolution to AML. Two patients (2.2%) experienced secondary neoplasia.

Conclusions: In young adults the risks of thrombosis and progression to MF/leukemia appear reduced when compared to older population. Thrombotic events are frequently a presenting sign with a higher incidence of arteriosus event. In our study we didn't find any factor able to predict a worse prognosis in these patients, maybe due to the low rate of observed events and the concomitant spreading use of cytoreductive therapy in our cohort.

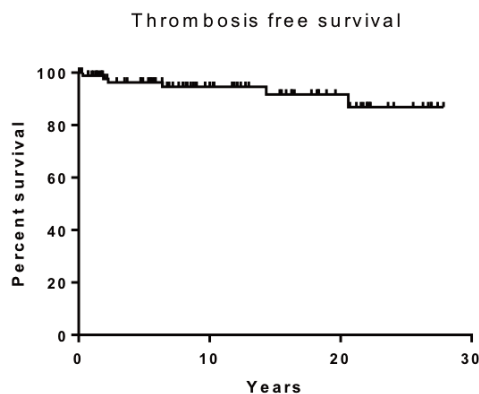


Figure 1.

Myeloproliferative Neoplasms 3

P159

THE MYELOPROLIFERATIVE NEOPLASMS UNCLASSIFIABLE: A SINGLE CENTER EXPERIENCE

V. Accurso¹, M. Santoro¹, A.D. Contrino¹, M. Sardo¹, S. Raso¹, F. Di Piazza², A. Perez², A.M. Florena³, A. Russo², S. Siragusa¹

¹Ematologia A.O.U.P Paolo Giaccone; ²Oncologia Medica A.O.U.P Paolo Giaccone; ³U.O.C. Anatomia Patologica A.O.U.P Paolo Giaccone Palermo, Italy

Background: According to the WHO classification, the designation of myeloproliferative neoplasms un-classifiable (MPN-U) should be used for cases with clinical, morphologic, and molecular features of MPN failing to meet the diagnostic criteria of aspecific entity. Reported incidence of MPN-U varies significantly in different studies with a range up to > 20%. However, most studies show an incidence of 10-15% or even less. When the 2016 WHO criteria have been applied, the incidence is reduced to <5%. **Aims:** In this report we study the clinical and laboratory characteristics of 34 consecutive cases of un-classifiable myeloproliferative disease afferent to the center for myeloproliferative diseases of our Hematology Unit.

Methods: From 2010 to 2018 our center has collected 514 cases of Philadelphia-negative Myeloproliferative Neoplasms (MPNs). Data from these patients were collected in a specific database and analysed with simple statistic tools.

Results: Among these 514 patients, 34 (6.61%) had a diagnosis of MPN-U, 17 male and 17 female. Median age at diagnosis was 60.78 years. 24/34 (70.58%) were JAK2 V617F positive, 2/34 were CALR mutated (5.88%), 2/34 (0.5,88%), MPL mutated and 6/34 (17.64%) resulted as triple negative (TN) for driver mutations. Data from the other groups of MPNs (PV, ET, overtMF and preMF) were analysed as well and compared to the ones from the MPN-U group.

Summary/Conclusions: Chronic myeloproliferative diseases unclassifiable (MPN-U) represent the 6.61% of the cases relating to our center. No significant difference at diagnosis has been highlighted regarding sex, age and mutational status between MPN-U and MPN classified according to WHO 2016. The exact incidence of MPN-U is unknown. It is probable that frequency varies significantly according to the experience of the diagnostician and the specific classification system and criteria used. It is possible that careful use of the WHO 2016 classification may significantly reduce the frequency of MPN-U.

P160

CARLETICULIN GENE MUTATIONS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA. A SINGLE CENTER STUDY

V. Accurso¹, M. Santoro¹, A.D. Contrino¹, M. Sardo¹, S. Raso¹, F. Di Piazza², A. Perez², A. Russo², S. Siragusa¹

¹Ematologia A.O.U.P Paolo Giaccone; ²Oncologia Medica A.O.U.P Paolo Giaccone, Italy

JAK2 (V617F) gene mutation is found in approximately 60% of patients with Essential Thrombocythemia, while 5-10% of JAK2 (V617F) negative ET patients carry MPL gene mutations. Mutations at the exon 9 of calreticulin gene have been identified in approximately 50% of patients with ET, unmutated for Jak2 and MPL. Primary aim of the current study was to evaluate the impact of gene mutations on clinical features of ET at diagnosis. A cohort of consecutive patients with a diagnosis of ET followed between January 2010 and June 2016 were considered. JAK2 (gene mutation was detected by PCR testing; MPL and CALR mutations were analyzed by direct sequencing methods. Thrombotic risk score was calculated according to ELN recommendations. Overall, 187 patients were included: 132 (70,59%) had JAK2 (V617F) gene mutation (JAK2+), 20 (10,7%) carried a mutation at exon 9 of CALR gene (CALR+), 4 (2,4 %) carried a mutation at codon 515 of MPL gene, 31 (16,58%) patients were not mutated for JAK2, CALR and MPL genes (triple negative). CALR+ subjects, compared to JAK2+ patients, had a younger age at diagnosis: median 61,5 year (24-87) in CALR+ patients vs 65 years (18-93, respectively). Patients with MPL mutation had a median age of 78 years while triple negative subjects had

a median age of 59 years (23-89). The mean score for thrombotic risk was 0 in CALR+ patients and 1 in JAK2+, MPL+ and triple negative patients. The distribution of International Prognostic Score for Essential Thrombocythemia (IPSET) categories was also statistically significantly different ($p=0.003$) for the three groups. The percentage of high-risk patients was 0 in CALR+ (0/12) group, 25, 60% (27/107) in JAK2+ group, and 18, 30 % (5/26) in the triple negative group. The IPSET model also stratified patients with statistically significant difference ($p=0.001$) among the three groups: the percentage of high-risk patients was 16, 66 (2/12) in the CALR+ group, 82, 35% (88/107) in the JAK2+ group, and 33, 33(9/29) in triple negative group. CALR+ patients belonged more frequently to the low /intermediate risk group than JAK2 +patients (80% versus 17, 5%, $p=0.05$). The incidence of thrombotic events at diagnosis of ET was 0 in the CALR+ group, 28,03% (30/107) in the JAK2+ group and 23,07% (6 /26) in the triple negative group. The median overall survival was not reached in any group. CALR+ patients with ET are phenotypically distinct from JAK2 + and triple negative patients. We can speculate a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET high-risk patients.

P161

THROMBOTIC RISK IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA WITH AGE LESS THAN OR EQUAL TO 60 YEARS

V. Accurso, M. Santoro, A.D. Contrino, M. Sardo, S. Raso, G. Tarantino, S. Siragusa

Ematologia A.O.U.P Paolo Giaccone, Italy

Current treatment in essential thrombocythemia (ET) and Polycythemia vera (PV) is primarily indicated for the purposes of preventing thrombotic complications, which might occur in 15–25% of patients. The traditional thrombotic risk stratification system, for patients with essential thrombocythemia and polycythemia vera, provides a high-risk and a low-risk category. The high-risk group includes patients over the age of 60 or previous thrombosis, low-risk group includes patients aged 60 years or less and without previous thrombosis. The aim of this study is to evaluate the frequency of patients with low thrombotic risk in a population of patients with essential thrombocythemia and with polycythemia vera and the frequency of thrombosis in patients aged 60 years or less. From 2010 to March 2019 were collected at the center for myeloproliferative diseases of our division of hematology of the polyclinic of Palermo 196 patients with ET (136 females and 60 males) median age 67.57 years (14.5 - 92.3) and 143 patients with PV (52 females and 91 males) with median age 62, 7 years (17.5 - 93.4). In the ET group the mutational state showed 137 patients with the V617f mutation of Jak2, 21 with Calr mutation, 4 with MPL mutation and 34 triple negative (TN). Patients with PV have the V617f mutation of Jak2 in 134 patients, in 4 patients the mutation of exon 12 of JAK2 is detected and 5 are TN. In the group of patients with ET at diagnosis 141 are at high thrombotic risk and 55 at low risk, in the group with PV 104 are high-risk and 39 low-risk. Patients with ET \leq 60 years are 66 (33.67%), the patients with PV \leq 60 years are 64 (44.76%). 14 patients / 66 \leq 60 years with ET show at diagnosis or subsequently, thrombosis (21.21%), while in patients with PV \leq 60 years thrombosis occur in 23/64 patients (35.94%). Current guidelines do not provide cytoreductive therapy in patients with low thrombotic risk, but as our experience shows a significant proportion of these patients will develop thrombosis; this occurrence poses significant therapeutic implications.

P162

LOW-DOSE ASPIRIN AND INCREASED THROMBOXANE IN PATIENTS WITH POLYCYTHEMIA VERA

E. Cacciola, E. Gentilini Cacciola, V. Vecchio, R. Cacciola

Haemostasis Unit, Department of Medical, Surgical Sciences, and Advanced Technologies "G.F. Ingrassia"; University of Catania, Haemostasis Unit Department of Clinical and Experimental Medicine, University of Catania, Italy

Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by increased thromboxane (TX) production and thrombotic risk. It is reported that serum TXB2 concentrations in PV patients are twofold higher than healthy controls and that low-dose aspirin (ASA) therapy reduces the risk of major vascular events by 50 to 60%. This

"window therapeutic" may be a consequence of extraplatelet reduced effect of ASA, particularly in the vessel wall. Therefore, we evaluated platelet count, hematocrit (HCT), β -thromboglobulin (β -TG) and platelet factor 4 (PF4), as markers of platelet activation, TXB2, as primary indicator of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity, and tissue factor (TF), as indicator of vascular activation. We studied 60 patients (38 men, 22 women; mean age 51 years, range 32-70) with PV according to WHO criteria. The mean duration of disease was 12 years. All patients were on ASA 100 mg once daily. All patients were on phlebotomy. None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelet count and HCT were measured by automated analyzer. β -TG and PF4 were determined by ELISA. TXB2 was measured by radioimmunoassay technique. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100). TF was measured by ELISA. The mean platelet count was $430 \pm 170 \times 10^9/L$. The mean HCT value was $42 \pm 3\%$. All patients had high β -TG and PF4 (110 ± 45 IU/ml vs 20 ± 11 IU/ml and 45 ± 21 IU/ml vs 6 ± 2 IU/ml, respectively) ($p < .0001$ and $p < .0001$, respectively), high TXB2 levels (1.700 ± 1.990 nmol/L vs 800 ± 280 nmol/L) ($p < .0001$), shortened C/EPI closure time (T, unit: s, n.v. 84-160 s) (55 ± 10 s), high TF (250 ± 180 pg/ml vs 4.8 ± 2.5 pg/ml) ($p < .0001$). These findings might provide a rationale to assess a novel dosing strategy that may improve the efficacy of ASA in PV.

P163

CLINICAL BENEFIT OF AZACITIDINE IN PATIENTS WITH MYELOPROLIFERATIVE NEO-PLASMS IN ACCELERATED PHASE

G. Guaragna, E. Rinaldi, M. Girasoli, A. Spina, M.R. Coppi, M.C. Brocca, D. Pastore

UOC Ematologia - Ospedale "A. Perrino", Bari, Italy

Myeloproliferative neoplasms (MPNs) in accelerated phase (MPNs-AP), are associated with a poor response to therapy and very short survival. Several reports have suggested clinical activity of hypomethylating agents in these patients. We conducted a retrospective data collection of 8 consecutive patients with MPNs-AP treated with azacitidine at our institution and evaluated their clinical outcomes. We identified 5 patients with initial myelofibrosis (DIPSS high risk in 4 patients and Intermediate-2 risk in 1 patient), 2 patients with initial Essential Thrombocythemia and 1 patient with initial Polycythemia Vera who were treated with azacitidine 75 mg/m² per day for 7 consecutive days every 28 days. MPN-AP was defined as MPN with 10%–19% blasts in the peripheral blood or bone marrow (BM). Median age at presentation was 71.2 years (range 65-79), 4 males and 4 females (1:1), karyotype was abnormal in 1 pts (complex), not done for puntio sicca in 4 patients and normal in the other ones. All patients were previously treated with hydroxyurea, except for 1 with myelofibrosis treated with ruxolitinib. Median number of cycles was 8.6 (2-27). The median overall survival (OS) was 11.5 months (range 3-44) and actually 3 patients are alive and still receiving azacitidine. Azacitidine was generally well tolerated by patients with MPNs-AP and demonstrates potentially promising clinical activity. Prospective clinical studies combining azacitidine alone or with other clinically active agents are needed to improve overall outcome.

P164

NEXT GENERATION SEQUENCING IN ESSENTIAL THROMBOCYTHEMIA

M. Santoro, G. Costanza, A.D. Contrino, F. Di Piazza, A. Perez, M. Sardo, V. Accurso, A. Russo, S. Siragusa

Dept. of Surgical, Oncological and Stomatological Disciplines, University of Palermo, Italy; Hematology Division University Hospital Policlinico "Paolo Giaccone", Palermo, Italy, Palermo, Italy; Department of Surgical, Oncological and Stomatological Disciplines, Section of Medical Oncology, University of Palermo, Palermo, Italy

Next generation sequencing has recently improved the diagnostic tools in oncology and hematology and has risen attention in the field of diagnosis of myeloproliferative neoplasms (MPN). Although triple-negative MPNs seem to be the most important field of application for NGS, using a myeloid-gene panel diagnostic tool, it is known that ad-

ditional genes mutations have a prognostic impact also in MPNs with known driver mutation. We used NGS to examine the molecular setting of genes involved in myeloid diseases of 14 patients diagnosed with low-risk ET between October 2017 and January 2019 and followed at our center. Samples used for the exam were portions of the peripheral blood samples obtained at diagnosis for conventional biomolecular study of JAK2-CALR-MPL asset. Between the 14 patients, we found 211 non-driver gene mutations. Of these, 24 were certain pathogenic mutations and 45 were probably pathogenic, while other mutations were classified as not surely associated with clonality and/or other hematologic malignancies. JAK2 V617F positive patients (7/14, 50%) carried the most of the non-driver mutations (45 mutations), CALR-mutated patients (2/14, 14%) carried 6 mutations, the only MPL-mutated patient carried 1 non-driver-gene mutation, while the 4 triple-negative patients carried 17 mutations in myeloid non-driver genes. If we just consider the adverse mutations, mutated non-driver genes were 16, of which the most commonly mutated were TET2 (10 cases, 71,4%), SH2B3 (9 cases, 64,3%) and ASXL1 (7 cases, 50%). Data from this study confirmed the known high frequency of mutations of TET2 and ASXL1 in ET patients. In triple negative patients, NGS with myeloid genes panel permitted to fit the diagnostic criteria revealing a biomolecular marker of clonality.

P165

POLYCYTHEMIA VERA AMONG BLOOD DONORS: PRELIMINARY RESULTS FROM A SINGLE-CENTER SCREENING STUDY

M. Santoro, E. Scirè, S. Rizzo, C. Rizzo, F. Di Piazza, A.D. Contrino, V. Accurso, A. Russo, S. Siragusa

Dept. of Surgical, Oncological and Stomatological Disciplines, University of Palermo, Palermo, Italy; Transfusional Medicine Unit, University Hospital "Paolo Giaccone", Palermo; Department of Surgical, Oncological and Stomatological Disciplines, Section of Medical Oncology, University of Palermo, Italy

Polycythemia Vera (PV) is a Philadelphia negative Myeloproliferative Neoplasm (MPN) characterized by trilinear myeloproliferation in bone marrow and high haematocrit values in peripheral blood at complete blood count. PV may be diagnosed in asymptomatic patients or present itself with systemic symptoms (due to cytokine release and blood hyperviscosity) and/or arterial or venous thrombosis. Virtually all patients with PV have a known mutation of JAK2. Patients with PV have moderate to high risk to develop a thrombotic complication (or to have a relapse if they already had one). Early diagnosis of PV in asymptomatic patients may reduce the risk of developing thrombosis applying the indicated prophylaxis with low-dose acetylsalicylic acid (ASA) and keeping the hematocrit level lower than 45% as for international guidelines. Blood donors referring to the Transfusional Medicine Unit of the University Hospital "Paolo Giaccone" in Palermo were screened for haematocrit values and people with HCT = or >50% on more than one examination were sent to hematologic evaluation between March 2018 and March 2019. 93 donors have been called for hematologic visit, of which 38 (40.4%) actually underwent the evaluation. Median age is 51 years and all the persons were male, reflecting the physiological prevalence of male population between donors and higher values of HCT in this sex. 33 (89.2%) were considered suitable for continuing blood donations. 3 (8.1%) patients were positive for JAK2 V617F and were diagnosed with PV according to the 2016 WHO diagnostic criteria. 1 patient was not diagnosed with a hematologic disease, but further examinations led to metabolic syndrome and was temporarily ruled out from the donors. Three patients were diagnosed with PV between the 37 donors with haematocrit higher than 50% that were screened. This is a result of note, because early diagnosis in this patients allowed us to prescribe ASA prophylaxis and phlebotomies with target HCT 45%, thus considerably reducing their risk for thrombosis complications. No female donor was screened, probably because a lower HCT value has to be chosen. We look forward to reach a higher number of screened donors to make data more suitable for further analysis.

P166

AGGRESSIVE B-CELL LYMPHOMA IN PATIENT WITH MYELOFIBROSIS RECEIVING JAK2/1 INHIBITOR RUXOLITINIB: A CASE REPORT

E. Rinaldi, G. Guaragna, G. Quintana, A. Spina, F. Merchionne, D. Pastore

Haematology, A. Perrino Hospital, Brindisi, Italy

Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) have a greater incidence of hematologic and non hematologic malignancies compared to the general population. In particular the association of MPN and lymphoproliferative neoplasms (LPN), coexistent or sequential, is known. Conversely, there are few data in literature about this association in the era of the JAK2 inhibitors. We report a case of a 60 years-old man referred to our institution with JAK2V617F mutated primary myelofibrosis intermediate-2 IPSS risk. At the time of diagnosis, CT scan demonstrated splenomegaly (30 cm) but not lymphadenopathy. Therefore the patient started treatment with JAK2 inhibitor ruxolitinib 20 mg BID. After six months, splenomegaly was reduced (19 cm) and B symptoms improved. The treatment with ruxolitinib was well tolerated, without hematological and not hematological adverse events. After 15 months the patient developed progressive anemia. Bone marrow biopsy confirmed myelofibrosis with grade 3 reticulin fibrosis (MF3) with normal male karyotype. FDG-PET detected as well as metabolically active multiple lymph nodes also hypermetabolic lung, liver and gastrointestinal lesions. Biopsy of abdominal lymph node showed infiltration of diffuse large B-cell lymphoma (DLBCL). On the basis of these findings, a diagnosis of stage IVA, IPI 4 DLBCL non GBC was established. Ruxolitinib was discontinued. The patient was treated with 6 cycles of CHOP and 8 cycles of rituximab obtaining a complete response with negative FDG-PET. The treatment was well tolerated, except an increased transfusion requirement. He did not present infectious adverse events. After 4 months he presented an early relapse with cerebral localization. He started therapy with high-dose cytarabine and high-dose methotrexate intravenously and died after 20 days for progression disease. Few experiments showed that lymphoid neoplasia can be related to pre-existing B-cell clones in patients with MPN and underlined the possible role of immunosuppression of JAK2 inhibition. Controlled studies are needed to better identify the population with MPN at high risk of developing LPD and to drive the choice of the therapy in this subset of patients.

P167

A RARE CASE OF HAIRY CELL LEUKEMIA IN ASSOCIATION WITH MAST-CELL LEUKEMIA

M.B. Giannini, D. Cangini, C. Cerchione, V. Di Battista, A. Lucchesi, G. Musuraca, M. Ceccolini, R. Sonia, G. Martinelli

IRST-IRCCS, Meldola, Italy

The association of systemic mastocytosis (SM) with a non-mast cell hematological neoplasm represents a subtype of mastocytosis known as systemic mastocytosis with associated hematological non-mast cell disease (SM-AHNMD). Only two cases of hairy cell leukemia (HCL) associated with systemic mastocytosis have been published in the literature. Our case: 65-year-old followed for ten years for small and stable MGUS IgG K. At the beginning of January 2018, the patient developed mild pancytopenia which progressively increased in subsequent months without, however, further signs or symptoms of hematological or non hematological malignancy. In July 2018, the number of platelets was 67000/mcL, neutrophils 1010/mcL, and spleen volume had increased. Under suspicion of chronic lymphoproliferative disease, the patient underwent bone marrow biopsy (BMB), bone marrow aspirate (BMA) and karyotype. The BMB gave a diagnosis of systemic mastocytosis with associated hematological neoplasm, morphologically and phenotypically compatible with hairy cell leukemia (SH-AHNMD). Bone marrow granulopoiesis showed 85% atypical and disgranulated mast cells, with diffuse infiltration of small lymphocytes and reniform and clear nuclei. Monoclonal plasmacells were < 10%. The karyotype showed 20 normal metaphases and neither PDGF receptor or c-KIT mutations were present. According to WHO 2016 classification, diagnosis was mast-cell leukemia (MCL) without C findings associated with HCL. Although this is a very aggressive form of systemic mast cell disease, the patient did not show conventional symptoms (hives, asthma, enteritis, gastritis, malignant hypertension, pathological fractures). CT

scans of the chest, abdomen and bone were negative. In September 2018, the patient underwent chemotherapy with chlorodesoxyadenosine 0.4 mg/m² for 5 days with good tolerance. Other courses of therapy were planned but suspended because the patient developed a lung infection. There was peripheral blood complete remission, while bone marrow showed only partial remission of HCL. Despite the same quantity of mast cells in bone marrow, the patient continues to be asymptomatic.

Conclusions: According to WHO 2016 classification, mast cell leukemia is a highly aggressive disease. However, in the case of our patient, who is still symptom-free 6 months after the first therapy, a wait-and-watch approach would seem to be adequate.

P168

A RARE CASE OF ACUTE PROMYELOCYTIC LEUKEMIA ORIGINATING FROM POST ESSENTIAL THROMBOCYTEMIA MYELOFIBROSIS

E. Morsia¹, K.B. Garvey¹, S. Rupoli², G. Micucci¹, A.R. Scortechini², S. Ceglie¹, A. Olivieri^{1,2}

¹Università Politecnica delle Marche; ²Ospedale Riuniti di Ancona, Italy

We herein report a promyelocytic blast crisis in a CALR-positive post essential thrombocytemia (ET) myelofibrosis (MF). A 69 years-old male was diagnosed with ET in 1994 and treated with hydroxyurea and then with pipobromano. In February 2016, splanchnic vein thrombosis (abdominal pain, ascites and increase in transaminase) and progression to overt MF were found (occurred). Molecular studies for JAK2 V617F mutation and BCR-ABL fusion gene were negative. Ruxolitinib and vitamin k antagonists were given with reduction of spleen dimension, improvement of systemic symptoms and partial portal recanalization. In 2018, as soon as CALR mutation was available in our center, we demonstrated the presence of a type-2 mutation. In January 2019, the patient was evaluated for microcytic anemia and thrombocytopenia. Peripheral blood cytomorphology showed 12% promyelocytes with multiple Auer rods, whereas bone marrow biopsy was markedly hypercellular with diffuse sheets of immature myeloid cells (CD117-, CD34-, MPO+, CD68, CD15-), increased proliferation of eosinophilic precursors and grade 2 fibrosis. Flow cytometry features were suggestive for acute promyelocytic leukemia (APL) and the diagnosis was confirmed by cytogenetic [(t15;17) in 20% of metaphases] and molecular studies (PLM-RARA rearrangement). At this time, CALR type-2 mutation was still positive (remained). Implementation of molecular genetic results derived from NGS are under way (ongoing). The patient started therapy with all-trans retinoid acid (ATRA) and arsenic trioxide (ATO), which resulted in normalization of blood cell counts and bone marrow morphology after 25 days. The patient is currently receiving a planned course of consolidation therapy. APL comprises 10% of cases of de novo acute myeloid leukemia (AML) and rarely can occur as a secondary leukemia after therapy for another malignancy. Moreover, this case was especially atypical because rare cases of APL have been reported in the literature as leukemic transformation (BP) of Ph-neg Myeloproliferative neoplasm (MPN). As CALR type-2 mutation was present both in the original chronic phase MPN and in BP, we discuss if that transformation may occur in a hematopoietic stem cell CALR type-2 mutated (ancestral to PML-RARA) or may arise by another independent clone that had cooperated as a secondary mutations is a multistep disease.

Hodgkin Lymphoma 2

P169

A COMPUTATIONAL DISSECTION OF TUMOR MICROENVIRONMENT REVEALS A PROGNOSTIC ASSOCIATION BETWEEN PD-L1, M2-LIKE MACROPHAGES AND CLINICAL OUTCOME IN CLASSICAL HODGKIN LYMPHOMA

G. Opinto¹, M.C. Vegliante¹, S. Ciavarella¹, S. De Summa², G. Loseto¹, C. Minoia¹, A. Scattonè³, A. Negri¹, A. Guarini¹

¹Hematology and Cell Therapy Unit, IRCCS-Istituto Tumori 'Giovanni Paolo II', Bari; ²Molecular Diagnostics and Pharmacogenetics Unit, IRCCS-Istituto Tumori 'Giovanni Paolo II', Bari; ³Pathology Department, IRCCS-Istituto Tumori 'Giovanni Paolo II', Bari, Italy

Classical Hodgkin's lymphoma (cHL) is a complex ecosystem composed by large mono- and multinucleated Hodgkin Reed-Stemberg (HRS) cells, and an extensive inflammatory infiltrate of both stromal and immune cells. This latter participates to the immune-evasion by strengthening the programmed death ligands (PD-Ls)/PD-1 axis. In a recent study, digital image analyses revealed that, beyond tumor cells, the majority of PD-L1 is expressed by macrophages in close proximity to HRS cells. Such observation on few cHL samples requires remarkable analytical efforts for extensive validation and clinical application. We applied the deconvolution approach CIBERSORT to dissect cHL microenvironment and investigate prognostic associations between PD-L1 and abundance of tumor-infiltrating non-malignant cell types. A 1,028-gene matrix was generated to distinguish among 17 different cytotypes, and CIBERSORT was run on a gene expression profiling (GEP) dataset (GSE17920) of 130 cHL pre-treatment biopsies. In silico stratification of cases was performed according to PD-L1 expression, proportions of putative tumor-infiltrating cell types and clinical outcome to ABVD regimen.

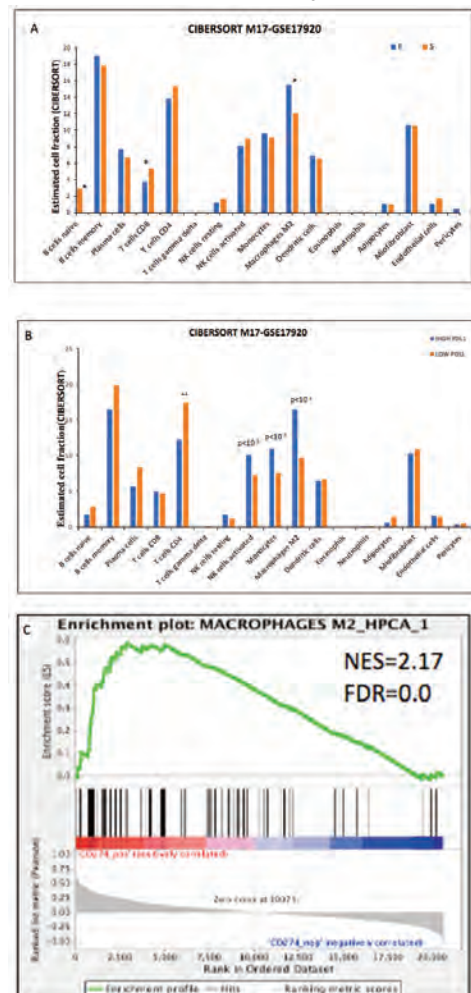


Figure 1.

Gene Set Enrichment Analysis (GSEA) was used to investigate the correlation between CD274 (PD-L1) gene and additional genes expressed by prognostic tumor-infiltrating cell subsets. CIBERSORT revealed a significant association between therapeutic failure, higher proportions of M2-like macrophages and lower infiltration by naïve B and CD8+ T cells (Figure 1A). When the cases were dichotomized according to the median value of PD-L1 transcript, the group with higher level of PD-L1 showed a significant predominance of M2-like macrophages, monocytes and activated NK cells (Figure 1B). GSEA revealed that those cases showing higher level of CD274 were significantly enriched in distinctive transcriptional programs of M2-polarized macrophages (Figure 1C). We applied a computational strategy to re-analyze GEP from whole-tissue biopsies of cHL and resolve “in silico” the cellular components of tumor microenvironment, identifying intriguing associations between PD-L1 expression, functionally specialized subsets of immune cells, particularly macrophages, and clinical outcome. Such preliminary results prompt the application of similar GEP-based methodologies to investigate larger retrospective or prospective cohorts of patients undergoing novel anti-PD-1 therapies for prognostic/predictive purposes.

P170

HODGKIN LYMPHOMA IN THE ELDERLY: A 10 YEARS MONOCENTER REAL-LIFE EXPERIENCE

E. Cencini, A. Fabbri, A. Sicuranza, A. Santoni, M. Bocchia

Unit of Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena, Italy

Background: older patients (≥ 60 years old) with Hodgkin lymphoma (HL) have a more aggressive disease characterized by adverse prognostic features, reduced tolerance to curative chemotherapy and less favourable outcome. Older HL patients treated with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) experienced lower dose intensity, higher toxicity and treatment-related mortality compared to younger patients. A randomized phase III trial comparing VEPEMB (vinblastine, cyclophosphamide, procarbazine, etoposide, mitoxantrone, bleomycin) with ABVD that enrolled fit elderly patients showed the same treatment-related mortality with reduced progression-free survival (PFS) for VEPEMB regimen.

Aims: we have reported our 10 years real-life experience in the management of elderly HL patients.

Methods: A cohort of 34 consecutive patients (22 were male), median age 70.5 years (range 60-91) diagnosed with classic HL between 2009 and 2018 was retrospectively analyzed. All patients completed staging with CT scan and PET. Geriatric assessment was performed in all cases and was used to choose treatment regimen. Out of 34 patients, 13/34 had early-stage HL and 21/34 advanced-stage HL. Induction treatment plan consisted of ABVD (21/34), CHLVPP (4/34), VEPEMB (8/34) and brentuximab (1 HIV positive case); if indicated, involved field RT as consolidation. Final response assessment was done according to the 2007 Revised Response Criteria.

Results: CR rate was 74%, median number of cycles was 4 (range 2-6). Grade 3-4 neutropenia occurred in 12/34 cases (35%), infectious complications in 9/34 cases (26%, hospitalization in 2 cases), pulmonary toxicity and nausea were mild to moderate, no treatment-related deaths were reported. After a median follow-up of 42 months (range 6-126) 25/34 patients (73%) were alive, cause of death was progressive disease (3 cases), second neoplasm (2 cases), cardiovascular disease (3 cases), cerebrovascular disease and HIV (1 case each). Median PFS and OS were not reached; estimated 3-y PFS and OS were 62% and 73%, respectively. Patients >70 years old experienced less favourable outcome with reduced efficacy and increased toxicity.

Summary/Conclusions: elderly HL treatment represents an arduous challenge, especially in a real-life setting outside clinical trials. Unfavourable prognostic features, comorbidities and reduced treatment tolerance make these cases an unmet medical need, even if prolonged survival is possible especially in fit patients.

P171

FLOW-CYTOMETRIC EVALUATION OF REED-STERNBERG CELLS ON LYMPH NODE SUSPENSIONS: A SINGLE CENTER EXPERIENCE

S. Bellesi, P.L. Puggioni, E. Maiolo, A. Cuccaro, F. Corrente, F. D'Alò, S. D'Innocenzo, I. Zangrilli, V. De Stefano, L.M. Larocca, S. Hohaus

Istituto di Ematologia Fondazione Policlinico A. Gemelli IRCCS Università Cattolica del Sacro Cuore, Italy

Flow-cytometric evaluation of Reed-Sternberg cells (RS) in Hodgkin Lymphoma (HL) has always been challenging. Fromm et al (Am J Clin Pathol 2014) described a 6-color assay that allows identification of cells with RS features. The aim of our study was to assess the diagnostic power of an 8-color RS detection tube including CD45 and PD-L1 as additional markers on lymph node suspensions in a single center experience. The suspensions were prepared from tissues obtained by surgical resection or endoscopic core biopsy. We used the MediMachine for mechanical disaggregation. Our 8-color tube was CD20V450/CD45V500/CD64FITC/CD30PE/CD40PECy5.5/PD-L1PECy7/CD95APC/CD3APCH7. In a diagnostic algorithm, it was performed in absence of pathological B or T cell population. By definition, all putative RS events showed expression of CD30, CD40, and CD95, with increased side and forward scatter, without CD64 expression. CD3 expression was variable and, if present, was due to T-cell-RS-cell rosettes. CD20 and CD45 were usually negative. Data were acquired by CytoFLEX Flow Cytometer and analyzed by Kaluza Software (Beckman Coulter). We considered a cluster of RS events as representative and quantifiable if more than 50 events were present. From June 2017 to march 2019 202 biopsies were evaluated in flow cytometry. Histological diagnosis was HL in 34/202 cases. Four cases had not been evaluated for the lack of sufficient material. Thirty cases were evaluated for the presence of RS cells. In 2 cases we found a RS cluster fewer than 50 events. In 9 cases without a RS cluster the number of totally registered events was too low. A putative RS population was observed in 19/30 cases (positive predictive value 63%). RS cells were always $<1\%$ of total events with a median percentage of 0.06% (0.009-0.6). The median absolute number of RS events was 397 (70-2861). The median number of total events were 341000 (170000-1500000). CD30 on RS population appeared to be heterogeneous, varying from dim to bright expression. CD40, CD95 and PD-L1 expression were always bright, separating RS cells from the other leukocyte populations. CD3 expression was heterogeneous. RS cells can be detected in lymph node suspensions by 8 color flow-cytometry. However, RS cell detection requires high analytical sensitivity and specificity that can be reached by increasing number of total acquired events. This assay allows the quantitative assessment of therapeutic target antigens, as CD30 and PDL1.

P172

ABVD CHEMOTHERAPY IN CHILDREN WITH ALL STAGES OF HODGKIN LYMPHOMA

A. Sau¹, S. Pascale¹, D. Onofrillo¹, F. Angrilli², A. Spadano²

¹Unità Operativa Semplice Oncoematologia Pediatrica, Ospedale Civile di Pescara; ²Unità Operativa Complessa di Ematologia Clinica, Ospedale Civile di Pescara, Italy

The worldwide incidence of Hodgkin lymphoma (HL) is approximately two to four new cases per 100000 population/year. While HL represents only approximately 4-5% of all cancers in children younger than 15 years of age, this increases to approximately 16% in adolescents, making HL the most common malignancy within this age group. With the current therapies, 5-year overall survival (OS) is greater than 90%, yet long-term OS continues to decline, from both delayed deaths from HL and late effects of therapy. Thus, the major focus in pediatric HL is the development of various strategies aimed at identifying the optimal balance between maintaining high rate of OS and avoiding the long-term morbidity and mortality associated with therapy. Although ABVD is standard chemotherapy used by most adult groups around the world, there is still considerable variation among pediatric groups as to the choice of a chemotherapy regimen. The aim of this prospective study is to evaluate safety and efficacy of ABVD with risk-adapted radiotherapy (RT) in pediatric HL. From 2011 to 2018, 22 consecutive patients with histologically confirmed classical HL were treated at our Institution with ABVD. The baseline patient characteristics is shown

on Table 1. Risk stratification based on adult criteria was adopted. Limited stage patients received 3-4 cycles of ABVD followed by IFRT (from 14.4 to 30 Gy). Advanced stage patients received 6 cycles of ABVD; IFRT to areas of residual CT or PET positive disease was recommended for patients with incomplete response. Patients were followed for late toxicity events. The media follow-up was 52,6 months (range 5-92 months). Overall, 19 of 22 patients achieved complete remission (CR), while 3 had a progressive disease (a patients died a few months after diagnosis) and one patient had a late relapse after 24 months. The OS was 100% for limited stage patients and 93.3% for those in advanced stages. The Progression Free Survival was 85.7% for limited stage patients and 80% for those in advanced stages. None of patient demonstrated late effects including secondary malignancy, growth abnormalities, cardiac and pulmonary toxicity. The three case of hypothyroidism occurred following neck irradiation at dose of 30 Gy. Additionally, in order to assess and reduce the late effects of the therapy, it is strongly advisable to maintain Observational Logs which would allow the patients to benefit from information about their screening schedule and specific follow-up.

Table 1. Patient characteristics.

Patients (total 22)	
Age (year) media (range)	13,9 (5-18)
Sex M	14
Sex F	8
LH SN	15
LH CM	4
Others	3
Stage I-II A/B	10
Stage III-IV	12
Bulk yes	4
Bulk no	18
Radiotherapy yes	11
Radiotherapy no	11

P173

NIVOLUMAB FOR RELAPSED/REFRACTORY HODGKIN LYMPHOMA: A REAL-LIFE EXPERIENCE IN CAMPANIA

F. Trastulli¹, O. Vitagliano², R. Della Pepa¹, C. Giordano¹, R. Rosamilio¹, L. Pezzullo³, F. Ronconi², F. Ferrara², F. Pane¹, M. Picardi⁴

¹Department of Clinical Medicine and Surgery, Federico II University Medical School Naples; ²Division of Hematology Cardarelli Hospital Naples Italy; ³Hematology and Hematopoietic Stem Cell Transplant Center A.U.O. San Giovanni di Dio e Ruggi D'Aragona Salerno Italy; ⁴Department of Advanced Biomedical Sciences Federico II University Medical School Naples, Italy

Classical Hodgkin Lymphoma (cHL) is characterized by an over-expression of PD-L1 and PD-L2. Several studies have been demonstrated that Nivolumab, a PD-1 blocking antibody, has a high ORR in relapsed or refractory (R/R) cHL. We present a retrospective analysis of 14 patients treated from in 3 centers in Campania. Patients were refractory to ≥ 3 lines of therapy including auto-SCT or couldn't undergo auto-SCT before Nivolumab. The primary end point was ORR after 6 months of therapy. Secondary end points included PFS, safety and feasibility as bridge to SCT. The response was assessed according to the Lugano Classification using 18F-FDG-PET/CT. Ultrasonography-guided core needle cutting biopsy was performed if needed. The median age was 41 yrs (range 20-54). Ten patients (71%) have stage ≥ 3 according

to the Ann Arbor Classification. The median lines of therapy before Nivolumab was 3 (range 3-4), 12 patients (85%) received a previous auto-SCT. At the time of the analysis, 4 patients were not able to be evaluated: 1 due to an early death unrelated to HL disease, 3 because didn't reach the time of assessment. Thus, ten patients (71%) were evaluated: 3 patients (30%) had a CR, 5 (50%) a PR, 1 (10%) had SD and 1 (10%) showed PD. The ORR after 6 months was 80%. Among the 8 patients who achieved a response, 1 patient underwent allo-SCT, 3 an auto-SCT while 4 continued. Among the patients who continued, no improve of response was observed: 2 patients (50%) remain in PR and CR after 27 and 28 cycles and are waiting for a SCT; 2 patients (50%) relapsed after 19 and 28 cycles. With a median follow-up of 10,2 months (range 1-30,7) the total PFS is 85%. The safety profile was acceptable with 3 patients (21%) which required delaying of administration: 2 due to liver toxicity, 1 patient due to grade 4 pulmonary toxicity. No hematologic toxicities of grade ≥ 3 was observed. Three patients underwent auto-SCT and one allo-SCT. Two patients are waiting for allo-SCT. All patients who underwent SCT are now in CR. The patient who received allo-SCT had an acute intestinal GVHD of grade 3 treated with steroid, no chronic GHVD was observed. Nivolumab represents candidate treatment for R/R patients and may serve as bridge to transplant without an increased risk of toxicity. The timing of best response in our cohort was after 12 cycles (6 months) and we suggest undergoing SCT immediately after the best response to reduce the impact of GHVD manifestation and to prevent the risk of progression

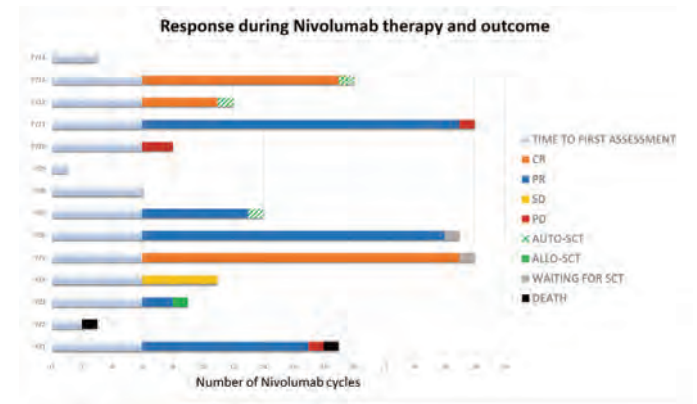


Figure 1.

P174

MAY TIGIT (T CELL IG AND ITIM DOMAIN) EXPRESSION BE A NEW THERAPEUTIC TARGET FOR HODGKIN LYMPHOMA (HL)?

O. Annibali¹, A. Bianchi², A. Grifoni³, V. Tomarchio¹, M. Tafuri¹, M. Verri², G.A. Crescenzi², G. Avvisati¹

Units of ¹Hematology, Stem Cell Transplantation and ²Pathology, University Hospital Campus Bio-Medico, Rome, Italy; ³Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology Athena Circle, La Jolla, CA, USA

The use of anti PD-1/PD-L1 resulted very effective in the treatment of R/R classical HL (cHL). TIGIT is another immune checkpoint receptor known to negatively regulate T cell functions. In this study, we investigated the expression of TIGIT in cHL microenvironment in order to find a potential new target for inhibitor therapy. TIGIT, PD-1 and PD-L1 expression was evaluated in 34 consecutive patients with cHL referred to Campus Bio-Medico University Hospital for diagnosis and treatment during the last eight years. TIGIT positivity was observed in 19/34 patients (56%); of these, 16 (84%) were also PD-1+. As for the 15 TIGIT- peritumoral T lymphocytes only 4 (27%) were PD-1+ while 11 (73%) were PD1- (P=0.001). Moreover, all these 15 TIGIT- patients were PD-L1+ (100%) while among the 19 TIGIT + patients, only 11 (58%) were PD-L1+ and the remaining 8 (42%) PD-L1- (P=0.004) (Figure 1). Furthermore, among the TIGIT + patients, 11 (58%) had advanced stages and 10 (52%) B symptoms. IPS ≥ 4 was observed in 8 patients and 6 (75%) were TIGIT +. This study indicate that the great majority of TIGIT+ patients are also PD-1+, while those who are TIGIT- are all PD-L1+. These preliminary results suggest the possibility

of a new immunotherapy in the treatment of R/R cHL by the use of anti-TIGIT MoAb.

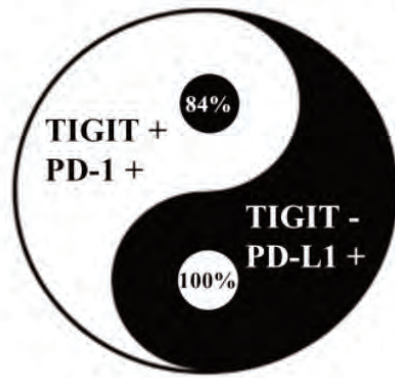


Figure 1. The Yin and Yang of check-point inhibitors.

P175

MORBIDITY AND MORTALITY IN LONG-TERM SURVIVORS OF HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE

F. Gaudio, T. Perrone, P. Curci, A. Giordano, P. Masciopinto, M.L. Di Noi, A. Piccolomo, F.E. Laddaga, G. Specchia

Ematologia Con Trapianto - Policlinico- Università degli Studi di Bari, Italy

Since the introduction of modern radiotherapy and combination chemotherapy, Hodgkin lymphoma (HL) has become a highly curable malignancy with 5-year survival rates of more than 80%. However, the life expectancy and quality of life of HL survivors are reduced by the occurrence of late adverse treatment effects. This retrospective study was focused on 96 patients with newly diagnosed HL treated at the University Hospital of Bari (Italy) between 2005 and 2008. Median age at diagnosis was 35 years (range 15-83), 33 pts (34%) presented advanced stage (III-IV), 43 (45%) bulky disease, 45 (47%) presented B symptoms, 26 (27%) extranodal disease. First line chemotherapy was ABVD in all the patients. The median number of chemotherapy lines was 1 (1-5), 49 patients (51%) had undergone radiotherapy. At the end of treatment, 75 patients (78%) were in CR, and 24 patients (25%) in PR; 18 patients (24%) relapsed after a median follow-up of 54 months (range: 12 – 62 months); 20 (21%) underwent autologous hematopoietic stem cells transplantation, 3 (3%) allogeneic stem cell transplantation. After a median follow-up of 12 years, 83 patients (86%) remain alive and 13 (14%) have died (4 of A second neoplasia, 1 of infection, 8 of the disease). The 10-year Kaplan-Meier survival estimates were 84%. Three women were pregnant and 3 healthy children were born. The most prevalent chronic conditions At last follow-up were: overweight/obesity (65%), elevated fasting glucose (38%), high total cholesterol (34%), and hypertension (25%). Results of this study offer indications about how long after the initial treatment the excess deaths from causes other than Hodgkin's disease begin to occur. However, challenges remain in establishing the optimal time to begin screening for potential late complications and in developing better surveillance guidelines. Further work is also needed to identify risk factors that may predict specific late effects.

P176

DIAGNOSIS OF MATERNAL HODGKIN LYMPHOMA FOLLOWING ABNORMAL FINDINGS AT NON INVASIVE PRENATAL SCREENING TEST (NIPT): REPORT OF TWO CASES

S. Elba, A. Castellino, R. Soriasio, C. Castellino, M. Bonferroni, M. Grasso, D. Mattei, N. Mordini, M. Foglietta, B. Mastruzo, R. Priotto, A. Zonta, D. Rapezzi, M. Massaia

AO Santa Croce e Carle di Cuneo; AOU Citta' della Salute e della Scienza, Torino, Italy

Sequencing cell-free DNA (cf-DNA) circulating in plasma of pregnant women is a noninvasive prenatal screening test (NIPT) for fetal chromosome abnormalities. However, cf-DNA could derive from fetus, placenta, maternal bone marrow or apoptotic cells. We report the cases of two pregnant women who showed unusual abnormal findings at NIPT leading to a diagnosis of Hodgkin lymphoma (HL). R.J. a 26 years-old girl performed for her decision NIPT at week 13 of pregnancy: abnormal findings, with multiple aneuploidies (trisomy 1,2,3,5,12,15,17, microdeletion 1p36) were in contrast with normal fetus development. Many differential diagnostic hypothesis were ruled out, including maternal hematological malignancy. Lymphopenia and multiple lympho-adenopathies at ultrasound were shown. At week 24, a biopsy of supraclavicular node was performed with a diagnosis of classical sclerodular HL. Staging with MRI was II A with a mediastinal bulky. ABVD chemotherapy was started at week 25. Normal delivery occurred at week 36, few days after the completion of third ABVD course, without complications neither for newborn nor for mother. A fourth cycle of ABVD was then performed, without significant treatment delay. Final PET scan and MRI were negative and patient received standard consolidation with involved field radiotherapy. She is in complete remission six months after the end of treatment. F.V a 29 years-old woman performed standard prenatal screening at week 13: abnormal findings not otherwise specified were found. Subsequently, she performed NIPT, which showed multiple aneuploidies that, in contrast with normal fetus development, suggested non fetal origin. Maternal malignancies were ruled out. Body-MRI showed a mediastinal mass and a needle-biopsy demonstrated a diagnosis of classic sclerodular HL, stage II A bulky. 4 ABVD courses were planned followed by radiotherapy. ABVD started at week 30. On day 13 of second ABVD natural delivery occurred, without complications neither for newborn nor for mother. She is planned to complete II ABVD in the next days followed by interim restaging with both MRI and CT PET. In conclusion, abnormal results of NIPT could disclose maternal malignancy and this evenience should be explained during pre test counselling. In the case of abnormal findings, a complete diagnostic assessment is recommended and, when reasonably safe, such as in HL diagnosis, treatment during pregnancy could be considered without major concern for mothers and babies.

Allogeneic and Autologous Transplantation 3

P177

CLINICAL OUTCOMES IN A CASE SERIES OF HSCT IN HODGKIN LYMPHOMA PREVIOUSLY TREATED WITH NIVOLUMAB

C. Cigana, A. Sperotto, M. Cerno, R. de Marchi, V. Radici, A. Geromin, R. Fanin, F. Patriarca

ASUIUD - Azienda Sanitaria Universitaria Integrata di Udine, Clinica Ematologica, Italy

Anti-programmed cell death protein 1 (PD1) monoclonal antibodies can be used as "bridge to" a subsequent allogeneic hematopoietic stem cell transplantation (HSCT) in patients with relapsed/refractory Hodgkin's Lymphoma (HL). This strategy has been reported to be effective, but a frequent onset of steroid-refractory Graft versus Host Disease (GVHD) was also reported. We report 6 patients affected by HL undergoing allogeneic HSCT after having been treated with Nivolumab. The 6 patients, median age 29 (range 18-48), had advanced HL relapsed after a previous autologous (5) or allogeneic (1) HSCT. They underwent a rescue therapy with 6 to 18 Nivolumab administrations, depending on time of response achievement and donor availability. Two patients received a thiotepa-fludarabine-cyclophosphamide conditioning, ATG-based prophylaxis and cells from unrelated donors, 4 patient received cells from an haploidentical donor using the "Baltimore" nonmyeloablative platform. Stem cells came from bone marrow (3) and peripheral blood (3). All the 6 patients achieved and maintained complete remission by PET-CT scans at a median of 8 months (3-15) after HSCT. Four patients developed acute GVHD on day +25, +32, +54, +107. GVHD was preceded by a prolonged fever without microbiological findings in 3 patients. Acute GVHD was refractory or progressed after first line 2 mg/kg steroid therapy in 3 patients and was responsive to second-line etanercept (1 patient) or third line ruxolitinib (2 patients). All the patients had CD3+ lymphocytes count < 200 mcL in peripheral blood on day + 180. The 2 patients with history of severe steroid refractory GVHD needing 2 subsequent immunosuppressant treatments died of CNS fusarium localization and pulmonary aspergillosis after 12 and 17 months after HSCT, respectively, in spite of GVHD control and HD complete remission. Both patients had received PBSC from unrelated donors and ATG prophylaxis. This case series confirms that Nivolumab as "bridge to transplant" is effective in appropriately selected patients. However, risk of acute GVHD and delayed immune reconstitution may require a careful consideration at the moment of planning the transplant. A possible advantage of post-transplant cyclophosphamide platform and haploidentical donors should be addressed in larger studies.

P178

RISK FACTORS FOR CMV REACTIVATION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION AND INITIAL EXPERIENCE WITH LETERMORIV PROPHYLAXIS

A. Sperotto, A. Candoni, M. Cerno, R. De Marchi, C. Cigana, V. Radici, M.L. Battista, F. Colasante, C. Rosignoli, V. Dubbini, R. Stella, A. Geromin, F. Patriarca, R. Fanin

Clinica Ematologica - CTMO, Azienda Ospedaliero, Universitaria, Udine, Italy

Cytomegalovirus (CMV) is recognized as an important cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT). Although ganciclovir and valganciclovir prophylaxis are routinely used in solid organ transplantation, this approach is limited after HSCT due to myelosuppression. In a phase 3 trial, prophylaxis with letermovir (antiviral agent that inhibits CMV replication) resulted in a significantly lower risk of CMV infection than placebo. We retrospectively analyzed patients submitted to HSCT from January '14 to December '18: the main end-point of the study was to identify patients at high risk of reactivation eligible to letermovir prophylaxis. Two hundred and sixty one patients were included: median age was 56 years (range 16 - 74); diagnosis: acute leukemia 148 (56.5%); lymphoma 45 (17.5%); chronic myeloproliferative disease 25 (9.5%); myelodysplastic syndrome 22 (8.5%); multiple myeloma 21 (8.0%). Donors: HLA matched related (MRD) 58 (22.0%); matched unrelated (MUD) 90 (34.5%); mismatched unrelated (MMUD) 66 (25.0%); haploidentical (haplo) 47 (18.5%). Re-

ipient (R)/Donor(D) CMV status: Rneg/Dneg 26 (10.5%); Rneg/Dpos 15 (5.5%); Rpos/Dneg 81 (31.0%); Rpos/Dpos 139 (53.0%). Eighty-four (32.0%) patients developed acute GVHD (aGVHD) and 55 were treated. CMV reactivation were documented in 117 patients (45.0%) at a median time of 29 days (1 - 130) from HSCT; early reactivation (< 100 days from transplant) was documented in 91 patients (77.5%). Forty-one patients (35.0%) had more than one reactivation. Seventy-six (65.0%) patients were treated with valganciclovir; 21 (18.0%) with ganciclovir and 20 (17.0%) with foscarnet. Eight patients (7.0%) developed a CMV disease: two (1.5%) died for CMV lung disease. In multivariate logistic regression analysis factors related to CMV reactivations were: donor HLA - mismatched (MMUD p =.04; haplo p=.03); ATG for GVHD prophylaxis (p=.04); CMV status (Rpos/Dneg p=.0006; Rpos/Dpos p=.0008); aGVHD (p value=.05). From January '19 to April '19, prophylaxis with letermovir has been employed in 7 patients: 3 haplo and 4 MMUD transplants. CMV status was: Rpos/Dneg - 4; Rpos/Dpos - 3. Letermovir was started at a median of 10 days (range 7 - 14) from re-infusion. No CMV reactivations were documented at a median follow-up of 45 days (3 - 60). Letermovir was well tolerated without any complications: this prophylaxis should be reserved to high risk patients such those transplanted from mismatched donors and those treated for aGVHD.

P179

AZACYTIDINE + DLI AS SALVAGE TREATMENT IN ACUTE MYELOID LEUKEMIA RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IMPROVING PROGRESSION FREE SURVIVAL

C. Liberatore^{1,2}, M.T. Lupo Stanghellini¹, M.G. Carrabba¹, C. Messina¹, L. Vago^{1,3}, F. Lorentino¹, F. Pavesi¹, S. Mastaglio¹, S. Markteli¹, A. Assanelli¹, F. Farina¹, R. Milani⁴, R. Greco¹, C. Corti¹, M. Bernardi¹, J. Peccatori¹, F. Ciceri^{1,2}

¹Haematology and Bone Marrow Transplant Unit IRCCS San Raffaele Scientific Institute; ²Vita-Salute San Raffaele University IRCCS San Raffaele Scientific Institute; ³Unit of Immunogenetics Leukemia Genomics and Immunobiology IRCCS San Raffaele Scientific Institute; ⁴Immunohematology and Transfusion Medicine Unit IRCCS San Raffaele Scientific Institute; Italy

Introduction: Azacytidine (AZA) alone or with donor lymphocytes infusions (DLI) is a possible treatment for acute myeloid leukemia (AML) relapsing after allogeneic hematopoietic stem cell transplantation (HSCT) due to anti-leukemic and immune-modulating effects.

Methods: We retrospectively analyzed patients (pts) with AML relapsed after HSCT and consecutively treated from 2012 to 2019 at our institution with AZA +/- DLI. Indications were either molecular relapse (evaluated by qPCR, multiparameter flow cytometry or chimerism analysis) and morphological relapse (marrow blasts <20% or >20% not eligible for intensive chemotherapy). AZA dosage was 75 mg/mq G1-7 q28 days. DLI was given at escalating dose every 2 cycles of AZA. Indication to DLI relied upon clinical-biological features of patient, disease and HSCT (active GvHD and HLA loss were contraindications).

Results: 22 pts were eligible. Clinical-biological features at baseline and relapse are reported in table 1. Median time from HSCT to relapse was 14 months (range 2.9-54). Relapses were 15 morphological and 7 molecular. 12 pts received AZA (group 1) and 10 pts AZA+DLI (group 2). Median number of AZA cycles was 3.5 (range 1-28; 3 in group 1 vs 4 in group 2). Median number of DLI was 1.5 (range 1-3). Overall complete response rate was 27% (6/22 pts), respectively 8% (1/12) in group 1 vs 50% (5/10) in group 2 (p=0.04). Median PFS was 4.9 months (range 0.7-57.3), respectively 4.4 months (range 2-17.4) in group 1 vs 5.7 (range 2.9-57.3) in group 2 (p=0.03). Median OS after salvage treatment was 13.2 months (range 1.5-75.7), respectively 7.3 months (range 1.5-72.5) in group 1 and 49.7 months (range 3-75.7) in group 2 (p=0.05). Median OS after HSCT was 29 months in group 1 and 108.5 months in group 2 (p=0.05). Most common grade 3-4 adverse events (AE) were neutropenia (59%), thrombocytopenia (41%) and febrile neutropenia (32%), without significant difference between groups. No pts died because of treatment. In group 1 GvHD worsened in 1 pts with previous chronic GvHD (8%, 1/12). In group 2 GvHD was diagnosed in 2 pts both without previous GvHD (20%, 1 acute and 1 chronic GvHD).

Conclusions: AZA +/- DLI is an effective treatment for AML relapsing after HSCT. Considering limits of retrospective study, AZA+DLI provides better PFS without increasing AE in comparison with AZA only.

Further studies are needed to better evaluate GvHD occurrence and the efficacy of pre-emptive therapy in molecular relapse setting.

Table 1. Clinical-biological characteristics at baseline and relapse.

Gender:	
- Male	13
- Female	9
Median age at HSCT	56 years (range 21 – 72)
Classification according to WHO 2016:	
- AML with inv(16) (p13.1q22)	1
- AML with mutated NPM1	2
- AML with myelodysplasia-related changes	8
- Therapy-related myeloid neoplasm	5
- AML not otherwise specified	6
ELN risk category:	
- Favourable	1
- Intermediate	8
- Adverse	10
- Unspecified	3
Median numbers of lines prior to HSCT	3 (range 1 – 7)
- Previous allogeneic HSCT	3/22 patients
Disease status pre-HSCT:	
- CR1	8
- CR≥2	5
- Active Disease	9
Conditioning regimens:	
- MAC	13
- RIC	9
Donor source types:	
- Matched Related	4
- Matched Unrelated	9
- Haploidentical	6
- Cord Blood Unit	2
- Mismatched Unrelated	1
GVHD prophylaxis:	
- PTCY-MMF-sirolimus	4
- PTCY-sirolimus	2
- ATG-CSA-MTX	6
- Others	10
Response to HSCT:	
- Complete Response	22
Type of relapse according to treatment group:	
AZA:	
- Molecular relapse	2
- Morphological relapse	10
AZA+DLI:	
- Molecular relapse	5
- Morphological relapse	5
GVHD before relapse:	
- Acute	10
- Chronic	9
- None	9
Pts with previous acute/chronic GVHD in treatment groups:	
- AZA	6/12
- AZA + DLI	7/10
Subsequent treatment after progression:	
- II allogeneic HSCT (haplo)	7 (6)
- Chemotherapy	1
- Other	2
- Supportive care	12

P180

PREDICTORS FOR PROLONGED GRAFT AND RELAPSE FREE SURVIVAL (GRFS) IN ALLOGENEIC STEM CELL TRANSPLANTATION FROM UNRELATED DONORS EMPLOYING AN ATG-BASED GVHD PROPHYLAXIS

C. Cigana, R. de Marchi, M. Isola, A. Sperotto, M. Cerno, V. Radici, F. Colasante, A. Geromin, R. Fanin, F. Patriarca

ASUIUD, Azienda Sanitaria Universitaria Integrata di Udine, Clinica Ematologica, Dipartimento di Scienze Mediche e Biologiche Università degli Studi di Udine, Italy

Stem cells from unrelated donors (URD) are currently reinfused in nearly half of the hematopoietic stem cell (HSCT) transplants performed in Italy. We analysed 271 consecutive HSCT from URD that were realized employing an uniform GVHD prophylaxis based on calcineurin inhibitors, methotrexate and anti-thymocyte globulin from January 2010 to December 2018 in order to identify predictors for long term outcome. HLA high resolution typing was performed at HLA-A, -B, -C, DRB1 loci. Median age was 55 years (range 18-71), 48% had a matched URD, 52% had a mismatched URD donor including 1 mismatch (44%) or 2 mismatches (8%) at HLA class I. Underlying diseases were mainly acute leukaemias (56.5%) and lymphomas (18%); 60% of the patients had an active disease, 50% had a Comorbidity Index \geq 3. Armand risk was high or very high in 70% of patients. A myeloablative conditioning regimen was used in 26% of the patients. GVHD prophylaxis was based on Cyclosporine in 69% of the patients and Tacrolimus in 31% of the patients, associated with methotrexate and anti-thymocyte globulin in all the patients. Grafts came from PBSC in 248 patients (91%). Patients received a median dose of CD34 positive cells of

$6.9 \times 10^6/\text{Kg}$ and a median dose of CD3 positive cells of $21.9 \times 10^7/\text{Kg}$. At a median follow of 36 months (range 1-106), 3 -years NRM, OS, and graft and relapse free survival (GRFS) were 26%, 51% and 34%, respectively. Cumulative incidence (CI) of acute II-IV GVHD was 38%, while CI of acute III-IV GVHD and steroid refractory GVHD were 10% and 15%, respectively. CI of overall chronic GVHD was 34%, with 18% of moderate and severe forms. In multivariate analysis significant predictors for lower NRM were HLA full matching (HR, 0.54; 95% CI, 0.31-0.94; $p=0.029$) and myeloablative conditioning (HR, 0.33; 95% CI, 0.15-0.71; $p=0.005$), while active disease, treatment before HSCT with at least 2 lines and reinfusion with an higher number CD 3 positive cells (modelled as a continuous variable) were significantly associated with poorer GRFS (HR, 1.57; 95% CI, 1.10-2.24, $p=0.011$; HR, 1.64; 95% CI, 1.17-2.29; $p=0.003$; HR, 1.02, 95% CI, 1.00-1.03, $p=0.003$, respectively). We conclude that in a series of transplants that had mainly received PBSC a GVHD prophylaxis based on calcineurin inhibitors, methotrexate and anti-thymocyte globulin did not abrogate the negative impact of HLA mismatching on NRM, while advanced disease and content of CD3 positive cells in the reinfusion had a significant influence on GRFS.

P181

SAFETY OF VACCINES IN A COHORT OF ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

C. Targhetta¹, M.P. Simula¹, C. Depau¹, A. Vacca¹, E. Piras¹, T. Durzu¹, R. Rojas², V. Serreli¹, S. Cardani¹, G. Mele¹, G. LaNasa¹

¹S.C. Ematologia e CTMO, Ospedale Oncologico "A. Businco", Cagliari;

²U.O. Ematologia Ospedale Policlinico, Sassari, Italy

Introduction: We performed a farmacovigilance study about vaccine-related problems in a cohort of allogeneic Hematopoietic Stem Cell Transplant (HSCT) recipients followed in our clinic during a 1 year period.

Materials and Methods: From October 2017 to December 2018 we administered a list of vaccines to 51 HSCT recipients fulfilling the following criteria: CD4 Tcells >200/ μl , CD19 Bcells >20/ μl , anti-CD20 antibody infusion >6 months, IVIG therapy >2 months, no active Graft-versus-Host-Disease (GvHD), no chemotherapy or biological agents on going. Vaccines suggested were Influenza, Pneumococcal conjugate, Inactivated Polio Vaccine, Diphtheria, Tetanus, Acellular pertussis, Hepatitis A and B, Haemophilus influenzae B, Meningococcal quadrivalent, Human Papillomavirus, Meningococcal B, Measles-Mumps-Rubella, Varicella. Live vaccines were not recommended before 2 years after HSCT and in patients with chronic GvHD. All the patients were asked to take the list to the local health facilities in order to have the vaccines injected and a vaccination table arranged. We checked the vaccination tables at each visit and monitored potential side effects and GvHD status at 3, 6, and 12 months after the first vaccine injection.

Results: Thirty-six out of 51 patients were evaluable (table 1), 19 without GvHD and 17 with chronic GvHD. Median time between HSCT and first vaccine injection was 33,5 months (15-188). Median number of vaccines received was 9 (1-15), while median number of doses was 13 (1-34). Three out of 19 patients without chronic GvHD experienced fever after vaccine injections; 1 developed transient reduction of platelet count, 1 reported headache and otalgia, 1 transient joint pain; 1 patient presented mouth chronic GvHD and transaminase increase 3 months after the first vaccine dose so that cyclosporine dose had to be re-augmented. Four out of 17 patients with chronic GvHD experienced fever after vaccine injections, 1 reported reversible pain and paresthesia in the limb where the vaccines had been administered; 2 patients with mild chronic GvHD of the mouth presented hepatic flare 2 and 3 months after the first vaccine dose, respectively. In both cases a new increase of cyclosporin and methylprednisolone doses determined progressive normalization of liver enzymes.

Conclusions: These data show that vaccines were globally well tolerated in HSCT recipients, even when they suffered from chronic GvHD. However, close monitoring is warranted in order to better evaluate possible vaccine side effects in this setting of patients.

P182**LONG TERM FOLLOW UP OF PATIENT WITH ACUTE MYELOID LEUKEMIA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION**

F. Sorà, P. Chiusolo, E. Metafuni, L. Laurenti, A. Corbingi, S. Giammarco, I. Innocenti, F. Autore, A. Bacigalupo, S. Sica

Istituto di Ematologia, Università Cattolica del Sacro Cuore, Roma, Italy

Autologous stem cell transplantation (ASCT) is a valid therapeutic option for patient with good or intermediate risk acute myeloid leukemia (AML). Disease recurrence is the major cause of treatment failure and it usually occurs within the first 2 years. The aim of this retrospective study is to assess the outcome of patient receiving ASCT in our department from 1992 to 2014 for AML. The patients included in the study are 70: 39 (56%) males and 31(44%) females and the median age is 46 years (range 18-65). Cytogenetic analysis on bone marrow at diagnosis was normal in 28 patients, good risk in 4 patients, no evaluated for absence metaphases in 28 patients and not available in 10 patients. Disease status at the transplant was respectively Complete Remission (CR) in 63 (90%), Partial Remission (PR) in 5(7%) and Progressive Disease (PD) in 2 (3%) patients. The most common conditioning regimen was busulfan in conjunction with cyclophosphamide administered to 59 patients (84%), but the combination of busulfan and melphalan was also used, in 11 patients (16%). Four (5,7%) out of 70 patients developed a second neoplastic disease after a median of 7,5 years (range 0,3-8 years) after transplantation and the death was related to the secondary disease after a median time of 9,5 years (range 0,5-12). Overall Survival rates (OS) for all patients is 36% after a median follow of 4 years (0.5-25). OS rates is 68%, 62%, 50% and 42% at 1,2, 5 and 10 years respectively. The overall OS rate is 72% in the population alive after 5 years from transplant and the OS rate a 10 years is 83%. Leukemia free Survival (LFS) rate rates for all patients is 63% LFS rates is 86%, 86%, 72% and 63% at 1,2, 5 and 10 years respectively. The overall LFS rate and the LFS rate a 10 years are 86% in the population alive after 5 years from transplant. Eleven (16%) out of 70 patient received a salvage allogeneic transplant after leukemia relapse or secondary disease. Our findings demonstrate that nearly half of patients with AML are alive after 5 years post ASCT and that the patients survivors after 5 years have very high OS and LFS. Salvage allogeneic transplantation remains feasible in those who relapse. ASCT therefore remains a reasonable option for patients with AML, Prospective risk-adapted approaches that assign patients to ASCT based on disease-risk and MRD status are ongoing and may clarify the specific subpopulations of AML patients who could take advantage of this procedure.

P183**BUSULFAN FLUDARABINE (BU-FLU) COMPARED TO THIOTEPA BUSULFAN FLUDARABINE (TBF) FOR ALLOGENEIC TRANSPLANTS IN ACUTE MYELOID LEUKEMIA (AML) IN REMISSION**

F. Sora¹, P. Chiusolo, C. Di Grazia, A.M. Raiola, S. Bregante, N. Mordini, A. Olivieri, A.P. Iori, F. Patriarca, S. Grisariu, E. Terrazzi, A. Rambaldi, S. Sica, E. Angelucci, A. Bacigalupo

Istituto di Ematologia Università Cattolica del Sacro Cuore Roma; U.O. Ematologia Ospedale Policlinico San Martino Genova; Division of Hematology Azienda Ospedaliera S. Croce e Carlo Cuneo; Division of Hematology Azienda Ospedaliera Universitaria Ospedali Riuniti Ancona; Department of 'Cellular Biotechnologies and Hematology' Azienda Policlinico Umberto I Università La Sapienza Roma; Hematology, Medical Department (DAME), University of Udine; Head of Adult Bone Marrow Transplantation inpatient unit Hadassah University Hospital, Jerusalem Israel; U.O. Ematologia Ospedale San Gerardo Monza; Department of Oncology and Hemato-oncology University of Milan and Azienda Ospedaliera Papa Giovanni XXIII Bergamo

This is a multicenter retrospective comparison of two conditioning regimens in 428 patients with acute myeloid leukemia (AML) in first or second remission: busulfan and fludarabine (BU-FLU) versus thiotepa, busulfan and fludarabine (TBF).

Methods: Eligible for this study were patients allografted between January 2008 and April 2017, with AML in first or second remission: 206 patients received the BU-FLU regimen (busulfan 3.2 mg/kg/day x4; and fludarabine 40 mg/m²/day x4), and 222 received TBF (thiotepa 5mg/kgx2, busulfan 3.2 mg/kg/dayx3, fludarabine 50 mg/m²x3). The

two groups (BU-FLU and TBF) were comparable for age (p=0.3). The TBF group had more second remission (27% vs 10%, p=0.001), more secondary AML (29% vs 12%, p<0.01). The donor included HLA identical siblings, unrelated donors and haploidentical donors.

Results: With a median follow up of 467 and 534 days after transplantation, for BU-FLU and TBF respectively, the 5-year cumulative incidence of TRM was 19% for BUFLU and 20% for TBF; and the 5-year cumulative incidence of relapse was 31% and 14% respectively (P =0.0001). The 5-year actuarial survival was 51% for BU-FLU and 69% for TBF (p=0.0005). The survival advantage at 5 years, was seen also when excluding patients receiving haploidentical grafts (50% vs 66%, p=0.01). In a multivariate Cox analysis, after correcting for patients age, year of transplant, first or second remission, and donor type, the use of TBF reduced the risk of relapse compared to BU-FLU (RR 0.44, p=0.01) and the risk of death (RR 0.61, p=0.03). TRM was only affected by patients age over 50 years.

Conclusions: Superior survival of patients receiving TBF, as compared to BU-FLU appears to be due to reduced relapse, with comparable TRM. The survival advantage is independent of donor type.

P184**PROSPECTIVE STUDY OF PATIENTS WITH ACUTE LEUKEMIA TO EVALUATE THE PREDICTIVE VALUE OF CD3/T REGS RATIO ON THE INCIDENCE OF AGVHD AFTER MYELOABLATIVE ALLOGENEIC PB TRANSPLANTATION: AN INITIAL REPORT**

M. Delia¹, D. Pastore², P. Carluccio¹, A. Mestice¹, C. Brunetti³, P. Chiusolo⁴, R. Fedele⁵, M. Martino⁵, F. Zallio⁶, R. Dilonzo⁷, A. Vacca⁸, G. Pisapia⁹, C. Ingresso⁹, G. Saporiti¹⁰, E. Benedetti¹¹, M. Arpinati¹², G. Specchia¹

¹Hematology and Bone Marrow Transplantation Unit, University of Bari; ²Ematologia, Ospedale Perrino; ³Laboratorio di Tipizzazione Tissutale, Policlinico di Bari; ⁴Hematology, Policlinico Gemelli; ⁵BMT Ospedale Morelli; ⁶Ematologia, Ospedale SS Biagio e Arrigo; ⁷Ematologia, Ospedale Civile; ⁸BMT, PO Binaghi; ⁹Hematology and BMT Unit Ospedale Moscati; ¹⁰Hematology, Policlinic, University of Milan; ¹¹Hematology, AOU Pisana; ¹²Hematology and BMT, Policlinico S Orsola Malpighi

Background: The Tregulatory cells (Tregs) content in stem cells harvested from peripheral blood (PB) has been suggested to be correlated with aGVHD and immunological recovery after PB stem cell transplant. While it is well known that intra-tumoral or intrabone (in cases of acute leukemias) Tregs might be correlated with the worst outcome in solid tumors, and in the acute leukemia setting too, by promoting immune surveillance escape, little has been studied in terms of their contribution to the immediately post transplant phase after the infusion of the PB allo-graft.

Aims: For patients affected by AML or ALL in complete remission (CR) undergoing myeloablative fully matched related (MR) and unrelated donor (MUD) allotransplant, Tregs action should be studied in the hope of limiting other immunological elements (no CR; different types of conditioning and stem cell source), that inevitably act in the allo setting. Therefore, we have studied the Tregs content of the allo-harvest with the aim of assessing any correlation with the aGVHD (grade≥II) incidence and post-allo outcome.

Patients and Methods: From May 2015 to December 2018 we enrolled 91 consecutive patients at 13 Italian centers belonging to the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) affected by AML or ALL in CR who underwent MRD or MUD allotransplant and calculated the Tregs (CD4⁺/CD45RA^{neg}/CD127^{low}/CD25^{high}/DR⁺/CD39⁺) content in the PB stem cell harvest together with the CD3, CD4, CD8 and NK population. Patients characteristics are reported in Table 1. Results. aGVHD occurred in 12 patients (13%), predominantly involving the skin (64%; gut, 54%, liver, 25%). Transplant-related mortality at 100 days was 3% (3 of 91 patients), and in all the cases the mortality cause was aGVHD. At 100 days, 4 patients (5%) relapsed after transplant, while the cumulative incidence of relapse and OS at 3 years was 14% and 69%, respectively. Median CD3x10⁶/kg, Tregsx10⁶/kg and CD3/Tregs were 201, 1.4 and 52, respectively. According to median CD3/Treg value [52] we observed an aGVHD incidence of 31% in the >52-group while two cases (3%) of aGVHD were documented in the ≤52-group (p<0.001). Accordingly, OS was better in the ≤52-group patients (p=0.038).

Conclusions: Although confirming the impact on the post-allo out-

come, these preliminary results are clearly affected by the sample size, that will be integrated with further patients, permitting firm conclusions (expected end of study: 30 June 2019).

Table 1.

Patients characteristics	n= 91	%
Median age, range	48 [22-68]	
M/F	47/44	52/48
Disease status at allotransplant		
CR1	51	56
CR2	27	30
CR>2	2	2
missing data	11	12
Type of disease		
AML	75	82
ALL	16	18
Type of myeloablative regimen		
BuCY	17	19
BuFlu	24	26
TBF	39	43
TBI based	5	5
others	6	7
CMV risk		
low	23	25
high	39	44
very high	14	15
missing data	15	16
Sex match		
Donor female/recipient male	14	15
Other combinations	77	85
Type of donor		
MRD	35	39
MUD	56	61
GvHD prophylaxis		
ATG based	66	73
not ATG based	18	20

CR, complete remission; AML, acute myeloid leukemia, ALL, acute lymphoblastic leukemia; BuCY, busulfan + cyclophosphamide; BuFlu, busulfan + fludarabine; TBF, thiotepa, busulfan, fludarabine; TBI, total body irradiation; CMV, cytomegalovirus; MRD, matched related donor; MUD, matched unrelated donor; ATG, anti-thymocytes globulin

Acute Myeloid Leukemia 2

P185

MITOXANTRONE, ETOPOSIDE AND CYTARABINE (MEC) CAN INDUCE DEEP COMPLETE REMISSION AND IS AN EFFECTIVE BRIDGE THERAPY TO ALLOTRANSPLANTATION (SCT) IN REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA (AML) PATIENTS

G. Marconi^{1*}, A. Talami^{1*}, M.C. Abbenante¹, S. Paolini¹, C. Sartor¹, S. Parisi¹, S. De Polo¹, J. Nanni¹, L. Bertamini¹, S. Ragaini¹, M. Olivi¹, M.C. Fontana¹, M.T. Bochicchio², E. Ottaviani¹, C. Baldazzi¹, N. Testoni¹, G. Martinelli², M. Cavo¹, C. Papayannidis^{1°}, A. Curti^{3°}
*GM and AT equally contributed; °CP and AC equally contributed

¹Institute of Hematology "L. and A. Seràgnoli", Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna; ²Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola; ³Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Institute of Hematology L. e A. Seràgnoli

Introduction: Relapsed/refractory (R/R) AML patients are still a formidable clinical challenge. SCT represents the only curative option for these patients. Our study aims to investigate the effectiveness of MEC regimen as a rescue therapy for R/R AML patients and as a bridge to SCT.

Methods: Fifty-five consecutive adult AML patients were treated with MEC regimen from 2008 to 2018. We administered, from day 1 to day 6, mitoxantrone 6 mg/sqm/die, etoposide 100 mg/sqm/die and cytarabine 1000mg/sqm/die. The schedule was reduced in over 66 patients to 4 consecutive days. Data were collected retrospectively. Response was defined in accordance with 2017 ELN recommendations. CTCAE 4.03 was used to grade adverse events (AEs). MRD was assessed with WT1 or specific fusion transcripts.

Results: Median age at diagnosis was 51 years (range 17-72). At induction, almost half of patients received "3+7" (n=25, 45,5%), while fludarabine-based regimens were administered to 14 patients (25,5%).

In our set, PMN recovery (>500/mm³) after MEC was reached after 26 days (range 18-67). Febrile neutropenia was the most recurrent AE. Out of 80 graded AEs, 38 (47,5%) were grade 2, 27 (33,8%) grade 3, 9 (11,3%) grade 4 and only 3 resulted in death (3,8%). Overall, 25/55 patients (45,5%) achieved a complete remission (CR) after one MEC course. Twelve patients (21,9%) achieved MRD negativity and 13 patients (23,6%) obtained an MRD+ CR. Six patients (10,9%) had a Partial Response and 1 patient (1,8%) had Hematological Improvement. Four patients (7,3%) died during aplastic phase. Disease risk at diagnosis and R/R status did not influence the chance to obtain CR. In 12 patients, a second MEC was administered; four of these improved their response (3 patients obtained MRD – from MRD+ CR and 1 patient a PR). Subsequently, 32/55 patients (58,2%), received SCT; 15 (46,9%) directly after the 1st course of MEC, 9 (28,1%) after the 2nd MEC course and 2 patients (6,3%) after an additional course of post-remission chemotherapy. Only 6 patients (18,8%), not responsive to MEC, underwent SCT after an alternative rescue therapy. Median overall survival (OS) from MEC was 455 days (95% C.I. 307-602 days.); 1-year OS, 3-year OS and 5-years OS were 57,9%, 33,2% and 23,1%, respectively (std. error ± 0,067).

Conclusions: Our data demonstrated that MEC is an effective salvage regimen with affordable toxicity, particularly useful as a bridge to SCT.

P186

KEVETRIN MOLECULE ALTERS TRANSCRIPTIONAL PROGRAM AND CELLULAR METABOLISM OF ACUTE MYELOID LEUKEMIA CELL LINES

R. Napolitano, S. De Matteis, S. Carloni, S. Bruno, G. Abbati, L. Capelli, M. Ghetti, A. Ghelli Luserna di Rorà, D. Calistri, A. Cuneo, K. Menon, A. Lucchesi, G. Musuraca, G. Martinelli, G. Simonetti
 Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, University of Bologna and Institute of Hematology "L. e A. Seràgnoli", University of Ferrara-Arcispedale Sant'Anna, Innovation Pharmaceuticals

Tumor protein p53 (TP53) is the most frequently mutated gene in cancer and it regulates a number of genes involved in DNA repair, cell cycle, apoptosis and angiogenesis. Kevetrin is a small compound that showed activity against TP53 wt and mutant solid tumors. In the present study we addressed the response of AML cell lines to kevetrin treatment and investigated gene expression changes in TP53 mutant and wt models, to better understand the molecular and biological consequences of kevetrin exposure. TP53-wt MOLM-13 and TP53-mut KASUMI-1 were treated with kevetrin [15-60 ug/ml]. After 24 and 48h MTS and Annexin-V were performed. The TP53-wt cell line showed a sensibility only at the highest drug concentration with a decreased cell viability and a significant increase in Annexin V+ cells (54.95% vs 12.53% of the CTRL). The TP53-mut cell line presented a significant dose- and time-dependent cell growth inhibition and apoptosis increase with 79.70% of Annexin V+ cells at 60 g/ml (vs 13.18% of the CTRL). After 6 and 48 h of treatment at the highest kevetrin dose Gene expression profiling (GEP) was performed using Human Transcriptome Array 2.0. Short-term (6h) kevetrin treatment induced few alterations of the overall transcriptional program. After prolonged kevetrin exposure (48h) MOLM-13 cell line showed 1024 upregulated and 1563 downregulated genes, whereas KASUMI-1 presented increased expression of 325 and decreased levels of 1535 genes. Kevetrin targeted also a common core transcriptional program in the selected models, including 162 upregulated and 812 downregulated genes. Upregulated genes were mainly involved in transcription, nucleosome assembly and telomere organization, apoptosis, autophagy, NF-kB pathway and MAPK activity. Downregulated genes were mostly involved in cell cycle, DNA repair, biosynthetic processes, bioenergetics, translation, telomere maintenance and splicing. Among the most relevant genes we found critical regulators of myelopoiesis and leukemogenesis, and leukemia-related genes involved in unfolded protein response. GSEA showed that kevetrin-treated cells shared the downregulation of glycolysis, DNA repair, unfolded protein response and MYC target gene sets. Our results show kevetrin alters several key genes and cellular metabolism. This study could provide a rationale for an experimental trial in AML patients, especially TP53-mutated ones who actually have very few therapeutic opportunities.

P187

ACUTE MYELOID LEUKEMIA CELLS DISREGULATE NORMAL HEMATOPOIESIS BY RELEASING EXTRACELLULAR VESICLES

S. Trino, I. Laurenzana, D. Lamorte, M. Santodirocco, L. Di Mauro, L. De Luca, A. Caivano, P. Musto

IRCCS "Referral Cancer Center of Basilicata" (CROB); IRCCS Casa Sollievo della Sofferenza, Italy

Acute myeloid leukemia (AML) is an aggressive and heterogeneous clonal disorder of hematopoietic stem/progenitor cells (HSPCs). During leukemogenesis, AML cells progressively occupy and likely alter bone marrow (BM) niche where normal HSPCs reside. Despite recent improvements in therapeutic approaches, only a fraction of AML patients is cured. Therefore, it is crucial to identify new molecular mechanisms driving the AML pathogenesis. Extracellular vesicles (EVs) are bi-layer membrane particles that shuttle a complex molecular cargo which can be transferred to target cells and alter their functions. Tumor derived EVs promote a tumor-supporting environment in non-malignant cells favoring cancer proliferation. We investigated the effect of leukemia derived-EVs (LEVs) on healthy umbilical cord blood HSPCs to explore their potential role in the induction of leukemic like-phenotype. EVs were isolated from AML cell lines by centrifugation steps and analyzed for AML surface markers by flow cytometry. Next, they were co-incu-

bated with healthy CD34+ sorted cells. After 24 h, cell count, apoptosis, HSPC-specific cluster of differentiation markers (Karamitros et al. Nat Immunol 2018), CXCR4 expression, colony-forming unit and migration assays were assessed. Our data showed: i) LEVs displaying specific AML surface markers; ii) a similar number of CD34+ cells in LEV treated-HSPCs, including HSCs, compared with controls; iii) no differences in apoptosis between the two groups; iv) an increase of multipotent progenitor (MPP) associated with a reduction of lymphoid-primed multipotent progenitors (LMPP) and multipotent lymphoid progenitors (MLP) in treated cells; v) an increase of common myeloid progenitors (CMP), together with reduction of granulocyte macrophage progenitors (GMP) and megakaryocyte erythroid progenitors (MEP) in treated group; vi) a reduction of colony-forming ability, including erythroid and granulocytic colonies, in treated cells; vii) decreased levels of CXCR4 in LEV treated-HSPCs, that were accompanied by reduced HSPC migration mediated by SDF-1. Altogether, this study suggests that AML derived-EVs can modify BM niche by dysregulating normal hematopoiesis and by promoting an increase of MPP and CMP (Figure 1). Furthermore, they seem to induce less attraction of HSPCs in BM niche by interfering with SDF1/CXCR4 axis. Strategies to block EV production and secretion, as well as EV induced-reprogramming, could be a novel exciting therapeutic approach in AML.

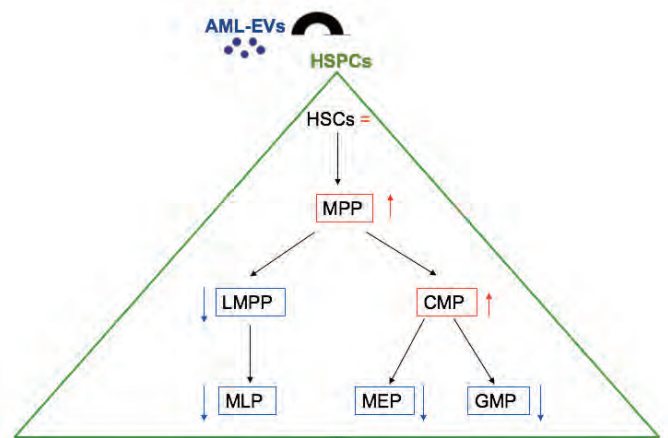


Figure 1. Effects of AML derived-EVs on normal hematopoiesis. Hematopoietic stem/progenitor cells (HSPCs) are a heterogeneous population including hematopoietic stem cells (HSCs), multipotent progenitor (MPP), lymphoid-primed multipotent progenitors (LMPP), multipotent lymphoid progenitors (MLP), common myeloid progenitors (CMP), granulocyte macrophage progenitors (GMP), megakaryocyte erythroid progenitors (MEP). The up and down arrows indicate an increase or a reduction of specific population, respectively. = indicates no change.

P188

OUTCOME OF RELAPSED APL PATIENTS IN THE ATRA AND ATO ERA: A SINGLE CENTRE EXPERIENCE

R. Frappampina¹, P. Carluccio¹, D. Pastore², A. Ricco¹, M. Delia¹, I. Attolico¹, T. Perrone¹, F. Albano¹, L. Anelli¹, L. Impera¹, G. Specchia¹

¹Policlinico Consorziale Universitario di Bari; ²Ospedale Antonio Perrino Brindisi, Italy

Background: The management and outcome of Acute Promyelocytic leukaemia (APL) has been revolutionized since the introduction of all-transretinoic acid (ATRA) and arsenic trioxide (ATO). Nonetheless, the recurrence rate remains 15-25%, mostly within 3-4y after the achievement of complete remission (CR).

Aim and Methods: We performed a retrospective analysis of 117 patients (Pts) affected by APL, diagnosed according to ELN criteria in our centre from 1998 to 2017. Treatment of 85(87.6%)pts was the combination of ATRA and Idarubicin (AIDA), and of 12(12.4%) was ATRA and ATO according to GIMEMA Protocols. We analyzed clinical and biological feature of 32(27%) Pts who relapsed after a median of 2y (range1.5-7.6).

Results: Clinical and biological features of relapsed patients were: 20 (62.5%) m and 12(37.5%) f, median age 45.5y. According to the FAB

classification 3(9.4%) were M3v; PML/RARA fusion gene type was bcr1 in 19(59.4%), bcr2 in 2(6.2%) and bcr3 in 11(34.4%). As to FLT3, 21(65.6%) Pts were negative, 6(18.7%) FLT3 ITD positive, 5(15.6%) FLT3 D835 positive. According to the risk stratification proposed by the PETHEMA/GIMEMA Groups, 12(37.5%) Pts were high risk, 14(46.8%) intermediate risk and 5(15.6%) low risk. The ATRA + ATO regimen was administered to 3(9.4%) patients and the combination of ATRA + chemotherapy to 29(90.6%). All pts achieved complete molecular and haematological remission (CMR) after induction and consolidation therapy. Then 15(46.9%) pts experienced haematological relapse, 11 (34.4%) isolated molecular relapse and 6(18.8%) molecular and extramedullary relapse (EMR). Among patients with EMR 3 patients had CNS involvement and 3 skin involvement. As 2nd line chemotherapy, pts with haematological relapse were treated with ATO+ATRA, 6 with molecular isolated relapse with ATO, 3 Pts with EMR (skin) with ATRA+ATO, 3 with EMR (CNS) relapse with ARACHD and ATRA. CMR after second line chemotherapy was obtained in 23(71.9%), 9(28%) not responder received other therapies; median OS was respectively of 6,4y(range 2,2-16,4) and 3,8 y(range 1,2-18).

Conclusions: In ATRA and ATO era, a high rate of CMR in APL pts relapsed after 1stline therapy. Isolated molecular relapses show a longer OS, underlying the importance of monitoring the molecular disease after CMR especially for intermediate and high risk patients. EMR is confirmed to be a rare event and CNS involvement to be associated with a shorter survival wondering for best strategies to prevent his recurrence.

P189

BITTER TASTE RECEPTORS ARE EXPRESSED AND FUNCTIONAL ON NORMAL CD34+ HAEMATOPOIETIC STEM CELLS

V. Pensato¹, V. Salvestrini¹, M. Cavo¹, A. Curti²

¹Department of Experimental Diagnostic and Specialty Medicine Institute of Hematology "L. and A. Seràgnoli" University of Bologna; ²Azienda Ospedaliero-Universitaria S. Orsola-Malpighi Department of Hematology and Oncology Institute of Hematology L. e A. Seràgnoli

Background: Haematopoietic stem cells (HSCs), from which all immune and inflammatory cells derive, are residing in bone marrow (BM). Findings suggest that HSCs, located in the BM and circulated in peripheral blood (PB), can sense the presence of danger or stress signals in the microenvironment. Bitter taste receptors (T2Rs) are typical G-protein coupled receptors normally located in the oral cavity. Recent studies showed that T2Rs are widely expressed in various tissue where they are involved in the regulation of physiological processes, thus suggesting a wider function in "sensing microenvironment". We recently reported that AML primary cells express fully functional T2R subtypes and, after their activation, leukaemia cells undergo down-regulation of genes involved in positive regulation of cell proliferation, migration, and cell cycle. Functional assays supported these **Results:** In the present work, we further investigated the T2Rs role by extending the analysis to normal HSCs

Methods: T2Rs expression was analyzed by Real-Time PCR and immunofluorescence. Functional assays were performed by culturing highly purified cord blood-derived CD34+ cells to the "bitter" agonist, denatonium benzoate (DEN). Cell viability and proliferation were analyzed by MTT assay. Apoptosis and progenitors subsets were evaluated by FACS analysis. Migration was determined by transwell assay.

Results: Cord blood-derived CD34+ cells expressed several T2R subtypes, with two significant differences compared to AML primary cells. Differently from AML cells, where the stimulation of T2Rs results in reduced viability and apoptosis induction, in normal CD34+ HSCs T2Rs activation with high dose of agonist does not affect viability and apoptosis. The presence of DEN in the transwell system does not affect the HSC migration, contrary to what happens for AML cells. However, DEN exposure significantly reduces HSCs clonogenic capacity.

Conclusions: Our results indicate that, although T2Rs receptor system is expressed and functional in both HSCs and AML cells, its activation exerts different effects in the two settings. These results need to further deepen but they may have implications for the discovery of novel pathways involved in the modulation of HSCs and leukemic cells and for the development of a new class of therapeutic molecules.

P190

BUFORMIN COMBINED WITH ASCORBATE SELECTIVELY INDUCES APOPTOSIS IN AML CELLS BUT NOT IN NORMAL BONE MARROW PRECURSORS THROUGH METABOLIC STARVATION

C. Banella, G. Guerrero, G. Catalano, D.F. Angelini, M.D. Divona, L. Battistini, M.T. Voso, F. Lo Coco, N.I. Noguera

Università di Roma Tor Vergata, Fondazione Santa Lucia, Italy

Acute myeloid leukemia downregulates glycolysis while mainly affects elderly subjects unfit for intensive chemotherapy. New therapies are often frustrated by clonal evolution and resistance. Agents interfering with altered metabolic pathways induced by cancer, including ascorbic acid (ASC), which is a potent pro-oxidant when used at high doses, and the anti-diabetic drug Buformin®, that completely shuts down mitochondrial contribution in ATP production, are currently tested in clinical trials. We sought to characterize metabolic pathways in different AML subsets, to assess the efficacy and mechanism of action of Buformin® (100 µM and 500 µM) in combination with ASC (1mM). Using the Seahorse XF Agilent, we compared the metabolism of normal promyelocytes with that of primary AML blasts (n=8 samples) and of different cell lines. We observed reduced respiratory capacity and mainly mitochondrial ATP production in primary AML. In Oci-AML3 and MV4;11 cell lines, ATP production decreased from 346 pmol/min to 63 pmol/min after 24 hrs of treatment. In Oci-AML3 cells, the glycolytic capacity decreased 4.7 times, explaining the higher sensitivity observed in Oci-AML3 cells versus MV4;11 and Oci-AML2. The analysis of ROS using Mitosox did not show an increase in H₂O₂ after treatment, probably due to the increase in the expression of ROS scavengers catalase and peroxidase. Primary blasts from eight AML patients, assayed for annexin and live/dead exclusion by flow cytometry, showed an increase in the apoptotic effect using the combination, as compared with ascorbate alone. The combination completely inhibited colony formation in MethoCult™ by OCI AML3 (Control 110; ASC 50; Buf + Asc: 14) and MV4;11 (Control 121; ASC 50; Buf + Asc: 20) cell lines, but not by normal bone marrow CD34+ cells (Control 111; ASC 149; Buf + Asc: 109). These data indicate that the combination is not toxic for normal cells and can be useful to target leukemic stem cells. Western blot analysis of BCL2 expression, on OCI AML2 (p<0,05), OCI AML3 (p<0,0001) and MV4;11 (p<0,0001) cell lines showed an evident decrease after treatment in a time dependent manner. Our data show that buformin combined with ASC decreases ATP production and downregulates glycolysis, adding to the apoptotic effect of ascorbate in primary blast from AML, sparing normal CD34+ bone marrow cells. The buformin-ascorbate combination could be an innovative therapeutic option for AML.

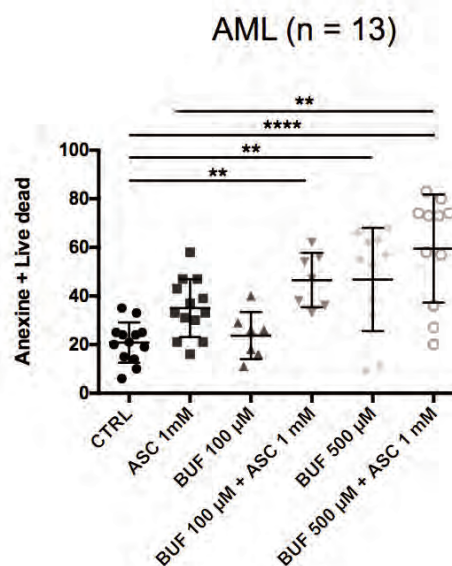


Figure 1. Apoptotic Efficacy of Buformin in Human Acute Myeloid Leukemia Cells. Cooperation with Ascorbic Acid. *p=0,05, **=p<0,005, **= p<0,0005**

P191

THE USE OF VENETOCLAX WITH HYPOMETHYLATING AGENTS (HMAs) FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA IN A REAL-LIFE SETTING: THE BOLOGNA EXPERIENCE

J. Nanni^{1*}, G. Cristiano^{1*}, G. Marconi¹, S. Parisi¹, C. Sartor¹, E. Ottaviani², L. Baldini¹, N. Testoni¹, C. Baldazzi¹, S. Paolini¹, M. Cavo¹, C. Papayannidis^{1°}, A. Curti^{2°}

*JN and GC equally contributed; ° CP and AC equally contributed

¹Department of Experimental, Diagnostic and Specialty Medicine, Institute of Hematology "L. and A. Seràgnoli", University of Bologna, Bologna, Italy; ²Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Department of Hematology and Oncology, Institute of Hematology L. e A. Seràgnoli, Bologna, Italy

Background: Relapse or refractory (R/R) acute myeloid leukemia is an unmet medical need. Although, the oral anti-apoptotic B-cell lymphoma 2 protein inhibitor venetoclax has shown strong activity in R/R AML in controlled clinical trials, no data are available in the real-life setting.

Methods: This is a single-center, retrospective study. Adverse events (AEs) were graded according CTCAE v4.03. Survival is estimated with Kaplan-Meier method.

Results: Thirty consecutive patients have been prescribed Venetoclax from Jun 2018 to Apr 2019. Fifteen patients completed at least 1 course of venetoclax (range 1-7) and are considered in this analysis. Median age was 70 years, (range 24-80). At diagnosis, 8/15 patients (53.3%) had a secondary AML and 7/15 patients (46.7%) had de novo AML. Among 13 evaluable patients, 7 (53.8%) had normal karyotype, 1 (7.6%) had inv(16), 1 had inv(3) (7.6%), 2 (15.3%) had complex karyotype and 2 patients (15.3%) had other alterations. Three out of 15 patients (20.0%) had FLT3 internal tandem duplication and 1/15 (6.7%) had NPM1 mutation. Patients received a median of 2 therapy courses (range 1-6). Seven out of 15 patients (46.7%) and 12/15 (80%) received previous chemotherapy or HMAs, respectively. Venetoclax was combined with azacitidine in 3/15 patients (20.0%) and with decitabine in 11/15 patients (73.3%). Thirteen out of 15 patients (86.7%) received a maximum dose of 400mg of venetoclax and 2 patients reduced to 200 mg for concomitant Posaconazole administration. The median follow-up is 62 days. We reported 11 adverse events in 9/15 patients (60%); most common adverse events were cytopenia (3/15, 20%) and infection (2/15, 13.3%). Most of the AEs were moderate in grade (9/11 AEs grade I/II, 2/11 grade II/IV, 0 fatal); 6/15 patients (40%) had a dose reduction or interruption for an AE and no patient had permanent withdrawal of Venetoclax. The response at 2 months was evaluable in 9/15 patients. Three out of 9 patients (33.3%) had a complete response, 2/9 (22.2%) had hematological improvement and 4/9 (44.4%) had stable disease. Two out of 15 patients (13.3%) received an allogeneic hematopoietic stem cell transplant after Venetoclax therapy. Median Overall Survival was 196 days (95% C.I. 67.7-324.3).

Interpretation: These data confirm that venetoclax plus HMAs has an acceptable toxicity profile and promising activity for R/R AML patients in a real-life setting.

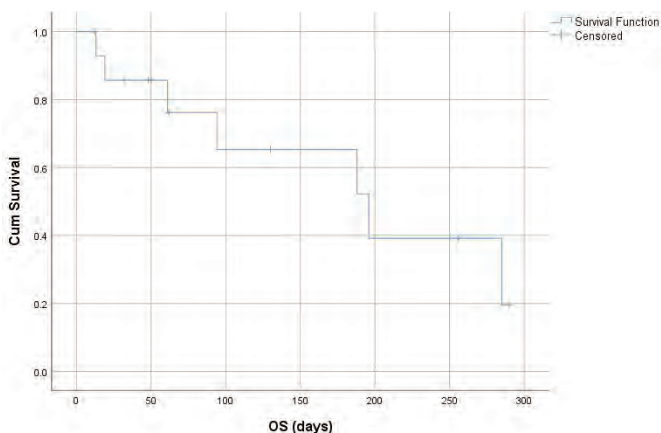


Figure 1.

P192

A SIMPLE CYTOFLUORIMETRIC SCORE MAY OPTIMIZE TESTING FOR BIALLELIC CEBPA MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

R. Marcolin, F. Guolo, P. Minetto, M. Clavio, F. Ballerini, L. Manconi, A. Carli, M. Passannante, A. Todiere, N. Colombo, E. Carminati, G. Pugliese, E. Tedone, P. Contini, R. Mangerini, A. Kunkl, M. Miglino, A. Cagnetta, M. Cea, M. Gobbi, R.M. Lemoli

Clinica Ematologica Dipartimento di Medicina Interna (DiMI), Università degli studi di Genova Policlinico San Martino IRCCS per l'Oncologia; Unità di Citofluorimetria, Dipartimento di Anatomia Patologica, Policlinico San Martino IRCCS per l'Oncologia

Acute myeloid leukemia with biallelic mutation of CEBPA (CEBPA-dm AML) is a distinct entity recognized by WHO 2016 classification, with a good prognosis according to European Leukemia Net 2017 risk stratification. However, testing for CEBPA mutation is challenging, due to the intrinsic characteristics of the genetic abnormality itself, that lacks hot spots and presents single nucleotide mutations. Therefore, this peculiar genetic abnormality cannot be studied with next generation sequencing technique and still requires Sanger sequencing. The association of recurrent mutations or translocations with specific immunophenotypic patterns has been already reported in other AML subtypes. The primary aim of this study was the development of a specific cytofluorimetric score (CEBPA-dm score), in order to distinguish patients who are unlikely to harbor the mutation. As secondary endpoint the impact of CEBPA-dm score on survival compared to other known variables was assessed. The correlation of a seven-point immunophenotypic score (CEBPA-dm score) with the presence of CEBPA mutation was analyzed in 50 young, de novo AML patients with normal karyotype and without NPM1 mutation (that is mutually exclusive with CEBPA-dm). One point each was assigned for expression of HLA DR, CD7, CD13, CD15, CD33, CD34 and one point for lack of expression of CD14. The surface antigens included in the score were selected according to previous reports on immunophenotypic features of AML patients with CEBPA-dm. Because of its high negative predictive value (100%), a CEBPA-dm score < 6 was able to identify those patients who were unlikely to have the mutation. Therefore, since immunophenotypic evaluation is always done in AML diagnostic work up, the application of this simple score as a screening test might prevent an inappropriate utilization of molecular assessment for CEBPA. In our series we observed that OS was not influenced by CEBPA-dm score. Multivariate analysis showed that CEBPA-dm ($p < 0.02$) and FLT3-ITD ($p < 0.01$) were the strongest independent predictors of OS, underlining that molecular aberrations are more relevant than the bare combination of surface antigens.

P193

EFFICACY AND SAFETY OF VENETOCLAX IN COMBINATION IN RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA: REAL LIFE EXPERIENCE OF "RETE EMATOLOGICA PUGLIESE" (REP)

V. Federico¹, M. Dargenio¹, C. Pasciolla², G. Guaragna³, R. Matera¹, L. Melillo⁴, M. D'Elia⁵, N. Cascavilla⁴, D. Pastore³, A. Guarini², G. Specchia⁵, N. Di Renzo¹

¹UOC Ematologia e Trapianto di cellule Staminali - PO "Vito Fazzi"; ²UOC Ematologia - IRCCS "Giovanni Paolo II"; ³UOC Ematologia -PO "Perrino-Di Summa"; ⁴UOC Ematologia e Trapianto di Midollo Osseo - IRCCS "Casa Sollievo della Sofferenza"; ⁵UOC di Ematologia con Trapianto - AOU "Policlinico"

Acute myeloid leukemia (AML) is associated with worse clinical outcomes, and often persist after conventional chemotherapy resulting in relapse. Our understanding of the genomic landscape of AML has improved prognostic accuracy and lead to the development of targeted therapies. The oral anti-apoptotic B-cell lymphoma 2 protein inhibitor, venetoclax, has shown promising single-agent activity in patients with AML and preclinical data suggested synergy between hypomethylating agents or low-dose cytarabine combined with venetoclax. Patients were enrolled into one of three groups: venetoclax and subcutaneous azacitidine 75 mg/m² [days 1-7 of each 28-day cycle] (Group A), venetoclax and intravenous decitabine 20 mg/m² [days 1-5 of each 28-day cycle] (Group B) and venetoclax with subcutaneous low dose cytarabine

(LDAC) 20 mg/m² [days 1-10 of each 28-day cycle] (Group C). Dose escalation was followed by venetoclax 400 mg per day orally in 28-day cycles. The study objectives were efficacy and safety of venetoclax in combination with hypomethylating agents or low dose cytarabine. Thirteen patients were enrolled in this observational study between February 2018 and February 2019, 7 patients in group A, 4 patients in group B and 2 patients in group C. Median age was 72 years (range, 39 to 78 years), the median of previous lines of chemotherapy was 1 (range 1-4). Sixty-two percent, had prior hypomethylating agents and 38% intensive chemotherapy treatment respectively. Nine patients (70%) had relapsed AML and 4 (30%) refractory. The median time from diagnosis to start Venetoclax was 11 months (range 5-78). Common grade 3 or greater adverse events were anemia (58%); febrile neutropenia (31%), thrombocytopenia (45%). No early death (30-day) was observed. Complete remission with or without complete blood count recovery were 31% and 23% respectively, (median time to first response, 2 months). After a median follow up 3 months (range 2-14), 8 patients were alive and continues treatment. One patient died in partial remission of pseudomonas sepsis and 4 patients of disease progression. This our promising results need to be confirmed in a larger series of patients. However this combination should be considered for this setting of patients not eligible for more intensive treatment.

Table 1.

	TOTAL (%)
Number of patients	13
Sex:	
• Male	7 (54%)
• Female	6 (46%)
Age, median (range)	72 years (39-78)
Cytogenetics:	
• Intermediate risk	9 (69%)
• Poor risk	4 (31%)
Molecular:	
• Favorable risk	5 (38%)
• Intermediate and Poor risk	8 (62%)
Baseline bone marrow blast count:	
• 20-30%	5 (35%)
• 31-50%	1 (5%)
• >50%	6 (50%)
• Median %-range	45 (20-80)
Baseline white blood cells (10x9/L), median (range)	8.360 (1.530-66.260)
No. of previous lines, median (range)	1 (1-4)
Number of cycles, median (range)	2 (2-7)
CR	4 (31%)
CRi	3 (23%)
PR	1 (8%)
ORR	62%
Sepsis	3 (24%)
• Escherichia bacteraemia	1 (8%)
• Pseudomonas bacteraemia	1 (8%)
• KPC bacteraemia	1 (8%)
Fungal infection	3 (24%)
• Candida Albicans	8 (16%)
• Aspergillus	1 (8%)

P194

ANTI-TUMOR ACTIVITY AND MECHANISMS OF ACTION OF PHYTOEXTRACTS ON ACUTE MYELOID LEUKEMIA CELLS

D. Lamorte, I. Laurenzana, S. Trino, I. Faraone, L. Milella, D. Russo, L. De Luca, A. Caivano, P. Musto

IRCCS "Referral Cancer Center of Basilicata" (CROB), University of Basilicata, Italy

Acute myeloid leukemia (AML) is an aggressive and heterogeneous disorder characterized by an abnormal proliferation and differentiation of myeloid precursors. Despite advances in understanding AML pathogenesis, the standard therapy remained unchanged over the past three decades. Novel therapies, including the refinements of conventional cytotoxic chemotherapies and targeted drugs have been developed in recent years. However, adverse events, toxicities and relapse are still problems. Thus, the development of more effective novel drugs having less side-effects is needed. Plant extracts represent new potential anti-tumor drugs thanks to their large structural diversity, low toxicity, favorable profile of absorption and metabolism. Of note, they affect malignant cell proliferation. We recently reported that extracts of *Azorella glabra* (AG) plant display anti-multiple myeloma activities in vitro, including apoptosis, cell cycle arrest and inhibition of cell-migration. We investigated the in vitro effects of AG extracts on AML and healthy cells. Twelve fractions were obtained from AG aerial parts by silica gel column chromatography. A FLT3-wild type leukemic cell line, KG1, and another carrying FLT3-ITD mutation, MV4-11, were used. Peripheral blood mononuclear cells (PBMCs) were isolated from 5 healthy donors. All the AG fractions, at different concentrations (10-50 g/ml), were co-incubated with AML cells or PBMCs for 24-72 hours. Viability, apoptosis and cell cycle were assessed by colorimetric assay and flow cytometry, respectively. We found that the majority of AG fractions induced a reduction of AML cell viability in a dose and time dependent way. In particular, two fractions showed major effects at low concentration. Interestingly, MV4-11 resulted more sensitive to AG extract action than KG1. In addition, in MV4-11, these two AG fractions reduced the viability of 50% at concentration lower than 10 g/ml (EC₅₀). The reduced viability was accompanied by an increased apoptosis and cell cycle arrest in G₀/G₁ phase in both AML treated cells. Importantly, AG fractions did not show toxicity towards healthy PBMCs. These data suggest AG extracts as potential anti-tumor natural agents. Further investigations are ongoing to identify specific bioactive compounds, to investigate the molecular mechanisms underlying anti-cancer activity and to define how these natural extracts could be used as an effective and well tolerated complementary approach to current therapies of AML.

P195

EFFECT OF THE CONVENTIONAL HIGH CLINICAL RISK FACTORS IN A SUBSET OF ACUTE MYELOID LEUKEMIA WITH FAVOURABLE CYTOGENETIC-MOLECULAR RISK (NPM MUTATED, FLT3-ITD UNMUTATED AND NORMAL KARYOTYPE)

M.V. Dubbini, D. Lazzarotto, M.E. Zannier, C. Fili, N. Rabassi, G. Facchin, C. Cigana, R. De Marchi, D. Griguolo, S. Frisoli, D. Schiavo, R. Stella, V. Radici, C. Rosignoli, P. Lauzzana, M. Girgenti, E. Simeone, A. Candoni, R. Fanin

Division of Hematology and Stem Cell Transplantation, Azienda Sanitaria Universitaria, Integrata di Udine, Italy

Introduction: Acute Myeloid Leukemia (AML) with NPM mutated (NPM+), FLT3-ITD unmutated (FLT3-) and Normal Karyotype (NK) are included in the favourable cytogenetic-molecular risk class according to the 2010 and 2017 ELN classifications. Today, in clinical practice, to define the AML prognosis, we focus mainly on cytogenetic-molecular risk regardless of clinical factors and specifically secondary AML and high blast cells count (BC) in peripheral blood (PB) at the disease onset.

Patients and Results: We analyze clinical characteristics, response to chemotherapy (Complete Remission-CR), relapse rate (RR) and outcome of a subgroup of 33 AML NPM+/FLT3-/NK cases that were homogeneously treated at our Center in the last 10 years. The median age was 61 years (range 20-72) and 24% of cases had more than 65 years. Five (15%) AMLs were secondary to a previous Myelodysplastic Syndrome and 40% (13/33) had more than 30.000/mm³ BC in PB at onset

(High Clinical Risk group-HCR). Overall 52% of AML cases were HCR at diagnosis. All 33 patients (pts) were treated with FLAI scheme as induction, followed by Ara-C+Ida and high dose Ara-C, as consolidation therapy. The CR after FLAI was 88% (29/33 cases); 9% of pts were resistant and DDI was 3%. After a median follow-up of 18 months (2-120), 55% of pts (18/33) are alive and 45% (15/33) died. The Relapse rate (RR) was 33% (10/30) and 67% (8/12) of relapsed pts had HCR at diagnosis. Allo-SCT rate was 36% (12/33) but 83% (10/12) of Allo-SCT were performed in HCR pts and mainly after relapse. The probability of OS at 12 and 24 months was 70% and 54%, respectively. The probability of DFS at 12 and 24 months was 63% and 51%, respectively. The OS (log-rank, $P=0,98$) and DFS (log-rank, $P=0,69$) did not differ between HCR group and pts without HCR factors but we underline that a significantly higher proportion of HCR pts received Allo-SCT (10/17 HCR cases vs 2/16 other cases, $P<0,05$) mainly after their first relapse.

Conclusions: In our experience, the AML with NPM+/FLT3-/NK have a 24 months DFS probability of 51% with relapse occurring mainly (67% of relapses) in pts with HCR (high PB-BC and secondary AML). For this HCR population early and close monitoring of minimal residual disease (quantitative NPM) should be performed in order to avoid cytological relapse and to promptly decide, for the MRD positive cases, a consolidation with Allo-SCT.

P196

INCIDENCE OF ACUTE PROMYELOCYTIC LEUKEMIA IN DIFFERENT AREAS OF BASILICATA. POSSIBLE INVERSE CORRELATION WITH ENVIRONMENT ARSENIC CONCENTRATION

M. Pizzuti¹, R. Galasso², S.P. Pascale¹, R. Nuccorini¹, S. Coluzzi¹, A. Amendola¹, M. Cimminiello¹, N. Filardi¹, A. Maturro¹, D. Vertone¹, I. Chitarelli⁶, C. Mannarella³, C. Bitetti³, B. Daraia³, A. Ciancio³, A. Fragasso³, G. Pietrantuono⁴, O. Villani⁴, G. Mansueto⁴, G. D'Arena⁴, A. Palma⁵, P. Musto⁴

¹UOC Ematologia AOR SAN CARLO, Potenza; ²UO Epidemiologia Clinica, Biostatistica, Registro Tumori Basilicata IRCCS CROB Rionero in Vulture; ³UO Ematologia Ospedale Madonna delle Grazie, Matera; ⁴UOC Ematologia IRCCS CROB Rionero in Vulture; ⁵CRM ARPAB; ⁶U.O.S.P. Centro Trasfusionale Lagonegro

Acute promyelocytic leukemia (APL) has shown a high sensitivity to Retinoic Acid and Arsenic Trioxide so it is often curable even without the use of chemotherapy. Many studies have highlighted the spatial and temporal clustering of APL, supporting the hypothesis of specific environmental risk factors. Since first GIMEMA reports, the border area between Basilicata and Calabria has been characterized by a higher incidence of APL. Aim of the study is to verify the distribution in Basilicata of APL cases diagnosed from 2005 to 2016. The territory of Basilicata has been divided into POIS (7 areas plus the two provincial capitals). In the 2005-2016 period 23 APL, 11 males aged 49 (35-77) and 12 females aged 45 (23-72) were registered by the Basilicata Cancer Registry. The ratio between the incidences Lagonegrese Pollino/Basilicata is equal to 2.09, Marmo Platano Melandro/Basilicata is 1.90 and Val D'Agri/Basilicata is 2.04. This increased incidence is currently not significant due to the low number of cases. However, the previous observation (1990-2005) concerning the border area with Calabria (Lagonegrese Pollino) has been confirmed for the 2005-2016 period and now it is highlighted as the "west area" has an incidence approximately double compared to the entire Basilicata. The cause of APL spatial clustering is not yet known. We have hypothesized that there may be an inverse correlation between incidence of APL and the environmental concentration of Arsenic (As). The concentration of As is today monitored in Basilicata along the course of all rivers. The potential toxicity threshold has never been exceeded, but there are clear differences among the different river basins. If we consider on Basilicata map the areas with the highest incidence of APL (blue color) and we indicate the As concentrations found in the years 2017-2018 we can see how concentration of As is lower where APL cases are more numerous. The analysis is partial for the small number of pts and for the different periods of observation of the diagnoses (2005-2016) and of measurements of As concentrations (2017-2018). However, the inverse correlation between APL and As concentration seems to be a stimulating hypothesis that deserves to be verified also in other Italian Regions.

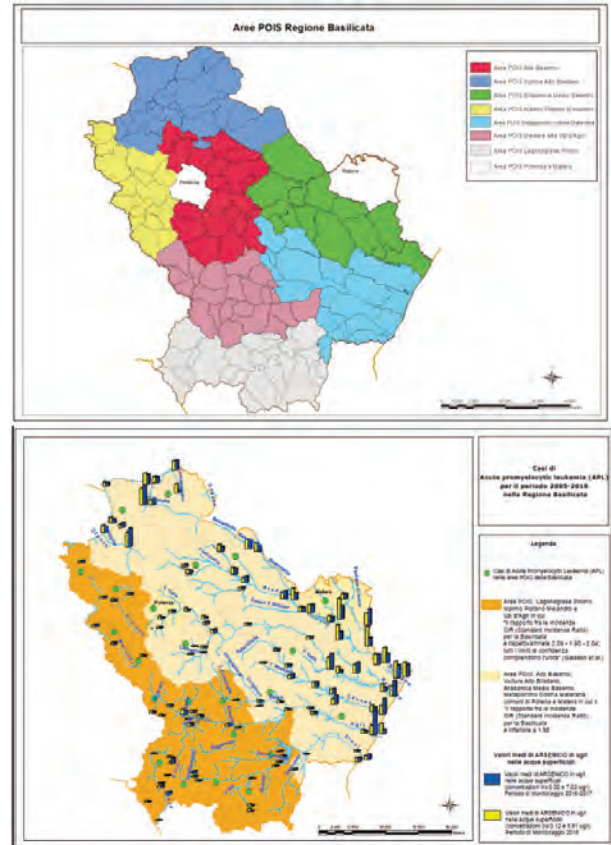


Figure 1.

Chronic Myeloid Leukemia

P197

SECOND GENERATION TYROSINE KINASE INHIBITORS AFTER IMATINIB FIRST-LINE: A 13-YEARS FOLLOW-UP EXPERIENCE

L. Rizzo, G. Caocci¹, E. Scalzulli, D. Alunni Fegatelli, M. Porrizzo, G. Colafigli, M. Molica, I. Carmosino, D. Diverio, R. Latagliata, R. Foà, M. Breccia

Hematology, Department of Translational and Precision Medicine, Policlinico Umberto 1, Sapienza University; ¹Hematology Unit, Businco Hospital, Department of Medical Sciences and Public Health, University of Cagliari, Italy

Background: Chronic phase chronic myeloid leukemia (CP-CML) patients failing first-line imatinib for toxicity or resistance require an effective second-line option. A “real-life” direct comparison of dasatinib and nilotinib second line is lacking.

Materials and Methods: We retrospectively analysed 131 patients followed between 2005 and 2018; all failed first-line imatinib either because of inefficacy (111, 84.7%) or for severe toxicity (20, 15.3%). As second-line treatment, these patients received dasatinib (72, 54.9%) or nilotinib (59, 45.1%), according to the baseline mutation profile, concomitant comorbidities and previous toxicity.

Results: One hundred and twenty patients were evaluable for molecular response at 6 months from the switch: 21 patients (31.3%) obtained a MR3, 9 (13.4%) an MR4-4.5 and 37 (55.2%) did not achieve a molecular endpoint (ratio >0.1-<1) with dasatinib. We found similar results in the nilotinib-treated patients (p=0.591): 14 patients (26.4%) improved to MR3, 5 (9.4%) to MR4-4.5 and 34 (64.2%) did not achieve a molecular endpoint (>0.1-<1). After 12 months of treatment, 108 patients were evaluable: 31 (47%) obtained a MR3, 12 (18.2%) a deep MR and 23 (34.8%) persisted with a BCR-ABL1 ratio >0.1-<1% with dasatinib, compared to 18 patients (37.5%) in MR3, 2 (16.2%) in MR4-4.5 and 22 (45.8%) with molecular residual disease >0.1-<1% with nilotinib (p=0.4806). After 24 months of continued exposure to 2 gen TKIs, 19 patients (37%) treated with dasatinib reached a deep MR with only 15 (30%) showing a persistent BCR-ABL1 ratio >0.1-<1%, compared to 11 patients (28%) treated with nilotinib who reached deep MR and 11 (28%) with molecular residual disease <1% (p=0.5). Non-hematologic treatment-related toxicities were observed in 48.8% of dasatinib-treated patients (grade ≥2-3 pleural effusions - recorded in 24 patients (33%) - being the most frequent) and in 21.4% of the nilotinib group (grade ≥2-3 thromboembolic events - observed in 6 patients (10%), p=0.003). At the last follow-up, 94 patients (71.7%) are still in treatment with similar efficacy between the two drugs (p=0.15).

Conclusions: In our “real-life” experience, dasatinib or nilotinib as second-line treatment seem to induce similar long-term molecular responses. Non-optimal responders could improve their baseline residual disease even after 12 months of continuous exposure to the same TKI. However, related specific toxicity should be considered according to baseline comorbidities to avoid long-term related side effects.

P198

PERFORMANCE OF EUTOS LONG TERM SURVIVAL (ELTS) SCORE AND EFFICACY OF FIRST LINE TREATMENT IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS: RESULTS OF THE CML ITALIAN MULTICENTER OBSERVATIONAL STUDY (CML-IT-MOS)

P. Pregno¹, F. Stagno², M. Breccia³, F. Castagnetti⁴, M. Bonifacio⁵, I. Capodanno⁶, G. Caocci⁷, B. Martino⁸, N. Di Renzo⁹, M. Pizzuti¹⁰, E. Lucia¹¹, E. Crisà¹², A. Gozzini¹³, M. Tiribelli¹⁴, G. Rege Cambrin¹⁵, S. Sica¹⁶, S. Galimberti¹⁷, M. Bergamaschi¹⁸, L. Levato¹⁹, A. Guarini²⁰, P. Mazza²¹, D. Pastore²², N. Cascavilla²³, C. Aguzzi²⁴, L. Luciano²⁵, E. Abruzzese²⁶, E. Di Bona²⁷, A. Lucchesi²⁸, F. Falzetti²⁹, G. La Barba³⁰, S. Rupoli³¹, A. Guella³², M. Nicolosi¹, F. Saccona³³, C. Monagheddu³³, F. Albano³⁴, F. Pane²⁵, G. Ciccone³⁵, G. Rosti⁴, M. Baccarani⁴, G. Saglio³⁵, G. Specchia³⁴

¹S.C. Ematologia, Città della Salute e della Scienza di Torino; ²Unità di Ematologia, Ospedale Ferrarotto; ³Istituto di Ematol e Biotech Cell Univ. La Sapienza - Policlinico Umberto I; ⁴Istituto di Emat e Onc Medica - Policlinico S. Orsola-Malpighi; ⁵UO di Ematologia - AOU Integrata Verona; ⁶UOA Ematologia AO Arcisp S. Maria Nuova; ⁷Div. di Ematologia - Ospedale Businco; ⁸U.O.A. di Ematologia - A.O. “Bianchi-Melacrino-Morelli”; ⁹Divisione Ematologia Osp Vito Fazzi; ¹⁰Div. di Ematologia - Ospedale San Carlo; ¹¹Ematologia e Immunematologia A. O. Cosenza; ¹²Divisione di Ematologia, Università Piemonte Orientale; ¹³Ematologia - Università degli Studi di Firenze - AOU Careggi; ¹⁴Clinica Ematologia e Trapianto Midollo Osseo - AOU Santa Maria della Misericordia; ¹⁵SCDU Medicina Interna AOU San Luigi Gonzaga; ¹⁶Cattedra di Ematologia Un Cattolica Policlinico Gemelli; ¹⁷UO Ematologia Uni Osp Santa Chiara; ¹⁸Dip. di Emato-Oncologia - Clinica Ematologica - AOU San Martino; ¹⁹Div Ematologia AO Pugliese-Ciaccio; ²⁰DIV ematologia IRCCS Osp Oncologico; ²¹S.C. Ematologia POC SS. Annunziata; ²²Div. Ematologia- Pres Osp. Perrino A.; ²³S.C. Ematologia IRCCS Osp Sollievo della Sofferenza; ²⁴S.C. Ematologia U., Città della Salute e della Scienza di Torino; ²⁵U.O. di Ematologia - II Università Federico II; ²⁶DIV Ematologia ASO S. Eugenio; ²⁷Div. Ematologia Os S. Bortolo; ²⁸S.C. Oncologia Medica Ist Sc Romagnolo per lo Studio e Cura dei Tumori; ²⁹Ematologia - AOU di Perugia - Ospedale S. Maria della Misericordia; ³⁰Div. Universitaria di Ematologia - ASO Spirito; ³¹Clinica di Ematologia - Dip. Scienze Mediche e Chirurgiche - Ospedali Riuniti - Università Politecnica delle Marche; ³²SS Ematologia - U.O. Medicina II - Presidio Ospedaliero Santa Chiara, 1 S.C. Ematologia, Città della Salute e della Scienza di Torino; ³³SSD Epidemiologia Clinica e Valutativa - CPO Piemonte. AOU Città della Salute e della Scienza di Torino; ³⁴Cattedra di Ematologia - AOU Policlinico di Bari, 25 U.O. di Ematologia - II Università Federico II, 4 Istituto di Ematologia e Oncologia Medica - Policlinico S. Orsola-Malpighi; ³⁵Dip di Scienze Biol e Cliniche Università di Torino, 34 Cattedra di Ematologia - AOU Policlinico di Bari, Italy

Background: The CML patients (pts) risk may be defined by ELTS or Sokal score, both validated in imatinib (IMA) era and it's interesting to compare IMA vs II Generation Tyrosine Kinase Inhibitors (IIGen-TKI) on Overall Survival (OS) in real life.

Aim: To assess performance of ELTS score in real life in a CML population treated in first line with IMA or IIGen-TKI and to compare response and OS according to therapy used. **Methods:** We retrospectively and prospectively recorded clinical and biological data on all newly diagnosed CML pts referred to 66 Italian Hematology Centers of the GIMEMA Study Group from January 2012 to December 2016.

Results: We enrolled 1111 newly CML pts with complete ELTS score: there were 99% chronic phase, 0.4% accelerate phase and 0.7% blast crisis. According to ELTS, 705 (63.5%), 279 (25%), 127 (11.4%) were low (LR), intermediate (IR) and high risk (HR). Among 1090 pts with complete Sokal score 405 (37.2%), 486 (44.6%), 199 (18.3%) were LR, IR and HR. Table 1 summarizes all data. Therapy was IMA in 517 (46.5%) pts vs IIGEN-TKI in 594 (53.4%). Major comorbidities were cardiovascular, lung, metabolic diseases and cancer, mainly in IMA group. Molecular response (MR) at 3-6-12-24 months confirmed that IIGen-TKI was able to obtain higher, earlier and deeper MR than IMA. Five years-OS by ELTS in LR, IR and HR pts treated with IMA or IIGen-TKI were 91.3%, 79.9%, 73.2% (p<0.001) vs 98.5%, 98.2%, 86.5% (p<0.0001), respectively. Five years-OS by Sokal in LR, IR and HR pts treated with IMA or IIGen-TKI were 94.3%, 79.9%, 73.2% (p<0.2935) vs 98.1%, 98.3%, 93.2% (p<0.0122) respectively. Stratifying pts by therapy, ELTS confirmed high prognostic value in IMA group, while ELTS in IIGen-TKI did not differentiate between LR and IR because OS was

very good in both groups. Cumulative 5 years-OS was 92.2%, statistically significant better with IIGEN-TKI than IMA (Crude HR=3.37, 95%CI 89.3-94.3); while comparing therapy with Cox model (adjusting for age, comorbidities, ELTS), OS was not statistically significant inferior with IMA vs IIGen-TKI (HR=1.60; 95%CI 0.83-3.11).

Conclusions: Our analysis showed that IMA treated pts were older and with higher number of comorbidities than IIGen-TKI group. Also in real life IIGen-TKI was able to obtain higher, earlier and deeper MR than IMA. Lastly, stratifying pts by therapy used the ELTS score did not differentiate OS between LR and IR in IIGEN-TKI treated pts, due to achievement of similar OS in both groups.

Table 1. Clinical features, molecular response at 3, 6, 12 and 24 months and overall survival of 1111 CML patients, by first line treatment.

IMA + IIGEN-TKI (n=1111)	IMA (n=517)	IIGEN-TKI (n=594)
Median age; 59 years	66 (55-75)	52 (41-62)
Charlson Comorbidity Index ≥ 1 (n=1089)	31.4%	16.3%
Molecular Response at 3 months	N evaluated= 418 (81%)	N evaluated= 416 (70%)
MMoLR; n (%)	20 (5%)	100 (24%)
MR 4; n (%)	7 (2%)	30 (7%)
MR 4.5; n (%)	4 (1%)	14 (3%)
MR 5; n (%)	4 (1%)	12 (2.8%)
Molecular Response at 6 months	N evaluated= 385 (74%)	N evaluated= 439 (74%)
MMoLR; n (%)	123 (32%)	210 (47.8%)
MR 4; n (%)	27 (7%)	73 (16.6%)
MR 4.5; n (%)	13 (3%)	42 (9.5%)
MR 5; n (%)	10 (2.6%)	32 (7.3%)
Molecular Response at 12 months	N evaluated= 357 (69%)	N evaluated= 437 (74%)
MMoLR; n (%)	186 (52%)	292 (67%)
MR 4; n (%)	75 (21%)	148 (34%)
MR 4.5; n (%)	43 (12%)	95 (22%)
MR 5; n (%)	30 (8.4%)	75 (17%)
Molecular Response at 24 months	N evaluated= 341 (66%)	N evaluated= 392 (66%)
MMoLR; n (%)	249 (73%)	319 (81.3%)
MR 4; n (%)	134 (39%)	189 (48%)
MR 4.5; n (%)	76 (22%)	127 (32%)
MR 5; n (%)	56 (16%)	106 (27%)
Overall survival		
Deaths (median FU 54 months) (n=53)	39 (5 CML related)	13 (5 CML related)
5 years-OS 92.2% (95% CI 89.3-94.3)	86%	97.0%
Crude HR (95%CI)	3.37 (1.82-6.21)	1
Cox model HR (95%CI) adjusted by age, comorbidity and ELTS	1.60 (0.83-3.11)	1

Transplant Unit, Azienda Ospedaliera Papa Giovanni XXIII; ¹⁰Hematology Unit, AZ OSP. CITTA' DELLA SALUTE E SCIENZA; ¹¹Department of Hematology, Spedali Civili di Brescia; ¹²Unita Operativa Ematologia, Ospedale Ca' Foncello; ¹³Haematology Clinic, Ospedali Riuniti di Ancona; ¹⁴Hematology Section, Azienda Ospedaliero-Universitaria S. Anna; ¹⁵Chair of Hematology, Department of Medicine and Experimental Oncology, University of Torino; ¹⁶Hematology Unit, "A.Busino" Hospital; ¹⁷Department of Hematology and Transplants, University of Siena; ¹⁸Division of Hematology and BMT, Department of Experimental and Clinical Medical Sciences, Azienda Ospedaliero-Universitaria di Udine; ¹⁹Hematology Unit, Sant'Andrea Hospital; ²⁰Institute of Hematology, Catholic University; ²¹Chair of Hematology, University of Verona; ²²Hematology Unit, Fondazione IRCCS Policlinico San Matteo; ²³Department of Biochemistry and Medical Biotechnologies, Federico II University; ²⁴Hematology Unit, Mauriziano Hospital, University of Turin; ²⁵Institute of Hematology, University of Bologna, Italy

Background: A major molecular response (MMR) by the first year of treatment is a key therapeutic CML goal. The clinical significance of higher transcript levels after the first year is still unclear and expert treatment recommendations (ELN, ESMO, NCCN) are partially conflicting. New data are strongly required.

Aim: to assess the clinical significance of MR2 (transcript 0.1-1%) at 1 year and beyond.

Methods: Retrospective analysis of 559 patients enrolled within 3 prospective trials (NCT00514488, NCT00510926, observational trial CML/023); ITT population of each study. Molecular responses at 12, 18, 24, 30 months were evaluated. Definitions: MR1, BCR-ABL1 >1%; MR2, BCR-ABL1 0.1-1%; MMR or MR3, BCR-ABL1 0.01-0.1%; MR4, BCR-ABL1 <0.01%; progression, according to ELN criteria; leukemia-related death (LRD): death after progression.

Results: Median age 52 years (18-84 years). High risk patients 22% and 15% according to Sokal and ELTS score, respectively. Median follow-up 76 months (66-99 months). The depth of molecular response improved over time: at 12 months, patients with MR1, MR2, MR3, MR4 were 8%, 25%, 38%, 30%; at 18 months, 5%, 14%, 37%, 43%; at 24 months 3%, 10%, 31%, 56%; at 30 months 2%, 9%, 27%, 62%, respectively. The OS and LRD probabilities were slightly lower in patients with higher transcript levels, but differences were not significantly different. Progressions were 15%, 6%, 5%, 4% in patients with MR1, MR2, MR3, MR4 at 12 months; 6%, 4%, 6%, 7% in patients with MR1, MR2, MR3, MR4 at 18 months; 4%, 7%, 4%, 4% in patients with MR1, MR2, MR3, MR4 at 24 months; 13%, 5%, 5%, 4% in patients with MR1, MR2, MR3, MR4 at 30 months (p=NS; more frequent in high risk patients). MR1 and MR2 patients at different milestones were subsequently switched to other TKIs in 67-77% and 21-25% of cases, respectively (switch not prospectively planned, mostly performed after further worsening, including cytogenetic and hematologic relapse). The time to MMR strongly predicted the achievement and the stability of MR4, but interestingly 39-46% of MR2 patients at 12, 18, 24, 30 months subsequently achieved a MR4 (55-72% of MR3 patients at same timepoints; p<0.001).

Conclusions: MR2 patients at 12, 18, 24 and 30 months had similar outcome to MR3 patients; in contrast, the probability of MR4 was significantly lower. The potential benefit of a treatment change should be investigated in a prospective trial. The biological reasons of this slow response need to be elucidated.

P199

CLINICAL SIGNIFICANCE OF MR2 AT ONE YEAR AND BEYOND: A GIMEMA CML WP ANALYSIS ON 559 CML PATIENTS TREATED FRONTLINE WITH IMATINIB MESYLATE

F. Castagnetti¹, G. Gugliotta¹, M. Breccia², F. Stagno³, A. Iurlo⁴, F. Albano⁵, E. Abruzzese⁶, B. Martino⁷, L. Levato⁸, T. Intermesoli⁹, P. Pregno¹⁰, M. D'Adda¹¹, E. Calistri¹², S. Rupoli¹³, F. Cavazzini¹⁴, D. Ferrero¹⁵, G. Caocci¹⁶, M. Bocchia¹⁷, M. Tiribelli¹⁸, E. Montefusco¹⁹, F. Sora²⁰, M. Bonifacio²¹, C. Elena²², S. Soverini¹, R. Foà², M. Cavo¹, F. Pane²³, G. Saglio²⁴, M. Baccarani²⁵, G. Rosti¹

¹Institute of Hematology, S. Orsola-Malpighi University Hospital; ²Chair of Hematology, "Sapienza" University; ³Chair of Hematology, University of Catania; ⁴Division of Hematology, IRCCS Maggiore Policlinico Hospital Foundation; ⁵Hematology - Dept. of Emergency and Organ Transplantation, University of Bari; ⁶Hematology, S. Eugenio Hospital; ⁷Az. Osp. Bianchi-Melacrino-Morelli; ⁸Section of Hematology, A.O. Pugliese-Ciaccio; ⁹Hematology and Bone Marrow

P200

PH+ STEM CELLS (SCS) IN CHRONIC MYELOID LEUKEMIA (CML): TO ERADICATE OR NOT TO ERADICATE, THIS IS THE QUESTION

E. Pungolino¹, M. D'adda², A. Trojani¹, A. Perego³, C. Elena⁴, A. Iurlo⁵, S. Malato⁶, M. Turrini⁷, G. De Canal⁸, L. Borin⁹, L.E. Bossi¹, M. Caramella¹, S. Artale¹⁰, F. Spina¹¹, M.L. Latargia¹², M. Anghileri¹³, P. Spedini¹⁴, M.C. Carraro¹⁵, B. Di Camillo¹⁶, D. Gramegna², M.L. Pioltelli¹, G. Rossi², E. Morra¹⁷, R. Cairoli¹

¹Department of Hematology ASST Grande Ospedale Metropolitano Niguarda Milano; ²Department of Hematology ASST Spedali Civili di Brescia Brescia; ³Desio Hospital Internal medicine-Hematology Desio; ⁴Department of Hematology Oncology Foundation IRCCS Policlinico San Matteo Pavia; ⁵Hematology Division Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan Milano; ⁶San Raffaele Scientific Unit Hematology and Bone

Marrow Transplantation Unit Milano; ⁷Valduce Hospital Dep. of Internal Medicine Como; ⁸Pathology Dep Cytogenetics ASST Grande Ospedale Metropolitano Niguarda Milano; ⁹San Gerardo Hospital Monza; ¹⁰ASST Valle Olona Sant'Antonio Abate Gallarate; ¹¹Fondazione IRCCS Istituto Tumori Division of Hematology Milano; ¹²ASST Valle Olona Ospedale di Circolo Busto Arsizio; ¹³ASST Lecco Lecco; ¹⁴ASST Cremona Cremona; ¹⁵Hematology and Transfusion Medicine L. Sacco University Hospital Milano; ¹⁶University of Padova Dep of Information Engineering Padova; ¹⁷Rete Ematologica Lombarda Milano, Italy

Currently, one of the major objective of CML treatment is to eradicate Ph+SCs for obtaining a stable Treatment Free Remission (TFR). Many revisions of literature aimed to recognise possible new effective targets of treatment and some new protocols are now ongoing (e.g with JAK2 or NFKB inhibitors). In line with that, we revised clinical and biological data in the PhilosoPhi34 protocol (EudraCT:2012-005062-34). On behalf of the Rete Ematologica Lombarda (REL), this study aimed to verify the rate of Bone Marrow (BM) residual CD34+/lin-Ph+ cells in CCyR CML pts at 3, 6 and 12 months (mos) of treatment with Nilotinib 300 mg BID and to perform the gene expression profiling (GEP) of the same cells. According to the study design, we analysed 78 CCyR pts at 12 mos. FISH analysis on BM CD34+/lin- cells showed that 0/68 evaluable samples (0%) tested positive (9 negative samples not evaluable) (Am J Hematol 2018). GEP was performed on 78 pts at diagnosis (majority of Ph+cells) vs 12 mos of treatment (majority of Ph-cells). Here, we focused on few genes (JAK2, ICAM1, NFKBIA, OLFM4), according to their potential impact on the molecular response (MR) and on the stem cell survival in the niche. We observed the overexpression of JAK2 at diagnosis (.024). In univariate analysis, Wald's test showed that higher expression of ICAM1 at diagnosis correlated with optimal response (MR1) at 3 mos (.027, odds:214%) and the lower expression of NFKBIA correlated with MR3 at 3 mos (.006, odds:-76%). Similar analysis showed that a higher expression of OLFM4 correlated with MR3 at 12 mos (.023, odds:294%). Multivariate analysis revealed that JAK2, ICAM1 and OLFM4 expressions increased the statistical significance of NFKBIA role (.002). As we know, this deep and faster response correlated with a higher probability of TFR but we also consider that ICAM1 and OLFM4 promoted the stem cell anchoring to the niche. In addition, we know that NFKBIA inhibited NFKB that promoted OLFM4 synthesis. Thus, if we use a NFKB inhibitor we could downregulate OLFM4 expression (Figure 1). In conclusion, our data suggest that the best MR, in the short term, correlates with the higher stem cell anchoring to the niche. In order to obtain a long term TFR, is it better to suggest a major Ph+ SC anchoring to the niche or the Ph+ SC exhaustion? Is it better to eradicate, inducing Ph+ SC differentiation, or is it better to anchor? A sequential protocol, with different inhibitors, could clarify this crucial point.

OLFM4 expression at diagnosis according to NFKB and NFKBIA expression at diagnosis.

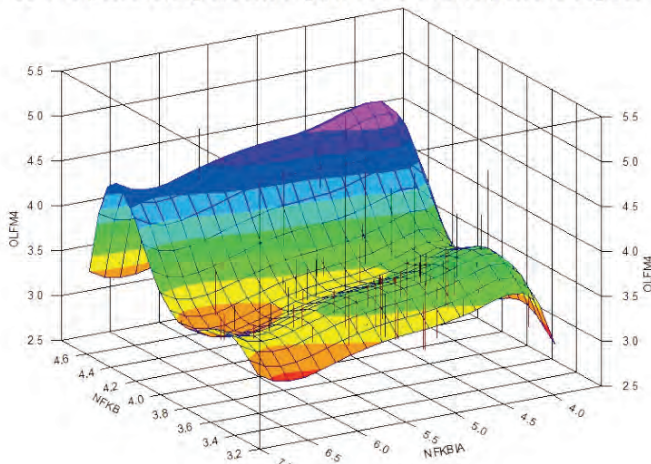


Figure 1.

P201

PRIMARY RESULTS OF THE PHASE 4 BYOND STUDY OF BOSUTINIB FOR PRETREATED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

C. Gambacorti-Passerini, E. Abruzzese, G. Specchia, C. Abboud, B. Gjertsen, T.H. Brummendorf, B.D. Smith, P. Giraldo-Castellano, U. Stromberg, S. Saussele, N. Bardy-Bouxin, A. Viqueira, E. Leip, J. Leone, G. Rosti, J. Watts, F. Giles, A. Hochhaus

University of Milano-Bicocca; S. Eugenio Hospital, Tor Vergata University; University of Bari; Washington University School of Medicine; Haukeland University Hospital, Helse Bergen, and University of Bergen; Universitätsklinikum RWTH Aachen; Johns Hopkins Sidney Kimmel Comprehensive Cancer Center; Quiron-salud Hospital; University of Uppsala and Department of Hematology, University Hospital; University Hospital Mannheim, Heidelberg University; Pfizer International Operation-Oncology; Pfizer SLU; Pfizer Inc; Pfizer Inc; University Hospital, University of Bologna; University of Miami; Developmental Therapeutics Consortium; Klinik für Innere Medizin II

Background: The tyrosine kinase inhibitor (TKI) bosutinib is approved for patients with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) resistant or intolerant to prior therapy and those with newly diagnosed Ph+ chronic phase (CP) CML.

Methods: The ongoing phase 4 BYOND study is further evaluating the efficacy and safety of bosutinib (starting dose 500 mg/day) for CML resistant or intolerant to prior TKIs. The primary endpoint in the Ph+ CP CML cohorts is cumulative confirmed major cytogenetic response (MCyR) by 1 year. This study did not include any formal sample size determination. No inferential analyses were planned.

Results: Of 163 patients who received bosutinib, 156 had Ph+ CP CML (46, 61 and 49 after 1, 2 and 3 prior TKIs, respectively); 53.2% were resistant to ≥1 prior TKI and 46.8% were intolerant to all prior TKIs. Across Ph+ CP CML cohorts, 51.9% of patients were male, and median age was 61 years (range 20.0–89.0). As of 1 year after last enrolled patient (median follow-up 30.4 months), 56.4% of patients remained on bosutinib. Median treatment duration was 23.7 months and median dose intensity after adjustment due to adverse events (AEs) was 313 mg/day. Of 144 evaluable patients with a valid baseline assessment, cumulative confirmed MCyR by 1 year was 71.5% (95% confidence interval [CI] 63.4–78.7). Cumulative complete cytogenetic response rate anytime on treatment was 81.3% (95% CI 73.9–87.3). Cumulative molecular response (MR) rates were high across lines of therapy (Table). 10 deaths occurred (5 on treatment; 1 due to disease under study); 1-year overall survival rate was 98.0%. No patient progressed to accelerated/blast phase on treatment. 25.0% discontinued bosutinib due to AEs and 5.1% due to insufficient response. The most common treatment-emergent AEs (TEAEs) were diarrhea (87.8%) and nausea (41.0%). Grade 3/4 TEAEs reported in >10% of patients were diarrhea (16.7%) and increased alanine aminotransferase (ALT; 14.7%). The only TEAE leading to discontinuation in >5% of patients was increased ALT (5.1%).

Conclusions: Most pretreated patients with Ph+ CP CML had MCyR by 1 year with bosutinib, and a substantial proportion achieved or preserved major and deep MR in all therapy lines. Results further support the use of bosutinib for Ph+ CP CML resistant or intolerant to prior TKIs.

Table 1.

Cumulative response rate any time on treatment, % (95% CI)	Ph+ CP CML, Number of prior TKIs		
	1	2	3
Evaluable, n	46	55	48
MMR	82.6 (68.6–92.2)	76.4 (63.0–86.8)	56.3 (41.2–70.5)
MR ⁴	69.6 (54.2–82.3)	61.8 (47.7–74.6)	39.6 (25.8–54.7)
No baseline MMR, n	25	28	26
MMR	76.0 (54.9–90.6)	64.3 (44.1–81.4)	38.5 (20.2–59.4)
MR ⁴	52.0 (31.3–72.2)	42.9 (24.5–62.8)	19.2 (6.6–39.4)

CI=confidence interval; CML=chronic myeloid leukemia; CP=chronic phase;

MMR=major molecular response; MR=molecular response; Ph=Philadelphia chromosome;

TKI=tyrosine kinase inhibitor

P202**WT1 (WILMS' TUMOR1) GENE EXPRESSION AS PREDICTIVE AND PROGNOSTIC MARKER IN CHRONIC MYELOID LEUKEMIA (CML)**

S. Coluzzi², T. Introcaso², F. Perutelli², S.P. Pascale, O. Villani², F. Guerrini², G. Bianchino², R. Nucorini², A. Matturro², I. Attolico², D. Vertone¹, N. Filardi², A. Amendola², M. Cimminiello², C. Barate², G. Pietrantonio², S. Galimberti², M. Pizzuti²

¹U.O. di Ematologia, Azienda Ospedaliera Regionale San Carlo, Potenza; ²Dipartimento di medicina clinica e sperimentale, Università di Pisa; IRCCS Crob Rionero in Vulture (PZ), Italy

In CML, about one third of patients (pts) reach a suboptimal molecular response nevertheless the use of TKIs. Physicians need to estimate the probability of failure; they use several risk scores systems. Nevertheless, the predictivity never reaches 100%. WT1 gene is located on the chromosome 11 and acts as oncogene. At diagnosis it is over-expressed in several hematological malignancies (~80% of Acute Myeloid Leukemias). Few data concern CML. Aim of the study: to evaluate retrospectively the WT1 expression in 58 CML pts and to assess its eventual ability of predicting treatment failure. Pts and Methods: WT1 and BCR/ABL quantitative PCR assays were performed at diagnosis, at 3, 6, and 12m of therapy on the RNAs extracted by peripheral blood samples of 58 CML pts in CP (33M and 25F, median age 58 yrs).

Results: At diagnosis, WT1 levels were not significantly different according to age, sex or to the baseline Sokal score, but we observed that WT1 levels show high inter-pts variability [median 61.8±307 (0-1805)]. During FU, we observed a reduction of WT1 expression. 15/58 cases presented a failure* during FU (*BCR-ABL1>10% at 6m of treatment, >1% at 12m, or confirmed loss of MR3 anytime) and WT1 expression levels at diagnosis were able to predict the occurrence of TKI change. In order to assess if its levels could condition the long-term EFS, a ROC analysis, based on WT1 levels at diagnosis was performed; we defined a cut-off value of 156 WT1/ABL1*10⁻⁴ (sensitivity: 69%, specificity: 76%) as value useful to discriminate pts with good or bad long-term outcome. According to this value, EFS resulted significantly longer for cases with low WT1 levels (85%) in respect of the high-WT1 subgroup (44%) (p=0.001) and WT1 expression values were able to discriminate the outcome in different Sokal subgroups. The WT1 negative prognostic power could be overcome by the type of TKI adopted (imatinib vs second-generation TKIs): in low WT1 cases the choice of a second-generation TKI as first-line treatment did not change the EFS length (3y-EFS: 82% for cases receiving imatinib vs 92% for those treated with nilotinib or dasatinib). In the high-WT1 cohort, the use of nilotinib or dasatinib significantly improved the 3y-EFS (61% second-generation TKIs vs 30% imatinib).

Conclusions: Our study suggests that WT1 could be considered an useful predictive and prognostic marker in CML, and that performing a RQ-PCR for its quantitation could help physicians to better plan the more correct treatment and FU.

P203**TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH SUSTAINED DEEP MOLECULAR RESPONSE WHO DISCONTINUED TYROSINE KINASE INHIBITOR TREATMENT. IS REAL LIFE BETTER THAN CLINICAL TRIALS?**

M. Ansuinelli, S. Rosati, C. Lisi, I. Carosino, D. Diverio, E. Scalzulli, A. Bruzzese, M. Porrazzo, E. Mariggio, S. Mohamed, M. Molica, L. Rizzo, M.L. De Luca, M. Mancini, M. Breccia, R. Foà, R. Latagliata
Ematologia Dipartimento di Medicina Traslazione e di Precisione Università "Sapienza" di Roma, Italy

Background: Several trials have explored the feasibility and safety of tyrosine kinase inhibitor (TKI) discontinuation in selected patients (pts) with chronic myeloid leukemia (CML). This is associated with a treatment-free remission (TFR) of 55-60%. Although this strategy is considered safe in clinical trials, its applicability in the real-life setting remains unsettled.

Materials and Methods: To evaluate TFR in a real-life setting, all 52 pts with CML who discontinued TKI therapy at our Center for any reason while in deep molecular response (DMR) were retrospectively analyzed.

Results: Twenty-nine pts (55.8%) were females; the median age at

discontinuation was 63 years (range 41-87). According to the Sokal score at diagnosis, 21 pts (40.4%) were low-risk, 25 (48.1%) intermediate-risk and only 2 (11.5%) high-risk. Twenty-one pts (40.4%) had received a previous treatment with alpha-interferon (IFN). At discontinuation, 43 pts (82.6%) were on first-line TKI treatment and 9 (17.4%) on second-line TKI treatment due to resistance/intolerance to first-line TKI. The last TKI prior to discontinuation was imatinib in 39 pts (75.0%), nilotinib in 9 (17.3%) and dasatinib in 4 (7.7%): the median duration of the last TKI treatment before discontinuation was 115.0 months (range 12.6-176.8). Treatment discontinuation was due to toxicity in 13 pts (25%), while in all other cases it was a shared decision with the patient. At discontinuation, all patients were in DMR [MR 4.0 in 15 pts (28.8%) and MR 4.5 in 37 pts (71.2%)], with a median duration of DMR prior to discontinuation of 88 months (range 25-170). Eleven pts (21%) relapsed, due to a loss of major molecular response (MMR), with a median time to relapse of 4.4 months (range 2.7-20.4): all patients restarted TKI treatment and regained at least a MMR. No progression to advanced phase occurred. The TFR at 12 and 24 months was 80.0% (95%CI 68.2- 91.8) and 73.5% (95%CI 59.6-87.4), respectively. At univariate analysis, no features showed a significant impact on TFR.

Conclusions: This real-life series confirms that TKI discontinuation is feasible and safe also outside protocols. Unlike clinical trials, the absence in the real life settings of fixed rules might lead clinicians to a more flexible evaluation of each patient for TKI discontinuation, increasing somewhat the accuracy of selection. This could explain the improved TFR in our cohort, compared to that of clinical trials.

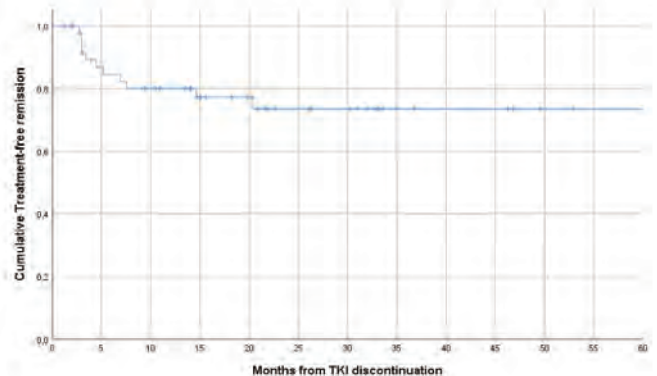


Figure 1. Treatment free remission of the whole cohort.

P204**HOW UGT1A1 GENOTYPE IMPACTS ON TKI EFFICACY AND SAFETY IN CHRONIC MYELOID LEUKEMIA PATIENTS**

A. Iurlo, C. Bucelli, D. Cattaneo, G. Levati, D. Tavazzi, D. Consonni, L. Baldini, M.D. Cappellini

Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, and University of Milan; Department of Clinical Sciences and Community Health, University of Milan, and Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico; Department of Epidemiology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico

Gilbert syndrome (GS) is a benign condition, characterized by intermittent unconjugated hyperbilirubinemia without structural liver damage, affecting about 10% of the white population. It is associated with variations in uridine-50 -diphosphate (UDP)-glucuronosyltransferase gene (UGT1A1). GS can affect pharmacokinetic properties of tyrosine-kinase inhibitors (TKIs), which are currently used for the treatment of chronic myeloid leukemia (CML). In particular, the A(TA)7TAA polymorphism has been associated with nilotinib-induced hyperbilirubinemia in CML patients. The aim of this study was then to assess if in CML-chronic phase patients treated with either first, second or third-generation TKIs, GS had an impact on clinical outcomes as cytogenetic/molecular response rates and progression-free survival, as well as on hematological or extra-hematological toxicity. We retrospectively

evaluated 105 CML patients (M/F = 61/44) treated with TKIs at our Institution. Median age at diagnosis was 51.4 years (range, 22.6-90.2). We performed PCR to identify variations in dinucleotide repeats in the UGT1A1 promoter region. Genotypes were assigned as follows: 6/6 [homozygous for (TA)6 allele] wild-type, 7/7 [homozygous for mutated (TA)7 allele] and 6/7 (heterozygous). Homozygous (TA)7 genotype was reported in 17 (16.2%) patients, 44 (41.9%) were heterozygous (TA)7 and the remaining wild-type. Concerning baseline characteristics, concomitant drugs were reported in 49 patients (46.7%), with 7 patients taking more than 5 medications. Efficacy and safety data are reported in Table 1. Comparing patients with or without GS, no difference was observed in the achievement of CCyR, MMR, DMR, hematological and extra-hematological toxicity, regardless of the TKI used. As expected, in homozygous (TA)7 patients a major rate of grade 3/4 hyperbilirubinemia was reported, without any difference from the TKI used. In clinical practice, GS is often underestimated, mainly because the specific text is performed only when bilirubin levels are significantly increased. However, it can be present also in patients with normal or borderline bilirubin levels. Interestingly, in our series GS has been detected in a higher percentage than expected (about 16.2% with homozygous genotype). In this study, even if homozygous patients showed a higher rate of grade 3/4 hyperbilirubinemia, GS did not seem to have a clinical impact on response rate and outcome in CML patients, regardless of the specific TKI used.

Table 1.

	All patients n=105	*6/*6 n=44	*6/*7 n=44	*7/*7 n=17
First line treatment, n (%)				
Imatinib	73 (69.5)	25 (56.8)	35 (79.6)	13 (76.5)
Dasatinib	14 (13.3)	9 (20.4)	3 (6.8)	2 (11.8)
Nilotinib	18 (17.1)	10 (22.8)	6 (13.6)	2 (11.8)
Response to first line treatment, n (%)				
CCyR	79 (75.2)	32 (72.3)	34 (77.3)	13 (76.5)
MMR	73 (69.5)	29 (65.9)	32 (72.3)	12 (70.6)
DMR	51 (48.6)	20 (45.4)	23 (52.3)	8 (47.1)
Switch, n (%)	47 (44.8)	20 (45.4)	19 (43.2)	9 (52.9)
Reason for switching, n (%)				
Primary resistance	18 (38.3)	9 (45)	7 (36.8)	2 (22.2)
Secondary resistance	14 (29.8)	4 (20)	6 (31.6)	4 (44.4)
Intolerance/toxicity	15 (31.9)	7 (35)	6 (31.6)	2 (22.2)
Response to second line treatment, n (%)				
MMR	35 (74.5)	14 (70)	14 (73.7)	7 (77.8)
DMR	17 (36.2)	4 (20)	7 (36.8)	6 (66.7)
Third-line patients, n (%)	10 (9.5)	4 (9.1)	4 (9.1)	2 (11.8)
Increased total bilirubin, n (%)				
Grade ≤ 2	26 (40.6)	8 (30.8)	10	8 (30.8)
Imatinib-treated	12 (46.2)	2 (16.7)	4 (33.4)	6 (50)
Dasatinib-treated	2 (7.6)	1 (50)	0	1 (50)
Nilotinib-treated	12 (46.2)	5 (41.7)	6 (50)	1 (8.3)
Grade ≥ 3	9 (34.6)	3 (33.3)	1 (11.1)	5 (55.6)
Imatinib-treated	4 (44.4)	1 (25)	0	3 (75)
Dasatinib-treated	2 (22.2)	1 (50)	0	1 (50)
Nilotinib-treated	3 (33.4)	1 (33.3)	1 (33.3)	1 (33.3)

Abbreviations: CCyR, complete cytogenetic response; MMR, major molecular response; DMR, deep molecular response.

P205

IDENTIFICAZIONE DI MIRNA E GENI TARGET NELL'ANALISI TRASCRITTOMICA DELLE CELLULE CD34+/LIN- DI PAZIENTI CON LEUCEMIA MIELOIDE CRONICA IN FASE CRONICA DOPO 12 MESI DI TERAPIA CON NILOTINIB

A. Trojani¹, E. Pungolino¹, A. Dal Molin², L.E. Bossi¹, G. Rossi³, M. D'Adda³, A. Perego⁴, C. Elena⁵, M. Turrini⁶, L. Borin⁷, C. Bucelli⁸, S. Malato⁹, M.C. Carraro¹⁰, F. Spina¹¹, M.L. Latargia¹², S. Artale¹³, P. Spedini¹⁴, M. Anghilieri¹⁵, B. Di Camillo², G. Baruzzo², G. De Canal¹⁶, A. Iurlo⁸, E. Morra¹⁷, R. Cairoli¹

¹Division of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy; ²Department of Information Engineering, University of Padova, Padova, Italy; ³Department of Hematology, ASST Spedali Civili, Brescia, Italy; ⁴Internal Medicine-Haematology, Desio Hospital, Desio, Italy; ⁵Hematology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; ⁶Division of Hematology, Department of Internal Medicine, Valduce Hospital, Como, Italy; ⁷Hematology Division, San Gerardo Hospital, Monza, Italy; ⁸Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano,

Italy; ⁹Hematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milano, Italy; ¹⁰Hematology and Transfusion Medicine, Sacco Hospital, Milano, Italy; ¹¹Division of Hematology - Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; ¹²ASST Valle Olona Ospedale di Circolo, Busto Arsizio, Italy; ¹³ASST Valle Olona Sant'Antonio Abate, Gallarate, Italy; ¹⁴Division of Hematology, Hospital of Cremona, Cremona, Italy; ¹⁵ASST Lecco, Lecco, Italy; ¹⁶Pathology Department, Cyto genetics, ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy; ¹⁷Rete Ematologica Lombarda, Italy

The PhilosoPhi34 (EudraCT: 2012-005062-34) study investigated the gene expression profiling (GEP) of bone marrow CD34+/lin- cells of 78 CP-CML patients at diagnosis and after 12 months of nilotinib treatment. Among these patients, we analyzed 21 patients with molecular response (MR) MR3.0 in Group 1 (G1) vs 3 patients with MR>10% IS at 3 months in Group 2 (G2) of nilotinib by microarray, respectively. We performed GEP analyses between G1 and G2 after 12 months of nilotinib using GeneChip HTA 2.0. SAM and Benjamini-Hochberg procedure were used to control the False Discovery Rate (FDR) applying a cut-off value of 0.05 to select for significant differential expression. 243 transcripts genes (72 of which annotated as coding mRNAs/miRNAs) resulted significantly differentially expressed. Clustering analysis was performed, applying complete linkage hierarchical agglomerative clustering on Euclidean pairwise distances between genes and samples. Interestingly, one subject belonging to G1, clustered with G2 (Figure 1). Among the 72 selected genes, we focused on 8 miRNAs and their target genes which were also differentially expressed between G1 and G2 with high FC. We identified that MIR4736 (FC=2,71), MIR548AC (FC=4,32), MIR548AN (FC=10,89), MIR548O2 (FC=16,98), and MIR548X (FC=21,96) were upregulated whereas MIR1224 (FC=-6,27), MIR4271 (FC=-4,35) and MIR3665 (FC=-2,08) were downregulated in G1 vs G2 after 12 months of nilotinib treatment, respectively. MIR3665 regulated COL18A1, MED1, and CDHR5, MIR548AN targeted RIT2, NOD2, PLEKH1, and PALM, MIR548AC modulated RIT2, FBXO34, RAB33B, MED1, CCDC34, and GCSH, MIR548X targeted RIT2 and MED1. MIR4736 targeted PALM and PSKH1, and MIR4271 modulated THRA. Of note, COL18A1 (involved in angiogenesis) and RAB33B (member RAS oncogene family) were downregulated in G1 with FC=-5,3 and FC=-3, respectively. We focused on genes involved in immunity: ISG15 (FC=-2,6), PYCARD (FC=-3,04), NOD2 (FC=-10,85), MSR1 (FC=-1,93) and HLA-DQB1 (FC=-5,81) were underexpressed whereas IL23R (FC=1,45) was overexpressed in G1 after 12 months of nilotinib, respectively. Interestingly, the intracellular NOD-like receptors encoded by PYCARD and NOD2 were involved in response to bacterial/virus infections and activated inflammasomes. Both genes were downregulated with high fold change in G1. NOD2 (FC=-10,85) was regulated by MIR548AN (FC=10,89) playing a crucial role in MAPK whereas PYCARD (-3,04) was involved in myeloid dendritic cell activation.

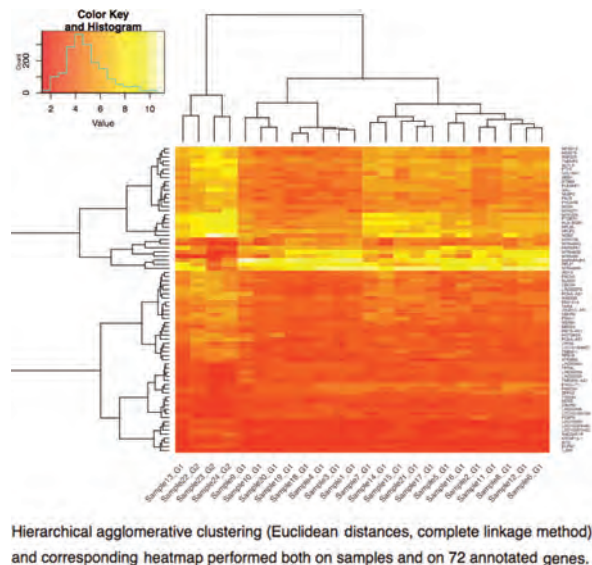


Figure 1.

P206

A PRACTICAL CHART TO EVALUATE THE CARDIOVASCULAR RISK IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS: A REPORT FROM THE APULIAN HAEMATOLOGISTS AND CARDIOLOGISTS NETWORK

I. Attolico¹, S. Cascella², S. Capalbo³, G. Spinosa³, G. Casavecchia⁴, A. Guarini⁵, P. Ditunno⁵, N. Di Renzo⁶, G. Reddiconto⁶, S. Cozza⁷, N. Cascavilla⁸, R. Spadano⁸, A. Russo⁹, S. Oliva¹⁰, G. Specchia¹, P. Colonna²

¹Hematology and Transplants Unit Policlinico of Bari; ²Cardiology Unit Policlinico of Bari; ³Hematology Unit Ospedali Riuniti; ⁴Cardiology Unit Ospedali Riuniti; ⁵Department of Hematology Hospital IRCCS Oncologico; ⁶Hematology Unit Vito Fazzi Hospital; ⁷Cardiology Unit Vito Fazzi Hospital; ⁸Hematology Unit IRCCS Casa Sollievo della Sofferenza; ⁹Cardiology Unit IRCCS Casa Sollievo della Sofferenza; ¹⁰Cardiology Unit Hospital IRCCS Oncologico, Italy

Background: Tyrosine Kinase Inhibitors (TKIs) have markedly increased the survival of CML patients (pts). Long-term therapy with Nilotinib and Dasatinib may result in cardiovascular (CV) complications. Use of Ponatinib can be complicated by CV events. Age >60 yrs and the CV toxicity of new generation TKIs are predisposing factors. Measures need to be implemented to minimize the onset of CV events. Aim of this study was to design a practical chart to evaluate CV risk in CML pts on TKIs.

Patients and methods: A group of hematologists and cardiologists designed a CV risk chart. Literature was reviewed and all risk factors were weighted and included. The new CV risk chart includes an 11 points medical history score (comorbidities, lifestyle habits, drugs...) and a 23 points score for ECG and echocardiogram. Pts scoring 0-2 are considered at low risk (LR), those with 3-6 at moderate risk (MR) and those with ≥7 at high CV risk (HR). Follow-up at 6 months was performed (average: 218±49 days). In 5 centers of the Apulian Hematologic Network, 69 pts treated with TKIs (Imatinib:13, Dasatinib: 14, Nilotinib: 27, Bosutinib:1, Ponatinib:14) were enrolled.

Results: The average CV risk score was 4.4±3.7, 29/69 pts showing LR, 22/69 MR and 18/69 HR. Clinical and cardiologic characteristics of the three groups were analyzed: age increased significantly in the 3 groups: 49.6±14.1(LR) vs 68.1±9.3(MR) vs 74.0 vs 10.7 y/o(HR) (p<0.0001); Left Ventricular Ejection Fraction (EF) (62.9±5.1 vs 61.0±5.1 vs 58.1±8.7%; p<0.05) and estimated Glomerular Filtration Rate (eGFR) (95.5±19.1 vs 84.0±26.1 vs 65.5±25.3 ml/min; p<0.0001) were significantly reduced in pts with MR/HR. We did not observe differences in terms of average CV risk (4.3±2.6 vs 4.4±4.0; p:n.s.), age (66.2±15.6 vs 60.8±16.0 y/o; p:n.s.), left ventricular EF (61.6±6.5 vs 60.9±6.5%; p:n.s.) or eGFR (73.4±26.3 vs 86.7±25.3 ml/min; p:n.s.) between pts treated with Ponatinib and pts treated with other TKIs. A CV event occurred in 6 pts during treatment, in 1 at 7 months of TFR after Nilotinib. Data are reported in the Table 1.

Conclusions: A practical chart for CV risk stratification in CML pts was designed. A statistical correlation was observed between the score and age, cardiac and renal function. CV events were observed in 7/69 pts, mainly MR/HR. This score could offer a useful tool to "personalize" cardiologic follow-up and CV prophylaxis or therapy in pts on TKI treatment.

Table 1. Patients and cardiovascular events.

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7
AGE (years)	81	79	51	67	69	54	61
SEX	F	F	F	F	M	M	M
TKI	IMA	PONA	PONA	PONA	PONA	NILO	NILO
SCORE	8	2	7	4	6	5	1
TIME FROM TKI START (months)	15	12	27	6	11	53	Event at 8 months of TFR after 60 months of treatment
EVENT	Congestive Heart Failure	Ischemic stroke	Ischemic optic neuritis	Atrial Fibrillation	Peripheral ischemia (gr 3)	STEMI	STEMI
ACTION TAKEN	Discontinuation	Discontinuation	Discontinuation	On treatment	On treatment	Discontinuation	In TFR

P207

CHRONIC MYELOID LEUKEMIA AS THE BEST MODEL FOR LEUKEMIA-DERIVED EXOSOMES ENRICHMENT: A NEW INFORMATIVE TOOL FOR THE DETECTION OF ACTIVE LEUKEMIC CELLS IN BONE MARROW

S. Bernardi^{1,2}, C. Foroni^{1,2}, C. Zanaglio^{1,2}, F. Re^{1,2}, N. Polverelli¹, A. Turra¹, E. Morello¹, M. Farina¹, F. Cattina¹, L. Gandolfi¹, T. Zollner¹, E. Accorsi Buttini¹, M. Malagola¹, D. Russo¹

¹Chair of Hematology, Unit of Blood Diseases and Stem Cell Transplantation, DPT of Clinical and Experimental Sciences, University of Brescia, ASST Spedali Civili di Brescia; ²CREA Laboratory (Centro di Ricerca Emato-Oncologica AIL), ASST Spedali Civili di Brescia, Brescia, Italy

Ph+ Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasia characterized by BCR-ABL1 fusion-gene, derived from the t(9;22) translocation. Tyrosine Kinase Inhibitors (TKIs) target BCR-ABL1 protein and induce major or deep molecular response in the most of patients. TKIs prolong the survival and allow the treatment free remission. However, many studies have demonstrated the persistence of leukemic cells in the bone marrow (BM) niche, even after treatment. The international standardized minimal residual disease monitoring for CML patients is based on the RT-qPCR quantification of BCR-ABL1 transcript in the peripheral blood cells. This method is not able to appreciate the activity of residual CML leukemic cells in the BM, which are the responsible of the molecular relapse in the patients undergoing TKIs discontinuation. Exosomes, carrying different information of the cell of origin, have garnered considerable attention in cancer research thanks to the discovery of their messenger role in cellular communication. Moreover, there are some evidences regarding the possibility to isolate different exosomes sub-populations targeting the antigens on their surface. In this study we aimed to explore the feasibility of the tumor-derived exosomes enrichment by an immune-affinity approach in onco-hematologic patients. Thanks to its characteristics, CML was considered the best model for this pilot study. We enrolled 10 CML patients in chronic phase (CP) and treated with TKIs. The quantification of BCR-ABL1 exosomal transcript was performed by dPCR according with the last evidences of its accuracy and sensitivity in transcript quantification. The possibility of the tumor-derived exosomes enrichment was confirmed quantifying an exosomal housekeeper and the presence of BCR-ABL1 transcript highlighted for the first time the presence of active leukemic cells in CML patients in CP. Thanks to these results, tumor-derived exosomes enrichment could be consider as a new tool for the identification of active leukemic cells and for the assessment of an innovative monitoring focused on the biological meaning of exosomes in CML and other hematological malignancies.

P208

THE ROLE OF NK CELLS IN CHRONIC MYELOID LEUKEMIA

S.P. Pascale¹, R. Nuccorini¹, A. Fortunato¹, S. Coluzzi¹, A. Amendola¹, M. Cimminiello¹, N. Filardi¹, A. Maturro¹, D. Vertone¹, I. Chitarelli², M. Pizzuti¹

¹UOC Ematologia AOR SAN CARLO, Potenza; ²U.O.S.P. Centro Trasfusionale Lagonegro, Italy

Chronic myeloid leukemia (CML) is a hematological cancer, characterized by a reciprocal chromosomal translocation between chromosomes 9 and 22, producing the Bcr-Abl oncogene. Tyrosine kinase inhibitors (TKIs) represent the standard of care for CML patients (pts) and exert a dual mode of action: direct oncokinase inhibition and restoration of effector-mediated immune surveillance, which is rendered dysfunctional in CML patients at diagnosis, prior to TKI therapy. The more recent goal of therapy in CML treatment is to induce a durable deep molecular response (DMR) as a prelude to successful treatment-free remission. The lack of overt relapse in such patients has been attributed to immunological control of CML thanks to an immune system re-activation and restoration of effector-mediated [natural killer (NK) cell and T cell] immune surveillance. Aim of the study is to investigate the role of NK cells at diagnosis and at achievement of DMR during TKIs treatment.

Patients and Methods: A total of 39 chronic phase CML pts were included in the study, 21 male and 18 female, with a median age of 64 (34-88). Pts were treated with imatinib (20), dasatinib (7), nilotinib (9)

and ponatinib (3) with a median follow up of 25 months (0-98). Basic NK-, B- and T-cell proportions were analyzed with the flow cytometry NAVIOS using the following antibodies: CD45, CD3, CD16, CD56, CD4, CD8, CD19. A total of 43 analysis were performed, 6 at diagnosis and 37 during treatment.

Results: We observed a lower number of NK cells in pts evaluated at diagnosis (median: 2%, range 0-3) than in pts during TKIs treatment. The proportion of NK cells increased at achievement of DMR, in fact pts in MR2-MR3 had a median of NK cells of 8% (range 0-31) while pts in MR> 4 a median value of 11% (range 2-49). The only pt in failure showed a NK value of 3%. Although the number of pts is limited, stratifying them based on TKIs, groups were not homogeneous but, in contrast to literature data, pts in treatment with imatinib showed an increased number of NK cells (median: 14%, range 4-49) compared to dasatinib group (median: 8%, range 0-42) and pts in therapy with nilotinib and ponatinib.

Conclusions: Our results, though limited to a small number of cases highlight the role of NK cells in sustaining remission and further analysis should be made considering how anti-leukaemia immune responses could help in the long term control of the disease, for example upon TKIs discontinuation.

P209

LONG-TERM (10-YEAR) OUTCOME OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH NILOTINIB 400 BID IN FIRST-LINE: FINAL RESULTS OF THE GIMEMA CML 0307 STUDY

G. Gugliotta¹, F. Castagnetti¹, M. Breccia², L. Levato³, T. Intermesoli⁴, M. D'Adda⁵, M. Salvucci⁶, F. Stagno⁷, G. Rege Cambrin⁸, M. Tiribelli⁹, B. Martino¹⁰, M. Bocchia¹¹, M. Cedrone¹², E. Trabacchi¹³, F. Cavazzini¹⁴, F. Porretto¹⁵, F. Sorà¹⁶, E. Usala¹⁷, F. Albano¹⁸, G. Martinelli¹⁹, S. Soverini¹, R. Foà², F. Pane²⁰, M. Cavo¹, G. Saglio²¹, M. Baccarani²², G. Rosti¹

¹Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna – Policlinico S.Orsola - Malpighi; ²UOC Pronto Soccorso e Accettazione Ematologica, Università degli Studi di Roma "Sapienza"; ³Unità di Ematologia, Ospedale "Pugliese-Ciaccio"; ⁴Unità di Ematologia, Ospedali Riuniti; ⁵Unità di Ematologia, "Spedali Civili"; ⁶Unità di Ematologia, Ospedali "S. Maria delle Croci"; ⁷Unità di Ematologia, Università di Catania; ⁸Unità di Medicina Interna 2- Ospedale S. Luigi Gonzaga; ⁹Clinica Ematologica - Policlinico Universitario, Università di Udine; ¹⁰Dipartimento Emato-Oncologia A.O. "Bianchi-Melacrino-Morelli"; ¹¹Unità di Ematologia, Ospedale "S. Maria alle Scotte", Università di Siena; ¹²Complesso Ospedaliero S. Giovanni Addolorata; ¹³Unità di Ematologia, Ospedale "Guglielmo da Saliceto"; ¹⁴Sezione di Ematologia, Arcispedale "S. Anna"; ¹⁵Ospedale "La Maddalena"; ¹⁶Università Cattolica del Sacro Cuore - Policlinico A. Gemelli; ¹⁷S.C. di Ematologia e Centro Trapianti di Cellule Staminali Emopoietiche, Ospedale "A. Businco"; ¹⁸Unità Ematologia e Trapianto, Università di Bari; ¹⁹Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori- IRST; ²⁰Azienda Ospedaliera Universitaria - Università degli Studi di Napoli "Federico II"; ²¹SCDU Ematologia e Terapie Cellulari- A.S.O. Ordine Mauriziano, Ospedale Umberto I; ²²Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Italy

In chronic phase (CP) chronic myeloid leukemia (CML) nilotinib (NIL) showed better efficacy compared to imatinib. The higher rates of deep molecular response with NIL may translate in more patients (pts) eligible for treatment discontinuation (treatment-free remission, TFR). On the other hand, cardiovascular toxicity may limit NIL use in selected groups of pts (e.g. elderly pts). Long follow-up is required for a proper evaluation of the benefit-risk ratio between efficacy (including TFR rates) and toxicity. However, only few data are currently available on the long-term outcome of NIL treated pts. The GIMEMA CML WP promoted in 2007 a phase II study with NIL first line (400 mg BID). Early and mid-term results have been already published. This abstract is focused on the long-term outcome of this study. The updated, final results (with 10-year minimum follow-up) will be available for presentation at the meeting. Seventy-three adult (≥18 years old) pts with newly diagnosed, CP-CML were enrolled at 18 GIMEMA Centers in Italy. Median age was 51 (18-83) years. After a median follow-up of 92 months, 70 (95.9%) pts are alive and progression-free. Three pts died (1 progression to blast phase; 2 CML-unrelated death). Failures according to ELN 2013 recommendation occurred in 5 pts (6.8%; 1 progression to BP; 2

BCR-ABL transcript level > 1% at 12 months; and 2 confirmed loss of MR3). At last contact, 45 (61.6%) pts were still on NIL (dose ≥= 600 mg/day in 29 pts; 400 or 450 mg/day in 12 pts; ≤= 300 mg in 4 pts). Reasons for permanent NIL discontinuation in the remaining 28 pts were: adverse events (17 pts, 23.3%); successful TFR (9 pts; 12.3%); failures (2 pts; 2.7%). The median age at CML diagnosis of pts obtaining the TFR was 47 (21-70) years. Fifteen athero-thrombotic events (ATEs) occurred in 13 (17.8%) pts (5 PAOD, 5 acute myocardial infarctions, and 5 others), after a total NIL exposure of 478 years (3.1 events/100 pt-years). The median age at CML diagnosis of these pts was 66 (43 - 83) years. No patient died for ATEs. Two pts with ATEs that discontinued NIL in deep-molecular response maintained the TFR.

In summary, these data highlight the efficacy of NIL in the long-term, with excellent overall survival. TFR was obtained in 15% of pts, particularly in younger ones. Some safety concerns remain, with ATEs occurring in 17.8% of pts (mainly in elderly pts), although none of these events was fatal.

P210

SKIN LESIONS DURING DASATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS

F. Tarantini, I. Attolico, M.A. Urbano, A.R. Russo Rossi, M.L. Di Noi, R. Frappampina, L. Anelli, A. Zagaria, E. Parciante, C. Schifone, G. Specchia, F. Albano

Department of Emergency and Organ Transplantation (D.E.T.O.), Hematology Section, University of Bari, Italy

Introduction: After the introduction of tyrosine kinase inhibitors (TKIs), chronic myeloid leukemia (CML) patients (pts) can now achieve long-term survival. Main safety concerns regarding each TKIs are pretty well known. Dermatological adverse events particularly are observed with all TKIs, but there are less evidences of cutaneous side effects associated with Dasatinib (DAS), and even fewer regarding an immune underlying activation over skin lesions/rashes associated with it.

Aim: We sought to define the prevalence rates of cutaneous side effects on DAS therapy and to investigate the clinical and pathological characteristics of these reactions in pts treated in our center.

Results: Among 67 CML pts, 4 (5.9%) showed skin lesions during DAS treatment. All cases except one showed cutaneous events during DAS as first line treatment. The median time to onset of skin lesions from the beginning of DAS treatment was 47 months (range 1-61). Moreover, at the time of the appearance of the skin lesions all CML pts except one achieved a deep molecular response; in fact this latter early showed dermatologic lesions after only one month from the DAS starting. The cutaneous manifestations were not generalized but mainly located in back, abdomen, thorax or lower limbs region. The CML pts did not show peripheral lymphocytosis at the time of skin lesions appearance. The histologic analysis of the skin lesion in all CML cases showed a lymphocytic infiltration of the dermis. DAS has a well known spectrum of side effects particularly due to its multiple kinase targets. The cutaneous lesions described in our four CML cases had various macroscopic aspects (even if basically a vesicular subcentimeter lesion is present), were clinically relevant (painful, itching) or mild, and they had the tendency to regress when were treated with steroids and/or temporary DAS discontinuation. The unusual T cytotoxic and NK lymphocytes cutaneous infiltrate demonstrated in our CML cases could be the expression of the lymphocyte expansion promoted by DAS. Moreover, the heterogeneity of dermatologic manifestations reported in our CML patients could be related to unknown factors specific for each CML patient.

Conclusion: Our work highlights that skin lesions may be associated with DAS treatment and they should not be confused as an expression of viral or bacterial infections but rather interpreted as the clinical expression of lymphocytosis promoted by this TKI.

Quality of Life, Pain Therapy and Home Care, Supportive Care 2

P211

PHYSICAL ACTIVITY AND QUALITY OF LIFE OUTCOMES IN MULTIPLE MYELOMA PATIENTS: A SYSTEMATIC REVIEW

M. Servadio, F. Cottone, P. Fazi, M. Vignetti, F. Efficace, On behalf of the GIMEMA WP Quality of Life

Italian Group for Adult Hematologic Diseases (GIMEMA)

There is mounting evidence indicating that physical activity (PA) is an important factor affecting quality of life (QoL) outcomes in cancer patients. We performed a systematic review to quantify the amount of evidence-based data available, on the effect of PA on QoL of patients with multiple myeloma (MM). We conducted a literature search in PubMed/Medline to identify studies published between January 2008 and April 2019, focusing on the impact of PA on either QoL or other patient-reported outcomes (PROs) in MM patients. The search strategy included all studies regardless of the design or number of patients included. Case reports, study protocols and abstracts were excluded. For each study, we analysed PA measures (e.g. total length and type of exercises), PROs and clinical variables, according to a pre-defined coding scheme. Two independent reviewers screened and analysed eligible studies and recorded information on PA, PROs and clinical variables. The systematic search yielded 34 records, but only three studies published between 2012 and 2015 were selected (one Randomized Controlled Trial (RCT), one single arm study and one retrospective study). These included two studies on newly diagnosed patients (patients undergoing induction therapies) and one on patients in stable disease (patients off treatment or on maintenance therapy). In all studies, PA was prescribed as intervention with pre-defined exercises. In the RCT, the PA intervention was 15 weeks long and it slightly influenced QoL, with a minimal effect on decreasing fatigue. In the remaining two studies, where PA interventions were 6 weeks and 6 months long respectively, PA improved QoL and significantly decreased fatigue over time. Whilst there is sparse evidence on the effects of PA in MM patients, the few data available suggest that PA interventions are associated to QoL improvements. There is urgent need of studies in this area to possibly further improve healthcare delivery in MM patients.

P212

“TAILORING A SPECIAL THERAPY BY PERTINENCE”: A GROUNDED THEORY (GT) MODEL DESCRIBING THE PROPOSAL OF A COMPASSIONATE USE (CU) THERAPY FOR PATIENTS WITH ADVANCED HAEMATOLOGICAL AND SOLID MALIGNANCIES

I. Capodanno, E. Bertocchi, F. Giovanardi, F. Nunziante, A. Noto, F. Sireci, S. Sacchi, S. Di Leo, F. Merli, L. Ghirrotto,

Azienda USL IRCCS

Background and Aims: Compassionate Use (CU) programs are pathways that allow cancer patients without medical treatment options to benefit from not yet approved therapy. Many studies highlight a complex interplay among medical needs, ethics, medical law and commercial interests. Up to now no qualitative studies about the perspective of patients and professionals exist. We aimed to model a qualitative explanation about the process of CU proposal to patients with advanced haematological and solid malignancies.

Methods: We carried out a Grounded Theory (GT) through semi-structured interviews, using Charmaz's constructivist approach. We involved 21 participants (patients, caregiver, haematologists, oncologists, nurses, data manager, pharmacists, and other stakeholders) from Haematology and Oncology Unit. We did data collection and data analysis concurrently; theoretical sampling allowed the definition of an explicative model of the process.

Results: We identified three main phases that outline the proposal of a CU therapy: “Giving a new opportunity”, “A special therapy”, “Ensuring pertinence”. First, the sense of uncertainty and clinical instability brings patients and physicians to build together a new therapeutic chance. Both Haematologists and Oncologists are in doubt about the efficacy, but they overbalance toward “trying” and therapy becomes “special” (phase II): it strengthens care relationship and generates “little

hopes”. Finally, the therapeutic proposal passes from hand to hand through different professionals who verify its pertinence. A lot of resources are employed in this process: different institutional filters go on while time is running out. Clinical and ethical debate among professionals is enhancing through responsibility and emotional load. We named the overall process: “Tailoring a special therapy by pertinence”.

Conclusions: Our model describes the process of CU proposal to patients with advanced haematological and solid malignancies, showing important areas for ethical interdisciplinary debate and target improvements. Results reveal how central is the role of the patient and how important is connecting patients' preferences and values with clinical pertinence.

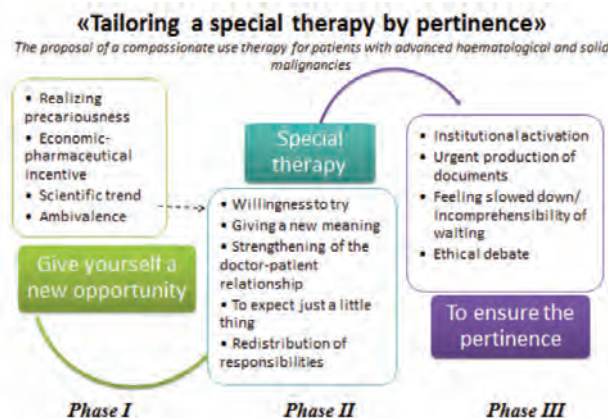


Figure 1.

P213

ROLE OF ERYTHROPOIESIS STIMULATING AGENTS (ESAs) IN SUPPORTIVE CARE OF LOW-RISK MYELODYSPLASTIC SYNDROMES

C. Cerchione¹, G. Martinelli¹, I. Soriente², A.M. D'Arco², P. Danise², G. Cerciello³, V. Di Battista¹, D. Cangini¹, M.B. Giannini¹, F. Pane³, F. Alfinito³

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; ²Hematology, AOU Federico II, Italy

Erythropoiesis stimulating agents (ESAs) are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs. Macrocytosis is one of the cytological hallmarks of dyserythropoiesis in MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non transfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDs, subgroup of Italian register, was performed. 183 patients, treated with standard-dose ESAs, have been retrospectively analyzed. Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.31 g/dl, mean serum EPO concentration: 51 mU/L, after a mean time from diagnosis of 6 months (r.1-118). ORR was 83.6% (153/183), no difference among WHO and IPSS subgroups was found: 132/183 (72.1%) achieved response after 3 months of treatment, while other 21/183 (11.2%) after 6 months. 19 patients with stable disease (non-responders, according to IWG criteria), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 83.2% exhibits again macrocytosis after 3 months, while 16.8% become normocytic. In the normocytic-responders group 89.8% exhibits again normocytosis, while 10.2% become macrocytic: in these patients, after 3 months, there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non-responders were

30/183 (16.3%): in the macrocytic non-responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 76% exhibits again macrocytosis, while 24% become normocytic. These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

P214

RED BLOOD CELL TRANSFUSION IN PALLIATIVE HOMECARE PRACTICE: OUR EXPERIENCE

A. Saeli¹, L. Maiorana, V. La Terra¹, A. Antolino², S. Cabibbo², G.O. Manenti², M. Poidomani², G. Garozzo²

¹SAMOT Ragusa Onlus, ²SIMT-UO Ematologia, Italy

Red blood cell transfusions (RBC) are commonly used in palliative care to treat anemia and its symptoms. We analysed our database on RBC transfusions in order to improve palliative care practice, evaluating which patients (Pts) could benefit more by RBC transfusion despite the potentially harmful risks. A retrospective analysis of data collected in 2018 on RBC transfusion practice in Pts assisted by SAMOT Ragusa Onlus was performed. In the province of Ragusa, that year, we executed 1150 home RBC transfusions: 91 outpatients (tot. 496 Pts), assisted in palliative care, received 194 RBC transfusions. General Practitioners (GPs) requested the blood components to the Hospital Immunohematology Service Staff, who evaluated the appropriateness of the request; our Doctors executed the transfusions at home. More than 90% of transfusions were performed using a biometrical system (Securblood, BBS). Average age is 74 years, 84% Pts have cancer. Average haemoglobin is 7.8 g/dL pre-transfusion, and 9.6 g/dL post transfusion. Median interval for the first transfusion is 48 days after activation of the service, median duration of assistance is 111 days. Death occurred after a median period of 40 days after the last transfusion. Karnofsky Performance Status (KPS) average is 36 before and 27 after; Palliative Performance Scale (PPS) average is 34 before and 27 after, transfusion. Analysis of Edmonton Symptom Assessment System (ESAS) before and after transfusion shows: a slight decrease of average after transfusion in pain, depression, illness and lack of appetite. Higher reduction is present for fatigue and drowsiness. A slight increase is present for dyspnea and anxiety. As a matter of facts, patients worsened progressively during assistance, however, our efforts are addressed to improve their quality of life through symptoms control. From this point of view, RBC transfusions have improved some symptoms such as fatigue and lack of appetite; and lessened pain, depression and illness. Although there were no adverse reactions, potentially harmful risks remain; furthermore, preliminary deeper analysis shows that only some patients and for limited time did have a real benefit from transfusion. Therefore, we are considering a future collaboration with GPs and Immunohematology Doctors, to better evaluate the use of alternative therapies, while reserving RBC transfusions for selected patients that might benefit from a significative improvement in their quality of life

P215

PEGFILGRASTIM VERSUS FILGRASTIM IN THE SUPPORTIVE CARE OF HEAVILY PRE-TREATED MULTIPLE MYELOMA IN TREATMENT WITH POMALIDOMIDE-DEXAMETHASONE

C. Cerchione¹, G. Martinelli¹, D. Nappi², A.E. Pareto², A. Lucchesi¹, G. Musuraca¹, S. Ronconi¹, F. Pane², L. Catalano²

¹Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; ²Hematology, AOU Federico II, Italy

Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than nonpegylated G-CSF. The objective of this study was to compare the efficacy and safety of pegfilgrastim in patients affected by heavily pretreated MM, treated with pomalidomide-dexamethasone, in order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospitalization duration. We enrolled 33 patients (19 M and 14 F) median age at diagnosis 69 years (r. 52-84),

and median age at start of treatment 76 years (r.56-90) treated with several lines of treatments (median 7, r. 2-11), every refractory to all the drugs previously received, received Pomalidomide-Dexamethasone (P 4 mg for 21 days, D 40 mg days 1,8,15,22, pegfilgrastim day +8) every 28 days, until progression. Since first course, received in domestic setting, with a very good compliance, patients performed blood counts once weekly and received, from day +8 to day +19, prophylactic oral chinolonic antibiotics and anti-fungal drugs. During neutropenia after first cycle, Filgrastim (5 gr/kg/day for 3 days) was given if neutrophils count was <1500x10⁹ cells/L. Median number of filgrastim administrations was 4.8 (r. 3-6); nadir neutropenia was registered after a median of 10.7 days (r. 7-14); median of nadir neutrophil count was 1.17 x 10⁹ cells/L (r.0.3 – 1.5), with maximum duration of 14 days. From the second course, all patients switched to prophylaxis with pegfilgrastim (6 mg), injected subcutaneously with a single administration on day +3 independently from the neutrophil count at that time. During pegfilgrastim, neutropenia was never longer than 8 days, with a consequent reduction of neutropenia-related infections. Median nadir neutrophil count, evaluated for every patients for at least three courses of therapy (r. 3-6) registered at day +11, was 1.39 (r.0.9-2.2). Only 4 patients needed a supplement of 3 administrations of filgrastim. Pegfilgrastim was well tolerated in all patients: main side effects in our patients were mild fever and bone pain (21.2%). In conclusions, in patients affected by heavily pretreated MM treated with pomalidomide-dexamethasone, pegfilgrastim seems to reduce the incidence of severe neutropenia and infections and may increase the possibility to maintain the scheduled time of treatment.

P216

G8 FRAILTY SCORE CORRELATES WITH THERAPEUTIC DECISIONS IN OLD (>75Y) PATIENTS WITH HAEMATOLOGIC MALIGNANCIES: A CROSS-SECTIONAL STUDY IN A COMMUNITY HOSPITAL.

M. Marchetti

Day Hospital Ematologico, SOC Oncologia, Asti, Italy

I percorsi terapeutici degli anziani con neoplasie ematologiche sono basati sulla vulnerabilità del paziente, pertanto è indispensabile scegliere uno strumento quantitativo per la valutazione di tali dimensioni. Il G8 (Subeyran 2008 JCO) è uno strumento di screening della fragilità geriatrica composto da 8 item e raccomandato dalla Rete Oncologica Piemontese per i pazienti neoplastici con >75 anni. La validità di questo strumento nella popolazione ematologica non risulta tuttavia ad oggi verificata. Lo studio si è svolto in un ospedale non-universitario che copre una popolazione di 200,000 abitanti nell'arco di 4 settimane arruolando 82 pazienti consecutivi con età >=75 anni (mediana 82 anni; range 75-96): 33% >85 anni e 11% > 90 anni. Il 22% dei pazienti era seguito per citopenie immuni e aplasia midollare mentre, il 42% dei pazienti riportava patologie linfoproliferative (12 MM/WD, 6 CLL, 7 linfomi alto rischio, 11 CLL/HCL) e il 36% neoplasie mieloidi (7 CML/CMML, 9 MPN, 11 MDS/LA). Nei 65 pazienti con neoplasie ematologiche lo score G8 mediano è risultato essere 13 e l'82% dei pazienti è risultato "fragile" (G8<14.5). Un'elevata correlazione (r>0.6) è stata rilevata tra G8 e tre dimensioni del questionario: BMI, movimento e valutazione soggettiva dello stato di salute. L'età e la comorbidità misurata dal CIRS hanno dimostrato scarsa correlazione con G8. La fragilità è risultata molto più frequente nei pazienti anemici (95% vs 70%) e il grado WHO di anemia ha correlato strettamente con l'item G8 di valutazione soggettiva dello stato di salute. Il 42% dei pazienti era in trattamento antineoplastico attivo (44% terapie orali) e il 12% aveva interrotto o completato un trattamento, mentre il 21%, generalmente neoplasie mieloidi, era assegnato a terapie "palliative" (HU, Ctx orale, epoetine). La porzione di pazienti assegnati a trattamento attivo era significativamente maggiore nei pazienti con score G8 maggiore: 43% se G8>11 vs 17% (chi-quadro<0.001). In particolare, il trattamento attivo era raramente assegnato ai pazienti con limitazioni cognitive e/o depressione moderata-severa (18% vs 58%, chi-quadro <0.001), mentre altri item del G8, quali l'età (<85 anni e la dipendenza motoria risultavano solo parzialmente predittive di un trattamento attivo. Lo screening della fragilità geriatrica G8 è fattibile nella pratica clinica ed è utile nei pazienti con tumori ematologici, ma occorre validare soglie di fragilità specifiche per i nuovi trattamenti antineoplastici orali e sottocute.

P217**PRESERVATION OF FERTILITY IN YOUNG PATIENTS AFFECTED WITH HEMATOLOGICAL MALIGNANCIES**

F. Rotondo, S. Tomassetti, M. Imola, A. Merli, A. Mianulli, A.L. Molinari, G. Tolomelli, V. Polli, D. Prota, B. Venturini, P. Tosi

U.O. Ematologia; Ospedale Infermi, Italy

Background: Chemotherapy and radiation treatment may impair ovarian and testis reserve, leading to reduced fertility potential and lower post treatment birth rates in male and female cancer survivors. Multi-agent chemotherapy and supportive care have improved survival rates in haematological malignancies so gonadal function and fertility have become important concerns before treatment of young people. Some options to preserve fertility include semen cryopreservation for adult men and embryo and oocyte cryopreservation for women. In order to set up an efficient policy of fertility preservation in our department, We retrospectively evaluated by clinical chart and phone survey patients diagnosed with Hodgkin lymphoma from 2008 to 2018.

Patients and Methods: Out of 101 patients aged 18-40, only 70 participated to phone survey, 30 males and 40 females.

Results: In male patients 28 out of 30 (93%) had undergone semen criopreservation and 2 births were registered, respectively after 39 months and 8 years after completion of chemotherapy. Conversely in female patients only 1 had undergone oocyte criopreservation but no birth was registered so far. 6 births in 39 patients (15%) were registered who did not undergo oocyte criopreservation (1 birth at diagnosis, 1 after 3 years from ASCT, 2 after 4 years from chemotherapy 1 after 2 years and 1 after 55 months). Data concerning young patients with non Hodgkin lymphoma will be soon available.

Conclusions: In our experience while preservation of fertility in male patients can be considered a routine practice, in female a standard policy is still lacking. Preservation of gonadal function must be considered an important goal for long term health of cancer survivors. Onco fertility requires cross disciplinary interaction between physicians in order to promote local programs for young patients with cancer. Onco fertility requires cross disciplinary interaction between physicians in order to promote local programs for young patients with cancer.

P218**NEW STRATEGIES TO ESTABLISH A CONTINUOUS HOSPITAL-TERRITORY CARE IN HEMATOLOGICAL OUTPATIENTS**

C. Girmenia, G. Pizzarell, C. Cartoni, S. Amadori

UOS Pronto Soccorso ed Accettazione Ematologica Policlinico Umberto I Sapienza University of Rome; Compass Pharmaceutical Sciences Consulting Srl Rome, Unità di Cure Domiciliari Ematologiche Policlinico Umberto I Sapienza University of Rome; AIL Associazione Italiana contro le Leucemie-Linfomi e Mieloma Rome, Italy

Effective hematological disease-modifying drugs have increased dramatically in the last years and new therapies are still being in development. Therapeutic progress has led to a definitive cure of a minority of diseases while in most of instances it transformed progressive hematologic malignancies into chronic diseases. These advances have generated a new landscape: increase of the overall survival, increase of elderly patients in active treatment, long-term outpatient management of chronically ill patients, often "quoad vitam" and frequently with permanent or temporary disabilities, increase of emergencies developing in the outpatient setting. The need to face these new and rapidly evolving situations is increasingly felt by health professionals, patients and health management. The traditional hospital clinic is a care modality that alone is no longer adequate to manage the out-of-hospital course of haematological diseases on territory. A project promoted by AIL is under development aiming at the identification of new care strategies to respond to the above-mentioned challenges. In particular, the aim of this project is to identify new models in the ambulatory care, in the home care and in the management of emergencies developing in the outpatient hematological setting. These models need to be tailored to the local healthcare realities in order to be adapted to the different needs, characteristics, population and resources. The project will be initially developed on restricted areas (e.g. provinces, districts of metro-

politan cities), and based on several innovative features: 1) Multidisciplinary. Various professionals are involved: hematologists, nurses, patient associations, health management, and others. 2) Data collection. Epidemiological, clinical, welfare, resources utilization, and other data will be analyzed. This analysis will allow describing the current welfare situation in that area, and the detection of associated needs. 3) Consensus conference. A local multidisciplinary group of experts will process the above-mentioned data based on their professional experience. The consensus conference method will thus make it possible to make up for the lack of other national or international health experiences and the lack of literature. The new models of hospital-territory care to hematological outpatients emerging from this process will be monitored over time, in order to introduce corrective measures for adjusting to a constantly evolving reality.

Infections

P219

CLINICAL CHARACTERISTICS AND OUTCOME OF WEST NILE VIRUS INFECTION IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES: A MULTICENTER STUDY

A. Visentin, V. Nasillo, M. Marchetti, I. Ferrarini, R. Paolini, R. Sancetta, G.M. Rigolin, F. Cibien, S. Imbergamo, M. Riva, C. Briani, S. Marinello, F. Piazza, F. Gherlinzoni, M. Krampera, R. Bassan, A. Cuneo, R. Marasca, G. Semenzato, L. Trentin

Hematology and Clinical Immunology unit, Department of Medicine, University of Padua, Padua; Venetian Institute of Molecular Medicine, Centro di Eccellenza per la Ricerca Biomedica Avanzata, Padua. Hematology section, Department of Medical and Surgical Science, University of Modena and Reggio Emilia, Modena; Hematology Unit, Cardinal Massaia hospital, Asti; Hematology unit, University of Verona, Verona; Hematology unit, Santa Maria della Misericordia hospital, Rovigo; Hematology unit, Ospedale dell'Angelo, Mestre-Venice, Hematology section, Department of Medical Sciences, University of Ferrara - Azienda Ospedaliera-Universitaria di Ferrara, Ferrara. Hematology unit, Ca' Foncello hospital, Treviso; Neurology unit, Department of Neuroscience, University of Padua, Padua; Unit of Infective and tropical diseases, Padua University Hospital, Padua, Italy

West Nile virus (WNV) is an arthropod-borne infection transmitted to human by mosquitoes. While most patients are asymptomatic and only 20% develop a flu-like syndrome, immunocompromised individuals are at high risk of WNV neuroinvasive disease (WNVND). However, only a few studies described WNV infections in patients with hematological malignancies. The aim of this study was to describe the clinical characteristics and outcome of patients with lymphoproliferative diseases (LD) who experienced WNV infections. 19 patients (11 chronic lymphocytic leukemia [CLL], 6 non-Hodgkin and 1 Hodgkin lymphomas, and 1 acute lymphoblastic leukemia) were recruited in a multicenter retrospective study among 6 Italian hematological units. Anti-WNV antibody and WNV-RNA were assessed in blood and cerebrospinal fluid (CSF) in all patients. The primary endpoint was the rate of WNVND. The secondary endpoint was overall survival (OS). The median age at infection was 64years, 11 patients were males, the median number of comorbidities was 2 (range 0-9), 15 received at least one treatment (range 0-3) and 10 had an active LD at WNV infection. The median time from LD diagnosis to WNV was 6years. All patients had fever (range 38.2-40 C), 16 asthenia and 9 arthralgia. 16, 11, 3 and 14, 7, 2 patients developed WNV-RNA, anti-IgM and anti-IgG antibody in the blood and CSF, respectively. 18 subjects developed CNS symptoms like confusion, amnesia or headache, but 14 (74%) fulfilled the criteria for WNVND (CNS symptoms + WNV-RNA in CSF). 12/19 patients were treated with polyclonal intravenous immunoglobulins. 6 out of 13 (46%) patients with WNVND completely resolved neurological symptoms. 2 CLL patients developed WNVND during treatment with kinase inhibitors: 2 ibrutinib and 1 rituximab-idelalisib. While patients taking ibrutinib, as first line therapy, developed anti-IgM WNV and resolved the infection, the relapsed subjects receiving rituximab-idelalisib had persistent viremia in CSF and persistent neurological impairment. After a median follow-up of 9.8 months 7 patients died: 5 due to WNVND, 1 of stroke and 1 of Richter syndrome. Among patients with WNVND the mortality rate was 39%. The median OS since WNV infections was 14.4 months for all patients but 8.0 months for WNVND. Since mildly symptomatic patients are rarely assessed for WNV infection, the number of cases could be biased. However, we observed that WNVND (72%) is common in patients with LD and associated with a high mortality rate.

P220

PROCALCITONIN IN THE DIAGNOSTIC WORK-UP OF FEBRILE EPISODES IN OUTPATIENTS WITH HEMATOLOGIC MALIGNANCIES

G. Ciotti, G. Maestrini, G. Morano, M. Breccia, R. Latagliata, G.A. Brunetti, C. Girmenia

UOS Pronto Soccorso e Accettazione Ematologica, Dipartimento di Ematologia, Oncologia e Dermatologia, Policlinico Umberto I, Sapienza Università di Roma, Italy

Fever is a major complication of outpatients with hematologic malignancies and the definition of the early diagnostic work-up for the appropriate management (choice of antimicrobial therapy, hospitalization, early discharge) is a challenging issue. C-reactive protein (CRP) and procalcitonin (PCT) have been proposed as useful biomarkers for the differential diagnosis of febrile episodes. PCT is a good biomarker of bacterial infection largely used in critical patients; however, it is not included in the standard work-up of fever in hematologic patients. Herein, we describe the results of a prospective experience on the use of CRP and PCT in the diagnostic work-up of febrile episodes in hematologic outpatients who attended our Hematologic Emergency Unit (HEU) during the period January-April 2019. At the admission to the HEU, all febrile outpatients underwent clinical evaluation, hematologic exams, radiologic exams, blood cultures and other microbiologic exams as indicated. PCT was measured by the point of care B•R•A•H•M•S direct Reader (Thermo Fisher Diagnostics S.p.A.). PCT values were defined as low (<0.5 ng/ml), + (>0.5-<1.5 ng/ml), ++ (>1.5-<5 ng/ml) and +++ (>5 ng/ml). Overall, 70 consecutive febrile episodes were observed. The underlying diseases were lymphoma (n:35), acute leukemia (n:15), multiple myeloma (n:8), chronic lymphoid leukemia (n:5) and other (n:7). Neutropenia (PMN<500/cmm) was present in 12 cases (17%). The distribution of the CRP and PCT values is detailed in the figure. In the 17 cases classified as fever of unknown origin, PCT was low in 14 cases (82%). In the 29 cases with a clinically documented infection (pneumonia in 23 cases), PCT was low, +, ++ and +++ in 18, 4, 3, and 4 cases, respectively. In all 13 cases of viral infection (influenza 10 cases), PCT was low. In 1 of 3 cases of gram-positive bacteremia PCT was positive, while in all 8 cases of gram-negative bacteremia PCT was positive (+, ++ and +++ in 2, 3 and 3 cases, respectively). CRP values did not correlate with any type of fever. Neutrophil counts did not correlate with the levels of CRP and PCT. Our experience confirms that PCT, but not CRP, is a useful biomarker in the early diagnostic work-up of febrile hematologic outpatients. Low levels of PCT in a clinically stable febrile patient may be considered a predictor of a low probability of gram-negative infection. It should be considered in the diagnostic work-up of febrile outpatients with hematologic malignancies.

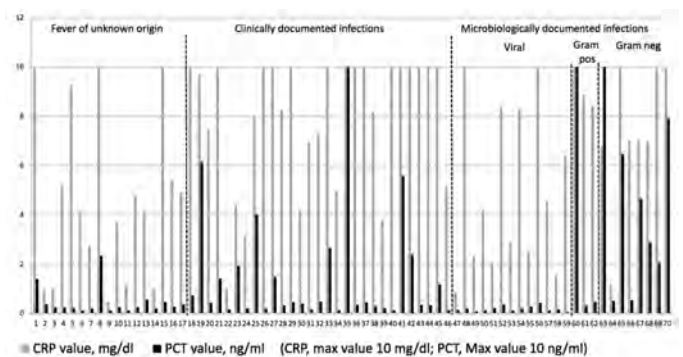


Figure 1. CRP and PCT levels in various types of febrile episode.

P221**BRINCIDOFIVIR IN ADULT PATIENTS WITH ADENOVIRUS INFECTION FOLLOWING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT**

F. Serio, R. Greco, D. Clerici, C. Oltolini, F. Giglio, L. Lazzari, M.T. Lupo Stanghellini, F. Farina, C. Liberatore, P. Angelillo, F. Lorentino, F. Pavesi, S. Mastaglio, S. Racca, M. Bernardi, C. Corti, J. Peccatori, F. Ciceri

IRCCS Ospedale San Raffaele, Milano, Italy

Adenovirus (Adv) infection is a recognized cause of morbidity and mortality following haematopoietic stem cell transplantation (HSCT). Brincidofovir (BCV, CMX001) is an orally bioavailable lipid conjugate of cidofovir that has activity against Adv. We present a retrospective analysis on six adult patients (median age 47 years; range 26-79) treated with BCV, used on a compassionate basis, for Adv infection after allogeneic HSCT. All patients were affected by high-risk hematological malignancies (five acute myeloid leukemia and one Hodgkin's lymphoma). Stem cell donors were family haploidentical for all patients. Stem cell source was T-cell replete PBSCs. All patients received a conditioning regimen based on treosulfan and fludarabine; five patients received thiotepa in addition. All patients received sirolimus and MMF as graft-versus-host disease (GvHD) prophylaxis plus post-transplant cyclophosphamide. All patients experienced grade III-IV acute GvHD. Viral load was detected by quantitative PCR, performed on clinical suspicion. All patients had Adv end-organ disease: 1 hepatitis; 4 colitis; 1 multi-site disease determining colitis, hepatitis and encephalitis. All patients received oral BCV 100 mg twice weekly, after initial cidofovir administration withdrawn for renal impairment. The median BCV treatment duration was 5 weeks (range 1-16 weeks). No severe adverse events occurred. Median post-transplant time to detection of viraemia was 44 days (range 15-271 days). Median peak viral load was 7,358,428 copies/ml (29,316-10,000,000 or more) on blood. On gut biopsies the median viral load was 474,187 copies/ml (range 60,300-10,000,000 or more), and immunohistochemistry was strongly positive in one case. In the other cases, it was difficult for pathologist to distinguish viral colitis from concomitant gut GvHD. All patients presented a median of 3 risk factors for Adv infection according to ECIL-4 criteria. All patients had a decrease in viral load and a clinical improvement following antiviral treatment. Only one patient, with persistent severe lymphopenia, experienced a recurrence of Adv infection after BCV withdrawal. These results confirm the activity of BCV against Adv. The longitudinal monitoring for Adv on blood, and routine search for Adv in patients undergoing gut biopsy, will allow early identifications of patients at risk for Adv infection.

P222**LOW RATE OF INFECTIOUS RELATED ADVERSE EVENTS, SEPTIC SHOCK AND MORTALITY IN ACUTE LEUKEMIA PATIENTS WITH POST-CHEMOTHERAPY SEVERE NEUTROPENIA, IN ABSENCE OF FLUORQUINOLONE PROPHYLAXIS**

E. Zappone¹, M. Bocchia¹, C. Betti², M. Defina¹, S. Ciofini¹, C. Zuanelli Brambilla¹, B. Mecacci¹, V. Sammartano¹, F. De Martino¹

¹UOC Ematologia Azienda Ospedaliera Universitaria Senese Università di Siena; ²Università degli Studi Siena, Italy

Fluorquinolone prophylaxis in severe prolonged neutropenia is widely used as standard care because it reduces the rate of infections, mortality and fever episodes. The increasing rate of quinolone-resistant strains and the risk of inducing multidrug-resistant pathogens represent a great challenge on the optimal management of such patients. Our center has implemented a highly efficient protocol for the prompt treatment of neutropenic fever in hospitalized patients, so we had the confidence to abandon fluorquinolone prophylaxis at the end of 2014 because of the increasing rate of local resistant strains and in order to reduce the incidence of multi-resistant bacteria colonization and infection. We report a real-life experience of 268 hospitalization for chemotherapy and subsequent prolonged neutropenia (>7days) in 125 adult acute leukemia or aggressive lymphoma patients, treated from January 2015 til march 2019, without fluorquinolone prophylaxis. The median age was 59, 56% were male and 44% female, 85 patients had acute myeloid leukemia (68%), 25 acute lymphoblastic leukemia and 15 aggressive lymphoma. The median length of neutropenia <500 N/ul was 15 days and that of very severe neutropenia <100 N/ul was 12

days, median length of hospital stay was 22 days. We registered 200 infectious episodes (74,6%): 73 were fever of unknown origin (36,5%) and 127 were documented infections (63,5%). Among documented infections 101 were bacteremia (53% gram negative and 47% gram positive), 27 were fungal infections (16 without concomitant bacteremia), 10 were organ infection (8 pneumonia) without documented bacterial, viral or fungal infection. Only 14 bacteremia were from multi-resistant bacteria, mostly ESBL positive E. Coli, only 1 Klebsiella KPC. Of the 200 infectious episodes 21 manifested septic shock (9,5%) requiring support with noradrenaline, none of them required treatment in intensive care unit (ICU). Three of the 125 patients died because of infection, 2 patients had angioinvasive pulmonary aspergillosis and 1 had severe acute respiratory distress with multiple pulmonary infiltrates without documented bacterial infection or probable IFI, this patient required ICU. The infection related mortality was 2,4%, much lower than what is reported in comparable setting. These results support our decision to safely omit fluorquinolone prophylaxis without harm in terms of infectious mortality and possibly with benefits in containing the risk of multi-resistant infections.

P223**OUTCOMES OF INFECTIONS BY MULTI-DRUG RESISTANT GRAM-NEGATIVE BACTERIA IN ACUTE LEUKEMIA TREATED WITH HIGH DOSE CHEMOTHERAPY**

D. Clerici¹, C. Oltolini², R. Greco¹, F. Pavesi¹, F. Giglio¹, S. Mastaglio¹, F. Lorentino¹, L. Lazzari¹, P. Scarpellini², J. Peccatori¹, M. Bernardi¹, C. Corti¹, A. Castagna², F. Ciceri¹

¹Haematology and Bone Marrow Transplant Unit, IRCCS San Raffaele Scientific Institute; ²Infectious Disease Unit, IRCCS San Raffaele Scientific Institute

Febrile neutropenia (FN) and sepsis are common and life-threatening complications in hematological diseases. This retrospective study was performed to investigate the impact of multi-drug resistant (MDR) Gram-negative bacteria (GNB) colonization and infection in patients affected by acute leukemias (AL), treated with high-dose chemotherapy (CT) in the Hematology and BMT Unit of our Institute during 2018. According to institutional guidelines, all patients received levofloxacin prophylaxis, active weekly surveillance for MDR-GNB colonization by rectal swab and standard treatment of FN. We collected bloodstream infections (BSI) during aplasia and analyzed the outcome of MDR-GNB infections. We performed 50 high-dose CT cycles (58% first line CT, 42% salvage treatment), in 34 patients (82% myeloid AL, 18% lymphoblastic AL). Three patients (9%) acquired colonization by MDR-GNB after first CT cycle [n=1 MDR Pseudomonas aeruginosa (Pa), n=2 Klebsiella pneumoniae (Kp); 1/2 KPC-producer, 1/2 OXA-producer]. Among non-carriers (n=31), we registered 10 BSI: 5 sustained by not ESBL-producer Escherichia coli (5/5 piperacillin/tazobactam-susceptible), 2 ampicillin-susceptible Enterococcus faecalis, 2 Enterococcus faecium (1/2 vancomycin-resistant) and 1 Streptococcus mitis. Among MDR-GNB carriers, only the patient colonized by KPC-Kp developed a BSI (after consolidation CT), caused by not ESBL-producer Kp (piperacillin/tazobactam-resistant, likely an AmpC-producer strain), treated with a 10-day course of ceftazidime/avibactam (C/A) plus amikacin as definite therapy (DT). This patient also received a 10-day course of C/A as empirical therapy (ET) for FN after induction CT. The patient colonized by OXA-Kp received C/A as ET for FN with de-escalation after 6 days. The patient colonized by MDR-Pa was treated with a 10-day course of ceftolozane/tazobactam plus fosfomicin as DT for pneumonia sustained by Pneumocystis jiroveci and MDR-Pa. Infectious-related mortality at 30 days was 0%. In conclusion, we observed a low rate of MDR-GNB colonization after induction CT, with only one episode of BSI by a beta-lactamase producer GNB in carriers after consolidation. Therefore, in our cohort the adoption of longitudinal active surveillance and the early initiation of targeted combined antimicrobial therapy in MDR-GNB carriers have successfully impacted on clinical outcomes, allowing the early recognition of patients at risk of MDR-GNB infections and the conclusion of intensive CT program.

P224

DOUBLE-FLUDARABINE BASED INDUCTION AND INFECTIVE RISK: THE BOLOGNA EXPERIENCE

C. Sartor¹, S. De Polo¹, R.E. Lewis², G. Marconi¹, S. Parisi¹, M.C. Abbenante³, G. Cristiano¹, J. Nanni¹, A. Talami¹, M. Olivi¹, S. Ragaini¹, L. Bertamini⁴, M. Ottaviani¹, L. Bandini¹, M.C. Fontana¹, G. Martinelli⁵, N. Testoni¹, C. Papayannidis¹, S. Paolini¹, A. Curti¹, M. Cavo¹, M. Stanzani¹

¹DIMES (Department of Experimental, Diagnostic, and Specialty Medicine) Institute of Hematology "L. and A. Seragnoli" University of Bologna; ²Clinic of Infectious Diseases Department of Medical and Surgical Sciences S. Orsola-Malpighi Hospital University of Bologna; ³Hematology Department "Casa Sollievo della Sofferenza" Hospital IRCCS; ⁴Division of Hematology University of Torino AOU Città della Salute e della Scienza di Torino; ⁵Hematology Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IRCCS

Background: Fludarabine-based regimens in acute myeloid leukemia (AML) have high complete remission (CR) rates but no proven survival benefit, prolonged hematological toxicity and possibly a higher infective risk.

Aims: To analyze the infective risk associated with double-FLAI5.

Methods: We retrospectively collected data of 40 consecutive adult patients with newly diagnosed AML treated with FLAI5 induction (Fludarabine 25 mg/sqm/die days 1-5, Cytarabine 2 g/sqm/die days 1-5 and Idarubicin 10 mg/sqm/die days 1-3-5); patients in CR proceeded to 2nd FLAI5. Incidence and type of infective events were collected in 36/40 patients and compared to a historical cohort of 39 patients treated with non-fludarabine regimens.

Results: Forty patients received FLAI5 from August 2015 to December 2018. Median age at diagnosis was 53 years (range 19-66). Seventeen of 40 patients (42.5%) were high risk (NCCN 2016). Thirty-five patients (87.5%) achieved CR after induction. Twenty-nine of 40 patients were evaluable for MRD analysis, 15/29 (52%) patients were MRD-. Thirty-two of the 35 patients in CR proceeded to 2nd FLAI5. At induction, patients recovered ANC > 500x10⁹/L after 21.5 days (range 13-32; controls 19 days, 13-25) from end of chemotherapy. Patients spent a median of 33 nights in hospital (range 14-61; controls 35 nights, 31-42; p ns). After 2nd cycle, median days for ANC recovery was 29 (range 16-45, controls 20 days, 18-25; p .001). Median patients hospitalization was 39 nights (range 26-55; controls 32.5 nights, 24-47; p .003). No difference was seen for antimicrobial prophylaxis in induction; patients and controls experienced respectively 33/36 (91.7%) and 36/39 (92.3%) infective events. Patients had a higher number of infections of unknown origin (15/33 vs 6/36, p .002), and lower incidence of Gram+ isolates (4/12 vs 15/22, p .002). Any-mold infection rate was comparable. Twenty-seven of 36 patients and 22/39 controls proceeded to consolidation. Anti-mold prophylaxis was different, 17/27 (63%) patients received posaconazole while 4/22 (18.2%) controls. Twenty-two of 27 patients (81.5%) and 17/22 (77.3%) controls had an infection. Three patients of 27 had a probable mold infection and 1 possible, compared to 1 proven mold infection and 1 possible in the 22 controls (any-mold infection 4/27 vs 2/22).

Conclusions: FLAI5 is associated with high CR rates, but prolonged neutropenia and hospitalization in 1st consolidation. This does not translate in a higher infection risk.

P225

PRIMARY VIGOROUS ANTI-INFECTIVE PROPHYLAXIS TO REDUCE THE RATE OF INFECTIONS AND RELATED CHEMOTHERAPY DISRUPTIONS IN PATIENTS WITH UNTREATED FOLLICULAR LYMPHOMA UNDERGOING RITUXIMAB PLUS BENDAMUSTINE TREATMENT

C. Giordano¹, R. Della Pepa¹, N. Pugliese¹, I. Cappuccio¹, G. Piccolo¹, F. Fanelli¹, M.G. Rascato¹, G. Campagna¹, S. Di Folca¹, A. Severino¹, M. Mascolo², M. Picardi², F. Pane¹

¹Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples; ²Department of Advanced Biomedical Sciences, Federico II University Medical School, Naples, Italy

Introduction: Routine anti-infective prophylaxis in induction treatment of follicular non-Hodgkin lymphoma (FL) is still controversial. We evaluated the impact of a vigorous primary prophylaxis in a prospective group in comparison with an historic cohort receiving prophylaxis on

demand.

Methods: From January 2015 to January 2019, consecutive untreated FL of Federico II Hematology with clinical indications to receive six 4-weeks cycles of Rituximab (R) (375 mg/m² i.v. on day 1) plus bendamustine (90 mg/m² i.v. days 1-2) had an intensified primary prophylaxis (IPP). IPP consisted in pegfilgrastim (6 mg, s.c) on day 4 of each cycle; trimethoprim-sulphamethoxazole (960 mg bid die, twice a week) and acyclovir (400 mg bid die) from day 1 until 6 months after the last cycle; lamivudine (100 mg die) for patients with serum HbCAb+ from day 1 until 12 months after. From January 2009 to December 2014 (historic cohort) patients with the same induction treatment, received a secondary prophylaxis with G-CSF and/or anti-infective drugs on demand. The primary endpoint was Grade 3 infection (NCI CTCAE v.5.0) and immune-chemotherapy disruption (premature stop and/or delay of at least 7 days) rate. The secondary endpoint was IPP-related side effects (grade ≥3 WHO toxicity criteria).

Results: A total of 111 patients (median age 63 y) were analyzed: 67 consecutive patients were enrolled in the prospective group while 44 in the historic cohort, with no difference in clinical characteristics. The median chemotherapy dose-intensity and median dose-density were, respectively, 83% and 93% (7 patients reduced dose and other 7 had a median delay of 12 days). All IPP-group patients had prophylaxis as previously outlined and no modifications were made. In the historic group 16 patients had a median of 6 G-CSF injections for ANC count <1000/mm³. The IPP-group had a lower rate of infections (Table) than the historic cohort (6% vs 34%, p<0.001) and a lower rate of infection-related chemotherapy disruptions [2/67 (3%) vs 12/44 (28%), p<0.001]. Prophylaxis was well tolerated with grade 3 bone pain toxicity due to pegfilgrastim in 5 patients vs 4 after G-CSF, managed with paracetamol.

Conclusions: Intensified primary prophylaxis with pegfilgrastim, acyclovir, trimethoprim-sulphamethoxazole and lamivudine seems to reduce the incidence of infection related chemotherapy disruptions for untreated FL enabling to maintain the planned treatment schedule reducing relapse risk.

Table 1.

	HISTORIC COHORT n= 44	IPP GROUP n= 67
INFECTION EPISODES^a		
FEBRILE NEUTROPENIA OF UNKNOWN ORIGIN	15 (34%)	4 (6%)
CLINICALLY DOCUMENTED INFECTION	5 (11%)	-
Site/source of infection:		
Upper respiratory tract	1 (2%)	-
Lower respiratory tract	2 (4%)	1 (1%)
Gastrointestinal tract	-	2 (3%)
MICROBIOLOGICALLY DOCUMENTED INFECTION	1 (2%)	1 (1%)
Bacteremia of Gram-positive		
Staphylococcus spp.	1 (2%)	-
Bacteremia of Gram-negative		
Enterobacteriaceae spp.	-	1 (1%)
CMV-DNA positivity	1 (2%)	-
VZV reactivation	3 (7%)	-
HBV-DNA positivity ^b	2 (4%)	-
HOSPITALIZATION DAYS, median (range)	7 (5-10)	5 (5-7)

Unless otherwise indicated data are the number of patients

^a Infection of grade ≥ 3 according to NCI CTCAE v.5

^b Acute hepatitis due to occult HBV infection; reactivation: 2/12 HBsAg-/HbCAb+ patients

CMV= cytomegalovirus

VZV= varicella zoster virus

HBV= hepatitis B virus

HBsAg= Hepatitis B surface antigen

HbCAb= hepatitis B core antibody

Historic cohort: secondary prophylaxis managed on demand i.e. after the first occurrence of neutropenia, fever and viral reactivations: 4 patients received trimethoprim-sulphamethoxazole after fever with signs of pneumonia, 3 patients received acyclovir after herpes virus infection, and 2 had lamivudine after HBV reactivation.

IPP: intensive primary prophylaxis was performed in every patient from the first cycle of immune-chemotherapy with pegfilgrastim, acyclovir, trimethoprim-sulphamethoxazole and lamivudine (HbCAb+).

P226

THE INCREASED CYTOMEGALOVIRUS (CMV) REACTIVATION INCIDENCE IN PATIENTS TREATED WITH BENDAMUSTINE-BASED REGIMEN CORRELATES WITH A SEVERE REDUCTION OF CIRCULATING CD4+ T LYMPHOCYTES

L. Pezzullo, A. Bruno, G. Cassiodoro, R. Bianco, G. Villani, E. Vaccaro, R. Rosamilio, M. Martorelli, R. Fontana, R. Guariglia, B. Serio, C. Selleri

Hematology, Department of Medicine and Surgery, Salerno University, Italy

Bendamustine (BENDA), alone or in combination with other drugs, is increasingly used in the treatment of hematological malignancy due to its safety and efficacy. Variable infection rates, severe CD4+ lymphocytes reduction, impaired CD4+ reconstitution, prolonged lymphopenia have been reported both after BENDA monotherapy and BENDA-containing regimens. Since January 2017, we monitored CD4+ lymphocytes counts and cytomegalovirus (CMV) DNA reactivation in 45 consecutive patients with B cell-NHL (16 female 29 male; median age: 70 years; range: 40-88 years) undergoing a BENDA-based treatment: thirty-three patients were treated with BENDA plus Rituximab (R-BENDA), 12 with R-BENDA plus Dexamethasone (RD-BENDA). 40 patients (90%) received a BENDA-based regimen as first-line therapy and were exposed with a median dose of BENDA dose of 150 mg/m² for each cycle (median number of courses: 4). Circulating T-cell subsets were determined by using flow cytometric analysis. The baseline median CD4+ lymphocytes count was 512 cells/mm³ (range 41-3226) and dropped to 77 cells/mm³ (range 7-250) 3 months after the start of BENDA courses (P value <0.004); CD4+ cell reduction was more pronounced in the RD-BENDA group (RD-Benda vs R-Benda group: 42 vs 110 CD4+ cells /mm³; p:ns). Thirty-four out of 45 patients were evaluable at the end of 6 BENDA courses: median CD4+ lymphocytes count was 87 cells/mm³ (range 15-281), 45 vs 120 CD4+ cells/mm³ in RD-BENDA vs R-BENDA group, respectively (p:ns). In 25 patients evaluable 3 months after the end of the therapy the median CD4+ lymphocytes count was 98 cells/mm³ (range 41-288), with 11 vs 120 cells/mm³ in the RD-BENDA vs R-BENDA group, respectively (p:ns). In 20 patients evaluable 6 months after the end of treatment, the median CD4+ lymphocytes count was 138 cells/mm³ (range 11-384 cells/mm³) with 38 and 192 cells/mm³ in the RD-Benda and R-Benda groups, respectively (p:ns). In 17 (37%) out of 45 patients we documented CMV reactivation (5 in RD-Benda group and 12 in R-Benda group, 42% and 35% of treated patients, respectively) but 5 out of 12 patients in the RD-Benda group that received CMV prophylaxis with valganciclovir 900 mg/3 times at week did not experience CMV reactivation. This single-center study provides further evidence that BENDA-based regimens in lymphoproliferative diseases are associated with a deep CD4+ lymphocytes reduction, lasting even 6 months after the end of therapy particularly in the RD-Benda group, and requiring a careful monitoring as well as a CMV prophylaxis to avoid opportunistic infections and CMV reactivation.

P227

LOCAL EPIDEMIOLOGY OF BLOODSTREAM SEPTIC INFECTION IN HEMATOLOGIC MALIGNANCIES :EXPERIENCE OF A SINGLE HEMATOLOGY WARD

C. Buquicchio¹, R. Miccolis¹, T.M. Santeramo¹, C. Germano¹, M. Leo¹, G. Lerario¹, V. Carluccio¹, G. De Santis¹, M. Santoro¹, D. Tatò², E. Capriuli², G. Tarantini¹

¹SC Ematologia con Trapianto, ²Laboratorio di Microbiologia, Ospedale Dimiccoli, Italy

Sepsis is one of major cause of mortality, besides disease progression in hematologic malignancies for prolonged neutropenia condition and a dysregulated host response to the infections. This study was conducted in a single hematology ward with autologous transplant unit of Barletta Dimiccoli Hospital from January 2018 until April 2019 and in cooperation with microbiologic lab of the same hospital, we respectively reviewed all bloodstream infections of 380 consecutive patients admitted to our ward. In all patients with fever was collected two sets of blood sample peripheral and central venous catheter if present. Of 380 consecutive patients, we found 35 bacterial bloodstream infection (BBSI) with blood culture positive. The BBSI patients characteristic were the following: 15 affected by Lymphoma (42%), 14 by acute leukemia (40%), 3 by Multiple myeloma (9%), 3 by others (9%), 17 were male (48%) and 18 female (52%), 15 with less of 60 years old (42%), 20

with age superior 60 years old (58%). The frequency of gram- BBSI was 48% (17 patients) and gram+ BBSI was 52% (18 patients). In gram-group higher frequency (70%) was of *E. Coli* (12 patients and 40% ESBL producing of those), then 3 cases of klebsiella (18%) of those 2 were ESBL+, 1 case of Acinetobacter, 1 Pseudomonas (each 6%). In gram+BBSI group *S.hominis* was present in 6 patients (33%), *S.haemoliticus* in 4 (23%), *Enterococcus faecalis* and *S. epidermidis* in 3 (17% each) and 1 patient with *S. aureus* and *capitis* (5% respectively).

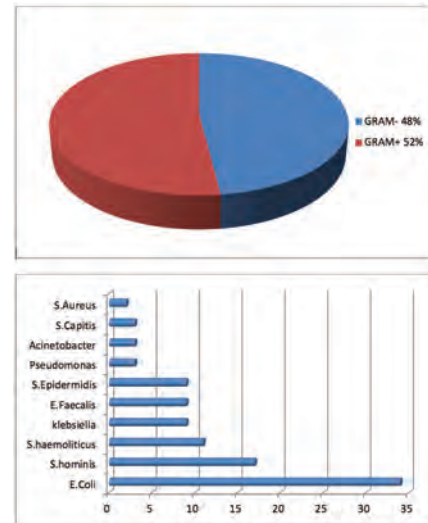


Figure 1.

Only 1 case with AML presented a polymicrobial BSI with Acinetobacter and *Candida glabrata* (3%). The majority of patients with BBSI (89%) presented a prolonged severe neutropenia (<500/microliter, >10 days). The mortality sepsis rate in this setting of BBSI was 25% (9 patients), it was higher for patients with AL (67%) than NHL (33%) and all with prolonged severe neutropenia (p 0.0034). Besides we comparing a previous experience conducted from 2013 to 2017 in our unit about septic patients with acute leukemia and we noticed a smaller reduction of sepsis incidence by gram negative from 70% to 63% and of antimicrobial multiresistance probably modifying our medical behavior (isolation patients multiresistant and avoiding empiric prophylaxis without fever or sign of infection). In accordance with recently literature we found still a small prevalence of gram-BBS in onco-hematologic patients in our ward.

P228

ISOLATED OCULAR TOXOPLASMOSIS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

R. De Marchi, C. Cigana, V. Radici, M. Cerno, A. Sperotto, F. Patriarca, E. Toffoletti, R. Fanin

Università degli studi di Udine, Italy

A 67 year-old woman, after 5 months from hematopoietic stem cell transplantation (HSCT) for myelofibrosis secondary to essential thrombocythemia (JAK2 and MPL wild type, CALR mutated), complained isolated and acute left blurred vision. HSCT was performed on June 2018 from unrelated donor HLA 8/8 matched from peripheral blood (CD34+ 5.8x10⁶/kg and CD3+ 52.2 x10⁷/kg). Reduce intensity conditioning was based on fludarabine and thiotepa. GVHD prophylaxis was performed with cyclosporine, metotrexate and ATG thymoglobuline. Patient and donor serological tests were positive for *Toxoplasma gondii* IgG but negative for IgM antibody. Not relevant infectious complications were reported. Hepatic venous occlusive disease developed after 10 days from HSCT was successfully treated with defibrotide. Unfortunately after 4 months her myeloproliferative neoplasms relapsed and cyclosporine was stopped on 11/12/18. There was no sign/symptom of neither acute or chronic GVHD. Left panuveitis diagnosis was performed by an ophthalmologist and endothelial deposits, presence of fibrin and cells in the vitreous chamber were evidenced while the fundus was not assessable. Blood PCR for Toxoplasmosis was negative as

well as TPHA, galactomannan antigen, HIV, CMV, HSV, VZV and EBV DNA. CD4 positive T cells was 105/microL (nv 500-1200/microL). Serological tests were positive for *T. gondii* IgG but negative for IgM antibody. An autoimmune/idiopathic cause was the first hypothesis and 1 mg/kg of steroids was started with slightest benefit after one week. After one month ocular paracentesis was performed and the PCR on aqueous humor was positive for *T. gondii*. A brain MRI was normal. *T. gondii* PCR on blood was repeated and it was still negative as well as the IgM antibody. We conclude for a reactivation of ocular toxoplasmosis. Sulfadiazine and pirimetamine therapy in association with steroids and folinic acid was started. After for 4 months the patient is nearly blind (VOS 1.5/10) from her left eyes and an acute peripheral nasal and temporal retinal necrosis were the outcome. At the time of the onset of infection, PCP prophylaxis with trimethoprim/sulfamethoxazole was ongoing. Toxoplasmosis is a well known, but relative rare opportunistic infection in HSCT patient and the main site of infection is the CNS, but we have to keep in mind the feasible involvement of the eye. Early diagnosis and treatment is of vital importance.

P229

EFFECTS OF DIFFERENT ORAL IRON FORMULATION SUPPORT ON SELECTION OF SOME PATHOGENS IN HUMAN GUT FLORA: MONOCENTRIC STUDY

G. Giordano, A. Lucchesi, M. Magri, G. Niro, A. Camperchioli, F. Vedruccio, A. Parente, M. Paolo, L. Petrella, M. Cutuli, R. Di Marco, M. Gasperi

Regional Hospital A. Cardarelli And Molise University - Faculty Of Medicine Campobasso, Irst Meldola, Italy

Background: Food and micronutrients support modify the composition of our intestinal flora.

Aim: Aim of this study is to see how different iron formulations used in human iron support are able to expand gut pathogen flora.

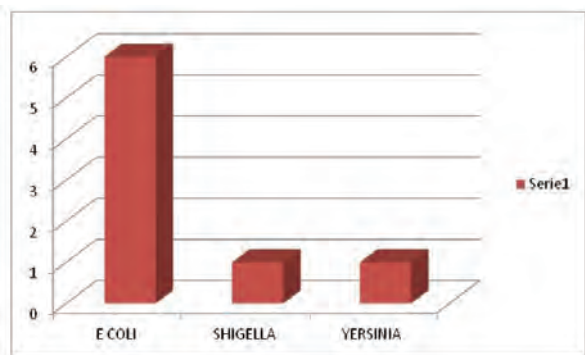


Figure 1.

Methods: 36 female patients (6 per group) with sideropenic anemia due to hypermenorrhoea received 60 mg/day of different iron formulations: sulphate, Sucrosomial®, heminic bisglycinated, bisglycinated chelated, micronized encapsulated. Median age of patients was 30y (R20-38). A stool culture test was performed in each patient at start of treatment and one month after. If at time 0 a culture was positive for a pathogen, patient was excluded from the study. Cultural stool test were performed for *E. coli* enteropathogen, enterohemorrhagic, salmonella, shigella, clostridium, yersinia enterocolica.

Results: Before treatment: 1 patient receiving iron sulphate had a yersinia positivity and 1 receiving heminic bisglycinated iron showed an *E. coli*. After treatment: 2 patients receiving heminic bisglycinated iron showed positivity for *E. coli* enteropathogenic, 1 for shigella; 4 patients receiving iron sulphate showed positivity for *E. coli* enteropathogenic; 2 patients receiving micronized encapsulated iron and 2 with bisglycinated chelated iron showed positivity for *E. coli* enteropathogenic, 1 with Sucrosomial® iron showed positivity for yersinia enterocolica.

Discussion: Different type of iron supplements differently affect pathogen gut flora selection. Molecular complexes iron carriers probably influence differently pathogen selection. The most represented selected pathogen flora is *E. coli* enteropathogenic.

PUBLISHED ONLY

PU01

THE INTERACTION BETWEEN HAEMATOLOGIST AND GENERAL PRACTITIONER IN A HOME CARE SETTING: REAL-LIFE CONSIDERATIONS EMERGING FROM A SINGLE-CENTRE EXPERIENCE

P. Alfieri¹, S. Consolini², F. Orlandini³, E. Tamagnini², C. Votino³, F. Merli¹

¹S.C. Ematologia, Azienda USL - IRCCS Reggio Emilia; ²Servizio Infermieristico Domiciliare, Azienda USL - IRCCS Reggio Emilia; ³Medicina Generale Azienda USL IRCCS Reggio Emilia, Italy

In order to maintain quality of life, prevent the discomfort of prolonged hospitalisation, driving distances, waiting times, and reduce healthcare costs, home care is considered a valid integration to the standard in-hospital services for unfit and frail patients with blood malignancies. In Italy domiciliary programs of supportive and palliative care for haematological patients have been launched since the late '90s under the auspices of local no-profit organisations. More recently, partly on the push of the new legislation on palliative care, some regional public health services have extended the already existing service of integrated home care for cancer patients (better known as ADI "Assistenza Domiciliare Integrata") to the haematology setting. According to the operating model implemented in our centre, a general practitioner (GP) who works side-by-side with a palliative care specialist (another GP with specific training in palliative care), a haematologist and a nurse fully dedicated to domiciliary activities guarantee the global management of haematological patients. Home care is currently offered to terminally ill patients (i.e. acute leukemia in the elderly) requiring exclusively palliation, control of severe symptoms and end-of-life care, to chronically ill patients (i.e. myelodysplastic syndromes) with the aim to provide supportive care outside the hospital and to actively treated patients (i.e. during chemotherapy for lymphoma/myeloma or after bone marrow transplant) with the purpose to anticipate discharge from hospital, reduce infectious risks and shorten recovery time. Blood and platelet transfusions, oral/subcutaneous/intravenous chemotherapies and antimicrobial therapy are routinely administered at home. Due to the highly specialistic context, the role of haematologist cannot be delegated on purpose to GP and palliative care specialist. Based on the experience gained from assisting more than 350 patients in 7 years, some key-points may be identified as an added value in the strategic interaction between haematologist and GP: investing on team networking and continuity of care, empowering families and caregivers, timing the appropriate place for the end-of-life care. This model of cooperation seems very promising in terms of feasibility, sustainability, safety and patient satisfaction. Other issues remain open, such as a network with hospices, the involvement of GPs in simultaneous care and 24/7/365 on-call support.

PU02

OBINUTUZUMAB WITH DHAP DID NOT IMPAIR PERIPHERAL STEM CELL HARVEST: RESULTS OF THE PHASE II PROSPECTIVE FIL GIOTTO TRIAL

L. Rigacci, S. Kovalchuk, C. Visco, R. Battistini, L. Arcaini, M. Gotti, R. Bassan, B. Puccini, M. Mian, L. Flenghi, S. Pozzi, F. Palombi, V. Zoli, A. Evangelista, C. Peracchio, M. Martelli

Ematologia e Centro Trapianti AO San Camillo Forlanini, Ematologia AOU Careggi, Ematologia Ospedale San Bortolo, Dipartimento di Ematologia ed Oncologia Fondazione IRCCS Policlinico San Matteo, UOC Ematologia dell'Ospedale dell'Angelo, Division of Hematology and BMT, General Hospital, Institute of Hematology, Ospedale S. Maria della Misericordia, UOC Ematologia, Azienda Ospedaliero Universitaria, UOSD Ematologia e Trapianti, Istituto Nazionale Tumori Regina Elena IFO, Segreteria Fondazione Italiana Linfomi, Hematology Unit, Department of Cellular Biotechnologies and Hematology, "Sapienza" University On Behalf of Fondazione Italiana Linfomi

Background: Salvage immune-chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard second line treatment for relapsed and refractory Diffuse Large B Cell Lymphoma (DLBCL). Second line immune-chemotherapy, rituximab plus platinum com-

pound or rituximab plus ifosfamide containing regimens, were able to obtain response and permit sufficient harvest of peripheral stem cell.

Aims: The aim of this phase II study (GIOTTO study, Eudract: 2013-004014-17) was to evaluate the efficacy and safety of new anti-CD20 antibody (Obinutuzumab) in association with DHAP as induction therapy before high dose chemotherapy with ASCT in patients with relapsed/refractory DLBCL. One of the secondary objective of this study was to demonstrate the capacity of hematopoietic cell mobilization of this new immune-chemotherapeutic regimen.

Methods: Obinutuzumab was administered on day 1,8,15 in association with DHAP in the first cycle and on day 1 in association with DHAP for the other three cycles. Peripheral stem cell collection (PBSCC) had to be performed after the second or third cycle between 12 and 14 days.

Results: From June 2014 to June 2017 29 patients were enrolled, according to clinical characteristics 17 patients were refractory to first line therapy and 12 relapsed after first line treatment. Seventeen patients underwent peripheral blood stem cell (PBSC) mobilization while 12 did not because of disease progression during salvage therapy. Eight out 17 underwent PBSCC after the second cycle and 9 after third cycle. The median number of harvested CD34 positive cells was $5,8 \times 10^6/\text{Kg}$ (range 2-12,24 $\times 10^6/\text{Kg}$) and the median number of aphereses was 2 (range 1-4). No patients needed plerixafor administration. Eight patients were transplanted with a median number of $3,85 \times 10^6/\text{Kg}$ CD34 positive cells and no engraftment failures were reported.

Conclusions: The use of Obinutuzumab did not impair peripheral stem cell harvest after salvage therapy in relapsed/refractory non Hodgkin's lymphoma.

PU03

CLL: COMMON LEUKEMIA-UNCOMMON PRESENTATION

F. Martini, N. Cecconi, G. Cervetti, S. Galimberti, E. Ciabatti, M. Petrini

Department of Translational Research on New Technologies in Medicine and Surgery Hematology Unit - A.O.U.P. Ospedale Santa Chiara, Pisa, Italy

We report the case of a patient who developed overt IgM related hyperviscosity syndrome after treatment for a diagnosis of chronic lymphocytic leukemia (CLL). A 47-year old wo-man was diagnosed as having stage IIB CLL (normal karyotype). Because of evidence for active disease, the patient chemotherapy was administered: 6 courses of Rituximab-Fludarabine-Cyclofosfamide followed by alemtuzumab and 6 courses of Rituximab-Bendamustine in 2014. Subsequently, IgM values progressively increased from 53 mg/dl to 6550 mg/dl, without any other significant evidence for active disease as defined by iwCLL guidelines. Bone marrow biopsy identified monoclonal lymphocytes (8-10%) with immunophenotypic and morphologic characteristics intermediate between CLL and LPL. B cell clonality testing by immunoglobulin heavy chain polymerase chain reaction (PCR) and flow cytometry (on bone marrow samples) were consistent with one clonal B cell population. Serum immunofixation revealed IgM k monoclonal gammopathy. MYD 88 L265P somatic mutation PCR assay was negative. The patient was treated with Idelalisib. Ritu-ximab was not administered because of patient intolerance (infusion reaction). IgM values decreased, however idelalisib was discontinued because of toxicity (recurrent oral mucositis) and Ibrutinib therapy initiated. Spleen size and IgM values increased gradual-ly. The patient began plasmapheresis because of symptomatic hyperviscosity. Ibrutinib was discontinued. One course of fludarabine, bortezomib and liposomal doxorubicin was administered, which caused severe toxicity (serious viral bronchiolitis infection). After chemotherapy discontinuation venetoclax was administered unsuccessfully: IgM values increased to 7990 mg/dl along with more severe symptomatic hyperviscosity, splenome-galy became symptomatic, bone marrow lymphoma infiltration increased to 50%, oeso-phagus wall's lymphocytic lymphoma infiltration was diagnosed to be the cause of pa-tient's dyspepsia. Splenectomy was recently performed because of symptomatic spleno-megaly. The association of CLL/SLL with serum monoclonal paraprotein is well recognized, however IgM paraprotein is usually low. Dominant symptomatic hyperviscosity is rare. IgM paraprotein seems not to affect patients' OS. The patient has higher risk marker: unmutated IgHV. P53 mutational status is ongoing.

Unfortunately our patient was refractory to chemotherapy, kinase inhibitor (idelalisib and ibrutinib) and bcl2 inhibitor (venetoclax), that have been shown effective in higher risk patients.

PU04

ABSTRACT WITHDRAWN

PU05

DIAGNOSIS OF PML-RARA GENE REARRANGEMENTS IN ACUTE LEUKEMIA: MOLECULAR CHARACTERIZATION BETWEEN QUALITATIVE PCR, REAL TIME MONITORING AND Q-LAMP ASSAYS

F. Culurgioni, E. Desogus, M. Usai, C. Musiu, S. Uda, G. La Nasa

Hematology and Stem Cell Transplantation Unit, Ospedale Oncologico "A. Businco", Azienda Ospedaliera Brotzu, Cagliari, Italy

Acute promyelocytic leukaemia is a rare variant of acute myeloid leukaemia characterised by a rearrangements between 15 and 17 chromosome with typical presentation, morphology, molecular pathogenesis and prognosis. Early therapy with all trans retinoic acid (ATRA) and arsenic demonstrated a significant reduction in early haemorrhagic death. Management of complications as thrombocytopenia, hypofibrinogenaemia and coagulopathy is need in the early stages to minimize this risk. Therefore, prompt initiation of therapy is indicated when APL is suspected until the diagnosis is confirmed or refuted. The comparison of 3 different diagnostic methodologies is the aim of our study as to verify the compatibility between the accuracy of the result, the times of execution of the diagnostic tests and above all the non-complexity in the execution of the same. During the last year, 6 suspected cases of acute promyelocytic leukemia afferent to our clinical unit were studied with 3 methods. In all cases, bcr1, bcr2 and bcr3 isoforms were studied, as qualitative PCR, real time PCR and Q-lamp technology were performed. All positive cases for t(15;17) translocation were confirmed by karyotype analysis with FISH assay. At diagnosis 6 patients in the last year with suspected APL have been analyzed. Qualitative PCR amplification, real time Q-PCR and Q Lamp technology were performed, and only in positive rearrangements FISH have been investigated. For Q Lamp technology assay, GUS beta as internal controls was used, while in qualitative PCR and real time Q-PCR Abelson gene was used. Not treated patients were enrolled for to investigate in t(15;17) translocation and Bcr1, Bcr2, and Bcr3 isoforms. The morphological and cytofluorimetric analyzes had been performed. On 6 patients the 33,3% were positive for t(15;17) respectively one bcr1 isoform and another bcr3 isoform. All diagnostic assay agree with the positivity and isoforms.

Conclusions: In t(15;17) study for APL leukemia at diagnosis, the Q-lamp with isothermal amplification method in unique set to provide improvements for the molecular diagnosis of APL leukemias. The Q-lamp with real time monitoring is reliable, rapid, the final result are available in 40 minutes or generally after 15, 20 minutes is possible to see as preliminary indications of positive samples. The simplicity of the method, its accuracy plays a role crucial in the molecular laboratory for one early response of t(15, 17) at diagnosis.

PU06

IGM MULTIPLE MYELOMA AND WALDENSTROM MACROGLOBULINEMIA: DIAGNOSTIC CHALLENGES AND THERAPEUTIC OPTIONS. A REPORT OF TWO CASES

S. Elba, A. Castellino, M. Bonferroni, C. Castellino, D. Mattei, N. Mordini, M. Foglietta, R. Priotto, D. Rapezzi, R. Sorasio, M. Grasso, M. Massaia

AO Santa Croce e Carle di Cuneo; AOU Città della Salute e della Scienza, Torino, Italy

IgM Multiple Myeloma (MM) and Waldenstrom Macroglobulinemia (WM) are different diseases, with common finding of IgM monoclonal gammopathy. However, clinical characteristics can overlap with challenging differential diagnosis. We described two cases of IgM neoplasm with the same histological bone marrow presentation but different clinical behavior, cytogenetics and biological assessment. On the basis of a comprehensive diagnostic work out, these patients were treated with different approaches. D.G. is a 70 years-old man who presented fatigue,

headache and arthralgia. Initial laboratories tests showed increased IgM level (7665 mg/l) with a M-spike electrophoresis of 4600 mg/L. Bone marrow biopsy resulted in a "small-cell" IgM MM, with an increased number of CD138+, CD79a +, free monoclonal Kappa light chain restricted, IgM +, cyclin D1 +, CD20 negative, CD56 negative plasma cells (70%). MYD88 L265P mutation was negative. FISH analysis showed translocation t(11,14). Low-dose CT scan excluded presence of lytic bone lesions, a denopathy and organomegaly. Since neoplastic clone was CD20 and CD56 negative, while CD138 and cyclin D1 positive, with the absence of somatic MYD88 L265P mutation and with a positivity for t(11,14) and in consideration of clinical presentation, a diagnosis of WM was unlikely and a IgM-k MM was defined. He received Bortezomib, Thalidomide and Dexamethasone induction followed by autologous stem cell transplant consolidation.

B.C is a 84 years-old man with a lot of comorbidities. He presented anemia and thrombocytopenia. Extended blood laboratories tests showed a M-spike at protein electrophoresis, with serum IgM level of 327 mg/L and elevated beta2-microglobulin level. Bone marrow biopsy showed an IgM MM, with an increased number of clonal CD138+, IgM +, cyclin D1+, MUM1+, CD56-/-, CD79 -/+, CD20-, CD19 -, kappa light chain negative plasma cells (30-40%). CT scan showed many enlarged lymph nodes and splenomegaly. Clinical features, enlarged lymph nodes and the absence of bone lesions, were suggestive of WM, in contrast to bone marrow results. MYD88 L265P mutation was researched in peripheral blood and bone marrow aspirate samples, resulting both positive. The patient received rituximab, cyclophosphamide and dexamethasone.

In conclusion, since the overlapping between IgM MM and WM, the correct diagnosis is possible only with a complete evaluation of clinical, molecular and radiological tests in order to choose the correct therapeutic behavior

PU07

THERAPY-RELATED RISK FACTORS CONTRIBUTING TO PEG-ASP HEPATOTOXICITY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

P. Minetto, F. Guolo, M. Clavio, N. Bisso, R. Marcolin, F. Ballerini, M. Miglino, M. Gobbi, R.M. Lemoli

Università degli studi di Genova, Ospedale Policlinico San Martino, Italy

The increased use of peg-asparaginase (PEG-ASP) in modern protocols for adult acute lymphoblastic leukemia (ALL) treatment has led to a significant improvement in clinical outcome. However there are some concerns about PEG-ASP-related severe adverse events. The aim of the present study was to identify therapy-related factors contributing to the development of hepatotoxicity in a cohort of adult ALL patients. Since 2013, 26 adult ALL patients received PEG-ASP in our Center. Median age was 47 years (range 19-76), 19 patients were treated front-line: 11 according to a full pediatric protocol (median age 36, range 19-56, receiving 2 PEG-ASP doses during induction), 5 patients according to a pediatric-like protocol for adult patients (median age: 47,5, range 39-76, receiving 1 PEG-ASP dose during induction). Ten patients received PEG-ASP as part of salvage therapy. Each course of therapy including PEG-ASP administration was analyzed as a separate episode, considering 49 events. Patient features, cumulative PEG-ASP doses, timing and doses of chemotherapy and concomitant medications were analyzed. No PEG-ASP doses reduction were scheduled according to baseline patients-related risk factors. Isolated hyperbilirubinemia was the most frequent adverse event observed (n. 24 grade II, N. 5 grade III, N. 2 grade IV). Grade III hepatotoxicity was observed in 5 patients; 3 patients experienced grade IV hepatotoxicity, with a clinical picture resembling sinusoidal occlusive disease. In univariate analysis age > 45 yrs or BMI >25 were not related with an increased incidence of grade III-IV hepatotoxicity, that was instead significantly affected by concomitant therapies. A cumulative dose of Idarubicin of at least 20 mg/sqm over the same episode, concomitant administration of vancomycin, synthetic penicillins + B-lactamases inhibitors and carbapenems all increased the risk of hepatotoxicity. Notably, none of the patients undergoing the intensive full pediatric induction experienced grade IV hepatotoxicity. A multivariate logistic regression analysis disclosed that administration of IDA and synthetic penicillins + B-lactamases inhibitors were independent predictors of grade III/IV hepatotoxicity (p 0.014, 0.041; Table 1). In our experience the toxicity profile of PEG-ASP in adult patients is

overall manageable. Our data suggest that the development of severe hepatic toxicity may be significantly influenced by chemotherapy schedule and specific concomitant medications.

Table 1.

Variables related to grade III/IV hepatic toxicity (EPISODE)				
	Grade III/IV hepatic toxicities (%)	HR	P (univariate)	P (multivariate)
Age >45 years	5/22 (23)	-	0.440	-
BMI >25	2/15 (13)	--	1.000	-
PEG-ASP ≥ 2000 UI/sqm x2/month*	3/24 (11)	--	0.440	-
Doxorubicin >90 mg/sqm*	4/23 (17)	--	1.000	-
Vincristine >2 mg/sqm*	7/26 (27)	4.20	0.055	0.278
Cyclophosphamide >750 UI/sqm*	5/23 (22)	--	0.454	-
Idarubicin >20 mg/sqm*	3/6 (50)	1.48	0.047	0.014
Cytarabine >1000 mg/sqm*	1/15 (7)	-	0.406	-
6-Mercaptopurine >1000 mg/sqm	0/12 (0)	-	0.173	-
Methotrexate >2500 mg/sqm*	0/9 (0)	-	0.322	-
Steroids	8/37 (21)	-	0.173	-
LMW Heparines	1/17 (6)	-	0.233	-
Vancomicine	4/7 (57)	1.85	0.009	0.070
Synthetic Penicillins + B-lactamases inhibitors	6/16 (38)	3.02	0.010	0.041
Azoles antifungals	6/23 (26)	--	0.125	-
Carabapenems	5/15 (33)	2.02	0.047	0.094
OVERALL	8/49 (16)	--	--	--

* cumulated dose during the single episode

PU08

ABSTRACT WITHDRAWN

PU09

CML PSYCHOEDUCATIONAL GROUP THERAPY

F. Dazzi², F. Simonetti¹, A. Stefanelli¹, P. Scarsini², E. Capochiani¹

¹UOC Ematologia Aziendale ATNO; ²UOC Psicologia clinica ATNO

Introduction: Chronic Myeloid Leukemia (CML) patients receive prolonged oral tyrosine kinase inhibitors (TKIs) treatment. TKIs showed improvement of the CML overall survival rate but could have negative effects on patients' quality of life. Therefore a more robustly medical information and psychological support improve quality of life and treatment adherence.

Tools: MPN-10-Clinical Interview - CBA H: psychophysical symptoms diagnostic evaluation to define the sample and multidisciplinary topics to be discussed.^{1,2} Operative Group after every educational session: arise qualitative data and thematical areas to work on to build together the shared knowledge.³

Method: First Individual Diagnostic Screening: Clinical Interview, CBA H, MPN-10, psychological-medical record. Seven Meetings: first Educational session 30 minutes, follows Operative Group 1 hour. Last meeting retest: CBAH and Qualitative Interview.⁴ After one month: presentation of study results and informative educational brochure

Results:

- State anxiety: normalized
- Health concerns: 60% sample increased
- Body awareness: increased
- Social impact of taking treatment: it organize daily routine and social activities
- Depressive reactions absent: just one patient shows depressive increase and anxious reduction

Conclusions:

The Psychoeducational Group effects are:

1. Decrease state anxiety levels through; information increased awareness sharing experience co-construction knowledge

- identify multidisciplinary specialist networking and referral useful for symptoms

2. Generate shared knowledge by specialist and patients for future patients the all material generated during the group activity is organized in an informative brochure that will be given to the future LMC patients

References

1. MPN-10: Adapted by R. Mesa et al Leukemia & Lymphoma, 2015
2. CBA H: A. M. Zotti, G. Bertolotti, P. Michelin, E. Sanavio, G. Vidotto, 2000
3. Operative Group: E. Pinchon-Rivière, 1940; J. Bleger, A. Bauleo, 1960
4. Qualitative Interview about satisfaction on topics discussed

PU10

ELOTUZUMAB, LENALIDOMIDE, AND DEXAMETHASONE (ELORD) AS SALVAGE THERAPY FOR PATIENTS WITH MULTIPLE MYELOMA: ITALIAN, MULTICENTER, RETROSPECTIVE CLINICAL EXPERIENCE WITH 300 CASES OUTSIDE OF CONTROLLED CLINICAL TRIALS

M. Gentile¹, G. Specchia², D. Derudas³, M. Galli⁴, S. Rocco⁵, C. Conticello⁶, C. Califano⁷, N. Giuliani⁸, S. Mangiacavalli⁹, E. Attingenti¹⁰, A. Lombardo¹¹, M. Brunori¹², E. Rossi¹³, E. Antoniolli¹⁴, R. Ria¹⁵, R. Zambello¹⁶, N. Di Renzo¹⁷, G. Mele¹⁸, G. Marcacci¹⁹, P. Musto²⁰, S. Capalbo²¹, N. Cascavilla²², C. Cerchione²³, A. Belotti²⁴, C. Criscuolo²⁵, G. Uccello²⁶, P. Curci², E. Vigna¹, D. Vincelli²⁷, A. Bonalumi²⁸, A. Siniscalchi²⁹, R. Stocchi³⁰, M. Martino³¹, S. Ballanti³², D. Gangemi³³, A. Gagliardi³⁴, B. Gamberi³⁵, A. Pompa³⁶, A.G. Recchia³⁷, G. Tripepi³⁸, F. Frigeri¹⁰, U. Consoli²⁶, S. Bringhen³⁹, E. Zamagni⁴⁰, F. Patriarca³⁰, V. De Stefano¹³, F. Di Raimondo⁶, F. Ferrara⁵, F. Morabito^{38,41}, M.T. Petrucci⁴², M. Offidani⁴³

¹Hematology Unit, Azienda Ospedaliera of Cosenza, Cosenza, Italy; ²Hematology Section, University of Bari, Italy; ³Department of Hematology, Businco Hospital, Cagliari, Italy; ⁴Hematology and Bone Marrow Transplant Unit, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; ⁵Hematology Unit, Ospedale Cardarelli, Napoli, Italy; ⁶Department of medical and surgical specialties, Hematology Section, University of Catania, Catania, Italy; ⁷Onc-hematology Unit, Ospedale Umberto I, Nocera Inferiore, Italy; ⁸Hematology Unit, Parma University Hospital, Italy; ⁹Hematology Division, Department of Hematology-Oncology, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy; ¹⁰Hematology Unit, Ospedale of Caserta, Italy; ¹¹Onc-hematology Unit Azienda Ospedaliera Santa Maria of Terni, Italy; ¹²Internal Medicine, Ospedale S. Croce, Azienda Ospedaliera Ospedali Riuniti Marche Nord, Presidio of Fano, Italy; ¹³Istituto di Ematologia, Università Cattolica, Fondazione Policlinico Gemelli IRCCS, Roma, Italy; ¹⁴Hematology Unit, Careggi Hospital, Florence, Italy; ¹⁵Department of Biomedical Science, University of Bari "Aldo Moro" Medical School, Internal Medicine "G. Baccelli", Policlinico, Bari, Italy; ¹⁶Department of Medicine, Hematology and Clinical Immunology Section, Padua University School of Medicine, Padua, Italy; ¹⁷Hematology Unit, Lecce, Italy; ¹⁸Hematology Unit, Brindisi, Italy; ¹⁹Hematology and Bone Marrow Transplant Unit, Istituto Nazionale Tumori, Fondazione "G. Pascale", IRCCS, Napoli, Italy; ²⁰Unit of Hematology and Stem Cell Transplantation, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy; ²¹Department of Hematology, Hospital University Riuniti, Foggia, Italy; ²²Department of Hematology and Bone Marrow Transplant, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; ²³Hematology Unit, IRCCS - Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola, Italy; ²⁴Hematology Unit, A.O. Spedali Civili, Brescia, Italy; ²⁵Hematology Unit, AO S.G. Moscati, Aversa (CE), Italy; ²⁶Hematology Department, G. Garibaldi Hospital, Catania, Italy; ²⁷Hematology Unit, Department of Hemato-Oncology and Radiotherapy, Great Metropolitan Hospital "Bianchi-Melacrino-Morelli", Reggio Calabria, Italy; ²⁸Section of Hematology, Department of Medicine, University of Verona, Verona, Italy; ²⁹Hematology Unit and Pathology Department, S. Eugenio Hospital Rome, Rome, Italy; ³⁰Hematology, DISM, University of Udine, Italy; ³¹Stem Cell Transplantation Unit, Department of Hemato-Oncology and Radiotherapy, Great Metropolitan Hospital "Bianchi-Melacrino-Morelli", Reggio Calabria, Italy; ³²Institute of Haematology and Stem Cell transplantation, Ospedale Santa Maria della Misericordia, University of Perugia, Perugia, Italy; ³³Hematology Unit, Fabrizio Spaziani Hospital, Frosinone, Italy; ³⁴Complex Operative Unit of Hematology, S.Maria di Loreto Nuovo Hospital, Napoli, Italy; ³⁵Hematology Unit, AUSL-IRCCS, Reggio Emilia, Italy; ³⁶Hematology Unit, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milano, Italy; ³⁷Biotech-

nology Research Unit, AO di Cosenza, Italy; ³⁸Nephrology Center of National Research Institute of Biomedicine and Molecular Immunology, Reggio Calabria, Italy; ³⁹Division of Hematology, University of Torino, AOU Città della Salute e della Scienza di Torino, Italy; ⁴⁰Seràgnoli Institute of Hematology and Medical Oncology, University of Bologna, Bologna, Italy; ⁴¹Hemato-Oncology department, Augusta Victoria Hospital of Jerusalem, Israel; ⁴²Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Italy; ⁴³Hematology Unit, AOU Ospedali Riuniti di Ancona, Italy

Elotuzumab in combination with lenalidomide and dexamethasone (EloRd) received marketing approval in Italy for relapsed or refractory multiple myeloma (RRMM) in April 2017. Here we report preliminary data of an Italian real-life experience on EloRd as therapy for RRMM patients treated outside of controlled clinical trials. The cohort included 300 RRMM patients from 41 Italian centers who received EloRd treatment according to marketing approval schedule between April 2017 and April 2019. Responsive patients had to reach at least a partial remission (PR). Baseline characteristics are shown in Table 1.

Table 1. Characteristics of the 300 patients at baseline.

	No. of patients (%)
Age, (years)	
<70	113 (37.7)
≥70	187 (63.3)
Sex	
Male	157 (52.3)
Female	143 (47.7)
Paraproteins (isotype)	
Immunoglobulin G	188 (62.7)
Immunoglobulin A	55 (18.3)
Immunoglobulin D	2 (0.7)
Light chain only	53 (17.7)
Non-secretory	2 (0.7)
Creatinine clearance (mL/min)	
≥60	214 (71.3)
<60	86 (28.7)
Stage ISS, (%) (n=238)	
I	91 (38.2)
II	95 (39.9)
III	52 (21.8)
Number of previous lines	
1	186 (62)
2	70 (23.3)
3	20 (6.7)
≥4	24 (8)
Previous ASCT	
No	185 (61.7)
Yes	115 (38.3)
Previous therapies	
Bortezomib	282 (94)
Lenalidomide	78 (26)
Status of the disease	
Biochemical relapse	56 (18.7)
Symptomatic relapse	171 (57)
Refractory to last treatment	73 (24.3)
Time from diagnosis to EloRd treatment (years)	
≥3.5	154 (51.3)
<3.5	146 (48.7)

Median age of the 300 patients was 74 years (range 44–91 years). One hundred and seventy-one patients (57%) showed a symptomatic relapse, 56 (18.7%) a biochemical relapse, and 73 cases (24.3%) were refractory to the last therapy. At last data collection (April 2019), the median number of courses administered was 8 (range 1–25). The overall response rate (ORR) was 77%, with 23 complete remissions (CRs) (7.6%) and 88 very good partial remissions (VGPRs) (29.3%). The median time to first response was 1.7 months, while the median time to best response 3.5 months. Common grade 3 or 4 adverse events were fatigue (20.7%), neutropenia (19%), pneumonia (17.3%), anemia (15.7%), and lymphocytopenia (12.7%). Infusion reactions occurred in 19 patients (6.3%) and were always of grade 1-2. Infusion reactions resolved in all patients and no case discontinued treatment. After a median follow-up of 12 months (range 1-27 months) 117 patients stopped treatment; 95 for disease progression, 11 for toxicity (7 cases infections, 2 lenalidomide-related severe skin rash, 1 dexamethasone-related psychosis, and 1 lenalidomide-related hepatotoxicity) and 12 patients died for causes unrelated to therapy. The 1-year PFS was 62.4%. Number of previous lines of therapy (1 vs >1 lines; p=0.006), time from diagnosis to EloRd start (≥3.5 years vs <3.5 years; p=0.018) and status of the dis-

ease at EloRd start (biochemical relapse vs symptomatic relapse vs refractory to last therapy; p=0.038) were significantly associated with PFS. The other baseline characteristics did not impact on the PFS. Our real world preliminary data confirm that EloRd is a safe and effective regimen for RRMM patients, particularly if used as first salvage regimen in patients with a biochemical relapse and with a MM diagnosis at least 3.5 years, basically resembling results obtained in controlled clinical trials.

PU11

RETROSPECTIVE EVALUATION OF THE PET/CT ROLE IN GASTROINTESTINAL INVOLVEMENT DETECTION IN MANTLE CELL LYMPHOMA

T. Skrypets^{1,2}, A. Sciacovelli¹, S. Ciavarella¹, L. Nassi³, G.M. Casaluci³, B. Puccini⁴, L. Mannelli⁴, A. Guarini¹, C. Minoia¹

¹Haematology Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; ²Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy; ³Haematology Department, Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara, Italy; ⁴Haematology, University of Firenze, Firenze, Italy

Background: Mantle cell lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma (NHL) which represents 5-7% of lymphoid malignancies. In 15-30% of cases by different data, MCL may affect the gastrointestinal tract (GI). It is unknown if the 18F-FDG-PET/CT is able to detect the GI involvement and eventually guide or substitute the endoscopic exams.

Aims: The main objectives of the study were to evaluate positive predictive value (PPV), sensitivity and specificity of the 18F-FDG-PET/CT in the detection of GI involvement in patients with MCL, in comparison to endoscopy with biopsy.

Results: The study was performed at the Hematology Centers of Bari IRCCS, Florence University, and Novara University. Sixty-seven patients with newly diagnosed MCL and pretreatment PET/CT were included. The median age was 70 (range 27-83) with male predominance (67%). The majority of patients (88%) presented a stage III-IV. GI tract involvement in different regions had been found in 27% of patients by PET/CT. Esophagogastroduodenal endoscopy (EGD) and colonoscopy were done for 38 (57%) and 23 (34%) patients, respectively. The false-negative rate of PET/CT in upper GI tract (EGD with biopsy) was observed in the 12.2% of cases, while positive upper GI tract lesions in 38.8% patients were false-positive, with a sensitivity of 53.85% (25.13% to 80.78% 95CI), a specificity of 72% (50.61% to 87.93% 95CI) and a PPV of 50% (30.89% to 69.11% 95CI). Most of them were gastritis and non-specific lesions. Comparing the PET/CT with colonoscopy and biopsy, we observed a false-negative rate of 6.1% and a false-positive rate of 5.5%, with a sensitivity of 66.67% (29.93% to 92.51% 95CI), specificity of 72% (50.61% to 87.93% 95CI), a PPV of 85.71% (46.19% to 97.67% 95CI). We could mention a bias in this group, because, if PET-CT was negative in the lower GI tract, not all patients have proceeded to colonoscopy.

Conclusions: Despite the fact that PET/CT takes a leading place in MCL staging, our data suggest that its sensitivity and specificity in GI tract involvement is quite low and not comparable to the endoscopic studies. Therefore, EGD still remains a «golden standart» in this group of patients. Taking into account all these points, we still need better confirmation on a larger cohort of patients, with centralized revision of images.

PU12

BLINATUMOMAB FOR THE TREATMENT OF RELAPSED/REFRACTORY B-ACUTE LYMPHOBLASTIC LEUKEMIA: REPORT FROM A SINGLE CENTER

S. Giammarco¹, P. Chiusolo², E. Metafuni¹, F. Sorà², L. Laurenti², I. Innocenti¹, F. Autore¹, A. Bacigalupo², S. Sica²

¹Fondazione Policlinico Universitario "A. Gemelli", IRCCS; ²Università Cattolica del Sacro Cuore, IRCCS, Italy

Blinatumomab is a bispecific T-cell engager (BiTE) antibody construct with dual specificity for CD19 and CD3, approved by FDA for the treatment of relapsed or refractory (R/R) precursor B-cell ALL, on the basis of results from two clinical trials, TOWER and ALCANTARA. We

collected data about 10 patients, treated at our Institution from September 2015 until now. There were 5 male and 5 female, with a median age of 49 years (range 28-70); 6 pts were positive for Philadelphia chromosome, 4 negative, with a normal karyotype, except one with trisomy 21, affected by Down syndrome; status disease was: morphological relapse in 4 pts; molecular relapse in 3 pts, refractory disease in 3 pts. All pts, except 1, received Blinatumomab as compassionate use. In each cycle, pts received treatment for 4 weeks. During cycle one, dose was 9 g/day for 1 week, then 28 g/day for 3 weeks to reduce risk of cytokine release syndrome. For subsequent 4 week cycles dose was 28 g/day, followed by two treatment-free weeks. Two out of 6 pts who relapsed after HSCT, received DLI during the two treatment-free weeks and after the end of treatment and another one before. All pts were submitted to central nervous system (CNS) chemotherapy prophylaxis. All 6 pts with bcr-abl transcript were treated with tyrosin-kinasi inhibitors (TKI), but in particular 2 patients continued ponatinib during Blinatumomab infusion. Three out ten pts (30%) completed all 4 cycle, without complications; treatment was interrupted during first cycle in two pts (20%) for onset of neurological symptoms, such as confusion, seizures, tremor and in other 2 patients (20%) for progressive disease, one for extramedullary disease. In the remaining four patients (40%), treatment is still ongoing. Among 6 pts relapsed after HSCT, DLI was performed in 2 pts during Blinatumomab treatment. After 6 infusion, DLI was stopped because of GVHD onset, which required steroid therapy with complete resolution. Five patients are alive and obtained CR with MRD negativity detected by flow cytometry and by PCR; in particular pts relapsed after HSCT, restored full donor chimerism. The remaining 5 pts died because of progression disease. No pts were submitted to HSCT after Blinatumomab. Blinatumomab represent a valid therapeutic option for pts with R/R B-ALL, with a good tolerability and efficacy. It is necessary to train medical staff, nurses and patient in the management of infusional pump kit and about prompt recognition of side effects.

PU13

INFECTIOUS RISK IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

G. Tarantino, G. Agliastro, P. Venza, F. Galbo, G. Sucato, M. Santoro, M. Carlisi, M.E. Mitra

Università degli studi di Palermo, Azienda Ospedaliera Universitaria Policlinico di Palermo, U.O. di Ematologia, Italy

The role of anti-infective prophylaxis in myelodysplastic syndromes (MDS) is still controversial, in fact there are no clear indications that determine its role. The infections have in myelodysplastic syndromes (MDS) an important role and condition the overall survival of these patients. The infectious risk varies according to the characteristics of the patient, intrinsic factors to the pathology, to stratification prognosis and the therapy that patients undertake. In our retrospective study were evaluated 49 patients (24 females and 25 males) with MDS stratified according WHO classification (2016) in a period between January 2016 and May 2018, to evaluate the incidence of febrile events and the anti-infective prophylaxis role. The median age was 72.6 years (73.1 years for patients with clinical history at least one infectious event; 72.2 years for patients without clinical history of infectious events). In our population only 25 patients (51%): 5 RCUD; 3 MDS-EB-1; 4 MDS-EB-2; 8 RCMD; 5 SMD-U developed infection. We globally detected and analyzed 54 infections. 24 of this occurred during severe neutropenia and were associated with bacterial etiology. Only 6 patients with bacterial infection had undergone antibiotic prophylaxis. Infections in our MDS patients occurred in 51% of the cases in according to literature, and also the 15% of infective episodes occurred at the disease onset. The therapy adopted, the advanced age and several comorbidities plays an important role especially in high risk patients. Our study and our data supporting the advantage of prophylaxis, confirm the significance of the levofloxacin in broad spectrum antibiotic prophylaxis. To stratify MDS patients according to prognostics scores and neutrophil counts over time, allows to define the infective risk and optimize timing of antibacterial prophylaxis. Anyway it would be desirable to be able to validate on a more extensive case series, in a randomized way prospective and with a multicenter study, the results we observed.

Table 1.

WHO 2016 classification	Number of patients with infection (%)	Sex		Median age (years)	Number of patients without infection (%)	Sex		Median age (years)	Total number of patients	
		M	F			M	F			
RCUD	5 (10,2%)	0	5	75,8	9 (18,4%)	6	3	70,1	14 (28,5%)	
WHO 2008 Subtype	AR	5 (10,2%)	0	5	75,8	6 (12,3%)	3	3	67,7	11 (22,5%)
	NR	0	0	0	1 (2%)	1	0	73	1 (2%)	
	TR	0	0	0	2 (4%)	2	0	76	2 (4%)	
MDS-EB-1	3 (6%)	3	0	75,3	2 (4%)	0	2	67,5	5 (10,2%)	
MDS-EB2	4 (8%)	1	3	64,2	1 (2%)	1	0	86	5 (10,2%)	
RCMD	8 (16,3%)	6	2	77,2	9 (18,4%)	3	6	71,3	17 (34,7%)	
SMD U	5 (10,2%)	3	2	69,6	3 (6%)	2	1	79,3	8 (16,3%)	
Subtype	LMMC (FAB)	4 (8%)	2	2	71,7	3 (6%)	2	1	79,3	7 (14,3%)
Totale	25 (51%)	13	12	73,1	24 (49%)	12	12	72,2	49	

PU14

COMPARISON BETWEEN SERUM AND URINARY METHODS IN THE ASSESSMENT OF MULTIPLE MYELOMA ACTIVITY

C. Cimmino¹, L. Catalano¹, M. Savoia², L. Vollono², N. Pugliese¹, M. Raimondo¹, I. Peluso¹, S. Vitiello¹, A. Leone¹, G. Delle Cave¹, M. Calabrò¹, F. Pane¹

¹AOUP Federico II, Ematologia; ²AOUP Federico II, Medicina di Laboratorio, Napoli, Italy

Background: Since 2001, the serum free light chain (sFLC) has been available and proved to be useful in Multiple Myeloma, also as potential substitute of 24-hour urine assessment, as it may solve some of the difficulties associated with this approach. Nevertheless, guidelines have not recommended the serum FLC assay as a replacement for urine assessment.

Methods: We evaluated retrospectively the performance of serum and urine measurements at diagnosis and after induction therapy in 14 light chain (LCMM) and 33 intact immunoglobulin myeloma (IIMM) patients evaluated from June 2016 to October 2018 in our institution. Urine and serum FLCs were measured using nephelometric assay.

Results: At baseline, all LCMM patients had measurable disease by serum FLCs, urine FLCs and urine immunofixation (uIFE), while 30 out of 33 IIMM patients had measurable disease by serum FLCs compared to 27 out of 33 by urine methods. At the end of induction therapy, the correlation coefficients between percentage change of serum FLC and urine FLCs ranged between 0.31 and 0.4. After treatment, the serum FLC involved (iFLC) remained elevated in 58% of IIMM patients, whereas urine FLCs and uIFE remained elevated in 39% of patients in this setting. Likewise in LCMM, iFLC remained elevated in 93% of patients, normalizing in only 1 out of 14 (7%), compared to 10 out of 14 (71%) and 8 of 14 (57%) patients in whom urine FLCs and uIFE remained positive. All patients with positive uIFE and urine FLCs at the end of therapy had also elevated serum FLC levels and only two patients, in the setting of IIMM, had positive urine FLCs, including one with uIFE positive, with normal serum FLC. Importantly, elevated serum iFLC after treatment was not associated with poorer progression-free survival (p=.092) as well as positive urine FLCs or uIFE were not.

Conclusions: In summary, we found a moderate agreement between methods for response assessment and, although there is a correlation between the changes of serum FLC and abnormal urine FLCs, the degree of correlation found was insufficient to consider the tests interchangeable. The FLC test provided greater sensitivity than urine FLCs and uIFE for monitoring, although it does not seem to have prognostic value over urine measurements. We conclude that improved sensitivity of sFLC assay over urine measurements seems not sufficient to recommend the former as unique parameter for monitoring patients with MM, especially in the setting of LCMM patients.

PU15

PROGNOSTIC IMPACT OF TUMOR-ASSOCIATED MACROPHAGES, LYMPHOCYTE-TO-MONOCYTE AND NEUTROPHIL-TO-LYMPHOCYTE RATIO IN NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

E. Cencini¹, A. Fabbri¹, L. Schiattone¹, V. Mancini², S. Lazzi², L. Leoncini², M. Bocchia¹

¹Unit of Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena; ²Section of Pathology, Department of Medical Biotechnology, University of Siena, Italy

Introduction: diffuse large B-cell lymphoma (DLBCL) is treated with rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP); 30% of total cases have relapse or refractory disease. Previous studies showed the microenvironment has a prognostic influence; among its cellular components, tumor-associated macrophages (TAM) surely play a leading role. TAM can be classified into 2 distinct types, M1 (anti-tumor activity) and M2 (pro-tumor activity). Another prognostic factor could be represented by lymphopenia, measured as lymphocyte-to-monocyte and neutrophil-to-lymphocyte ratio (LMR and NLR), that reflects a reduced host systemic immunity.

Methods: in this study we would like to evaluate the prognostic impact of total TAM, M1 and M2 subtypes, LMR and NLR in newly diagnosed DLBCL patients. The primary objective is progression-free survival (PFS). Secondary objectives are complete remission (CR) and overall survival (OS). We have retrospectively analyzed a cohort of 37 consecutive patients, treated between 2009 and 2013. Out of 37 patients, 28/37 (75.6%) received R-CHOP or CHOP-like regimens, 9/37 (24.4%) were treated with less intensive therapies. Treatment response was assessed according to 2007 revised criteria. Immunohistochemistry stainings were performed on paraffin-embedded sections with antibodies against CD68 (PG-M1 clone, 1:50, Dako) and CD163 (1:200, Novocastra-Leica). We have evaluated total TAM (CD68+ and CD163+) and different subtypes (CD68+, CD163+, CD68+/CD163+). We have divided our cohort in 2 categories according to Steidl scoring system; score 1-2 represented the group with low expression, while score 3 represented the group with high expression. TAM with the expression of both CD68 and CD 163 were considered as M2. For LMR and NLR we used previously published cut-off of 2.71 and 2.81, respectively.

Results: CR rate was 70.3%, 11 cases did not achieve an adequate response (5 PR, 4 SD, 2 PD). We did not report a significant correlation between total TAM, CD68+ TAM, CD163+ TAM, CD68+/CD163+ TAM, LMR, NLR and CR. We observed an association between lower expression of M2 TAM, higher expression of CD68+ TAM and improved PFS and OS, even if it did not reach a statistical significance.

Conclusions: our study suggests TAM CD68+ and especially M2 TAM could have a prognostic role for DLBCL cases receiving R-CHOP; the lack of statistical significance could be due to the small sample size and warrants future investigations.

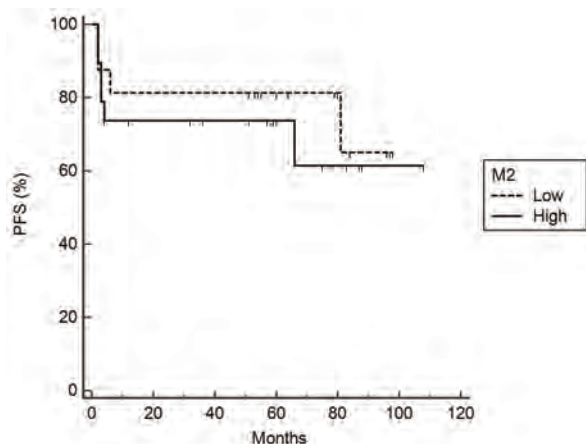


Figure 1.

PU16

IDELALISIB IN PATIENTS WITH REFRACTORY/RELAPESED FOLLICULAR LYMPHOMA REAL-LIFE EXPERIENCE OF RETE EMATOLOGICA PUGLIESE (REP)

G. Tarantini^{1,2}, S. Capalbo³, N. DiRenzo⁴, A. Guarini⁵, P. Mazza⁶, G. Specchia⁷, D. Pastore⁸, V. Pavone

¹SC Ematologia con Trapianto, ospedale Dimiccoli, Barletta; ²SC Ematologia con Trapianto, Ospedali Riuniti, Foggia; ³SC Ematologia con Trapianto, Lecce; ⁴SC Ematologia con Trapianto, IRCSS, Bari; ⁵SC Ematologia con Trapianto, Taranto; ⁶SC Ematologia con Trapianto, Policlinico di Bari; ⁷SC Ematologia con Trapianto, Brindisi; ⁸SC Ematologia con Trapianto, Tricase, Italy

Follicular lymphoma (FL) is the most common indolent lymphoma worldwide. R-CHOP and RB are considered the standard of care for first-line treatment. Treatment of relapsed/refractory patients remains an unmet clinical need. Idelalisib is an inhibitor of the PI3K, approved in Europe for patients with R/R FL who received almost 2 lines of treatment. We retrospectively assessed in real life analysis 31 patients affected by R/R grade I, II and IIIA FL, treated with Idelalisib 300 mg, until progression, toxicity or death, in 8 centres of REP from April 2018. In our cohort the largest proportion of patients was represented by stage III-IV disease (97%). Half of patients had \geq FLIPI2 score. 10 patients had grade 3 AFL (32%). 18 patients were treated with Idelalisib at disease relapse, only 13 patients were refractory to the last therapy. Median age was 70 year (51-87). The overall response rate (ORR) was 61%. CR was achieved in 8 patients (26%), PR in 11 patients (35%) and SD in 1 patient. 12 patients (39%) had PD. Among grade 3a FL patients (n=10) one patient achieved CR, while five patients (50%) obtained a PR with a median follow up of 20 months, 18 months median PFS was 68%. At the time of last follow-up (median follow up 16 months) seven patients (23%) discontinued Idelalisib, two because of toxicity (transaminitis) and five patients died due to lymphoma progression. No patient developed histologic transformation. The most common grade \geq 3 toxicity included Anemia (3%), thrombocytopenia (7%), neutropenia (3%) observed in 7 patients (24%), ALT or AST elevation (7%). 1 patient experienced grade 3 diarrhoea and 2 patients grade 3 oral mucositis. Idelalisib monotherapy was well tolerable with a good safety profile and few AES but appropriate monitoring is recommended in older patients. In our analysis idelalisib was a good treatment option in a group of patients heavily pretreated and with adverse prognostic factor as advanced stage, high Flipi score, grade 3A and for higher median age (70y). The ORR of relapsed pts was 33%, all of them were intermediate (9) or high (7) FLIPI score, none of them was Grade 3A. Univariate analysis related to outcome revealed no adverse significant prognostic factor except for previous number of chemotherapy lines. Our real life experience revealed an excellent result in terms of ORR and CR rate and consistent clinical benefit regardless of age. In our hands idelalisib monotherapy is relatively safe and real effective in R/R follicular Lymphoma also in high risk and older age patients.

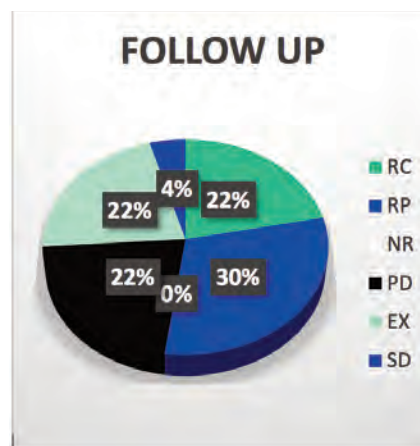


Figure 1.

PU17

R-IVAC AS THIRD-LINE REGIMEN IN REFRACTORY DLBCL: A SINGLE CENTRE EXPERIENCE.

M. Riva¹, G. Scapinello¹, A. Branca¹, M. Pizzi², A. Visentin¹, T. Berno¹, C. Gurrieri¹, F. Vianello¹, R. Zambello¹, L. Trentin¹, G. Semenzato¹, F. Piazza¹

¹Department of Medicine, Hematology and Clinical Immunology Unit, University of Padua; ²Cytopathology and Surgical Pathology Unit, University of Padua

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive but potentially curable B cell tumor, with an extreme biological complexity and different clinical outcomes. There is a lack of consensus regarding third-line regimen in patients with relapsed or refractory DLBCL, and treatment options are limited in this patient population.

Aims: We retrospectively analyzed outcome and toxicities in 16 refractory DLBCL patients (according to the definition of SCHOLAR study) treated with R-IVAC (Rituximab, Ifosfamide, Etoposide, Cytarabine) in the third-line.

Table 1.

N°16	Median (range)	Frequency (%)
Median age at R-IVAC, year	51 (22-69)	
Histology		
DLBCL GC		8 (50%)
DLBCL non-GC		6 (37,5%)
DLBCL NOS		1 (6,25%)
GZL		1 (6,25%)
BCL2 status in IHC		
pos		14 (87,5%)
neg		1 (6,25%)
nd		1 (6,25%)
Ki67% status	72% (35%-98%)	
Double expressor		3 (18,75%)
Triple expressor		3 (18,75%)
Cytogenetic complex		4 (25%)
normal		8 (50%)
nd		4 (25%)
Previous treatment	2 (2-4)	
IPI at failure		
high		13 (81,25%)
high-int.		2 (12,5%)
low-int.		1 (6,25%)
CNS-IPI at failure		
high		13 (81,25%)
int.		2 (12,5%)
low		1 (6,25%)
Response to R-IVAC		
CR		3 (3 (18,75%)
SD/PD		13 (81,25%)
ASCT		
yes		6 (37,5%)
no		10 (62,5%)
Response to ASCT		
CR		5 (31,25%)
SD/PD		11 (68,75%)
AEs after R-IVAC		
Infection		
• G3		5 (31,25%)
• G4		3 (18,75%)
Gastrointestinal disorders (G3-4)		
Hematologic toxicity		
• Cytopenia (Hb <10g/dL, ANC <1,5x10 ⁹ /L, Plts 100x10 ⁹ /L)		13 (81,25%)
• Therapy-related myeloid neoplasms (AML)		1 (6,25%)
lgG (g/L) after R-IVAC	3,5 (1,95-5 g/L)	

Methods: Patients characteristics are shown in Table 1. IPI at the end of two lines of therapies was: 13 high (81,25%), 2 high-intermediate (12,5%) and 1 low-intermediate (6,25%). According to the algorithm of Hans, 8 patients were classified as germinal center (GC) B-cell (50%), and 6 as non-GC (37,5%). Three patients were double expressors (18,75%) and 3 triple expressors (18,75%) lymphoma. Cytogenetics and FISH analysis performed at diagnosis in 11 patients (70%), revealed 4 cases with a complex karyotype (25%), 8 with normal (50%) and 4 with not-determined (25%). No double/triple hit translocations were observed. Response to therapy was assessed using the revised Lugano criteria.

Results: R-IVAC induced 3 CR (18,75%) and 13 SD/PD (81,25%); six patients (37,5%) underwent autologous stem cell transplantation (ASCT) after conditioning with FEAM. Responses were: before ASCT, 4 CR (1 CR after 10 cycles of Brentuximab vedotin in a CD30+ case), 1 SD and 1 PD; after ASCT, 5 CR and 1 PD. High rate of grade 3 and 4 hematological/non-hematological adverse events were reported after R-IVAC. With a median follow-up of 12 months, the 1-year OS and PFS of the entire population were 31% and 6%, with a median survival of

9,5 months. Median OS was 35 months in patients who were transplanted versus 2 months in those who were not (p=0.005, log rank). No differences in OS and PFS were observed according to the COO.

Conclusions: In our small cohort, R-IVAC as third-line salvage chemotherapy led to response and long-term survival in only 18,75% of our cases. These results are coherent with the common experience that standard chemotherapy should not be considered as a valid strategy in refractory DLBCL. Future standard treatments should rather employ combination of novel effective agents, upon risk-stratification of patients according to recognized clinical-biological prognostic factors validated in the R/R setting.

PU18

IBRUTINIB PLUS WITH VENETOCLAX FOR THE TREATMENT OF PRIMARY REFRACTORY MANTLE-CELL LYMPHOMA: A REAL LIFE EXPERIENCE

A. Fabbri¹, E. Cencini¹, A.G. Congiu², M. Miglino³, L. Rigacci⁴, M. Bocchia¹

¹UOC Ematologia Azienda Ospedaliero-Universitaria Senese & Università di Siena; ²UOC Ematologia Ospedale Policlinico S. Martino- IRCCS per l'Oncologia, Genova; ³Clinica Ematologica-Università di Genova- Policlinico San Martino- IRCCS per l'Oncologia-Genova; ⁴UOC Ematologia e Trapianti di Midollo Azienda Ospedaliera San Camillo-Forlanini, Roma, Italy

Introduction: Relapsed/refractory mantle-cell lymphoma (R/R-MCL) patients after intensive chemotherapy and autologous stem-cell transplantation (ASCT) have poor prognosis and few effective treatment options. The irreversible BTK inhibitor ibrutinib showed a promising median progression-free survival (PFS) with manageable toxicity. The BCL2 inhibitor venetoclax showed encouraging results in R/R MCL patients, even if heavily pre-treated. Preclinical models suggest a potential synergistic effect of dual BTK and BCL2 inhibition. Ibrutinib in association with venetoclax was successfully investigated in a phase II trial, with improved outcome of enrolled R/R-MCL patients compared to historical cohorts.

Methods: We report our multicentric experience on 4 patients with primary refractory MCL receiving daily oral ibrutinib in association with venetoclax. All patients started ibrutinib 560mg per day as monotherapy and subsequently added venetoclax at initial dose of 50mg per day, with weekly rump-up until a full dose of 400mg per day. Remarkably, 2 patients received 4 weeks ibrutinib single-agent, as previously published, while the other 2 cases received ibrutinib for more than 4 weeks before starting venetoclax, both with unsatisfactory response. Combined regimen was continued or programmed until allogeneic transplantation.

Aim: To evaluate efficacy and toxicity of ibrutinib plus venetoclax in a "real-life" setting.

Results: Patients were 40-61 years old, with a median of 2 previous treatment received. All cases were stage IV at diagnosis and chemorefractory; MIPI was high in 3/4 patients, bone marrow was involved in all cases, 3 cases presented with blastoid or pleomorphic variant, TP53 evaluation was performed in 1 case and resulted mutated. All patients achieved a response, CR rate was 50% (2/4 cases, MRD-negative CR by flow cytometry in 1 patient). Remarkably, allotransplant as consolidation was scheduled for all cases and it was recently performed in 2/4 patients (1 in CR and 1 in PR) while the other 2 patients are still on treatment. No tumor lysis syndrome occurred and overall toxicity was manageable, including grade 3 neutropenia (1 case), grade 1-2 diarrhea (2 cases) and skin rash (1 case).

Conclusions: ibrutinib plus venetoclax confirms synergy and represents a promising treatment option for R/R-MCL patients appearing safe and feasible in an outpatient "real-life" setting.

PU19

SUSTAINED REMISSION IN NEWLY DIAGNOSED STEROID NON RESPONSIVE ITP PATIENTS AFTER ELTROMBOPAG THERAPY DISCONTINUATION: A CASE SERIES

E. Rinaldi, G. Guaragna, M. Girasoli, D. Pastore

Haematologia, A. Perrino Hospital, Brindisi, Italy

The thrombopoietin receptor agonist (TPO-RA) eltrombopag is approved for second-line use in persistent and chronic immune thrombocytopenia (ITP). There are limited data on using eltrombopag in newly

diagnosed ITP despite about one-third of them do not respond to first-line therapy with steroids and/or IVIG. We report 3 cases of newly diagnosed steroid resistant ITP treated with eltrombopag that maintain sustained remission after discontinuation.

Case 1: A 30 years old man was hospitalized with severe thrombocytopenia (Plt 1000/ μ L) associated with hematuria but with normal renal function, autoimmunity and viral serology. He was diagnosed with ITP and started therapy with prednisone (1 mg/kg/day) and IVIG (0,4 g/kg/day for 5 consecutive days). After 4 days of treatment he developed acute renal failure which required hemodialysis. After twenty days of steroid therapy, severe thrombocytopenia persisted and renal function didn't improve. Therefore he started eltrombopag 50 mg daily with rapid increase of the platelets count. After twenty days of therapy, eltrombopag was stopped when platelet count was 321000/ μ L. After 4 years the patient has stable platelet count >100000/ μ L without therapy.

Case 2: A 83 years old woman with atrial fibrillation in treatment with direct oral anticoagulant (DOAC) presented with severe hemorrhagic thrombocytopenia. It was diagnosed ITP and she started treatment with prednisone and IVIG at standard doses. After 40 days platelet count did not improve. Since it was necessary to increase the platelet count rapidly and the clinical condition of the patient did not allow other treatments, she started eltrombopag 50 mg daily increased to 75 mg daily after 7 days. After 7 months of treatment platelets count was always >250000/ μ L. Then she stopped eltrombopag after tapering. After 1 year the patient has stable platelet count >100000/ μ L without therapy.

Case 3: A 72 years old woman was diagnosed ITP presenting with severe hemorrhagic thrombocytopenia (Plt 8000/ μ L). She started prednisone (1 mg/kg/day) and IVIG (0,4 g/kg/day for 5 consecutive days). After 5 weeks of treatment, there was no response. Then she started eltrombopag 50 mg daily with rapid increase of the platelets count. After 7 months of complete remission, she discontinued eltrombopag showing sustained response for 8 months without additional ITP therapy. Our findings highlight the potential value of TPO-RA in early treatment of patients refractory to standard first line therapy.

PU20

DIFFERENT IRON FORMULATION ADMINISTRATION SHOWS DIFFERENT DEMETILATION PATTERNS AND BONE MARROW MACROPHAGES POLARIZATION IN LOW RISK MYELODYSPLASTIC SYNDROMES (LRMDS)

G. Giordano, A. Lucchesi, M. Magri, G. Niro, A. Camperchioli, F. Vedruccio, A. Parente, M. Paolo, L. Petrella, M. Cutuli, R. Di Marco, M. Gasperi

Regional Hospital A. Cardarelli Campobasso and Molise University, Faculty of Medicine, Irst Meldola, Italy

Background: Iron status influences dna methylation. External factors as liposomes may switc-off bone marrow macrophages M1 polarization that is related to inflammatory status. These things are important in ineffective hemopoiesis in LRMDS.

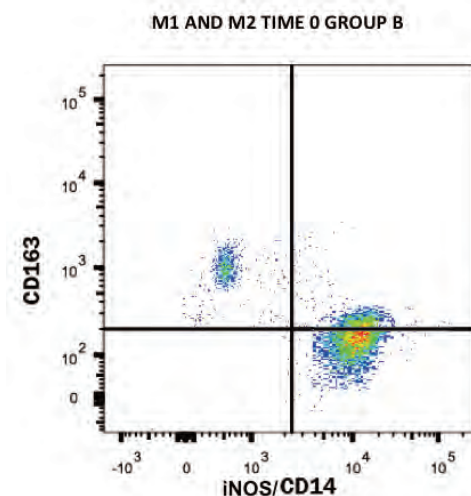
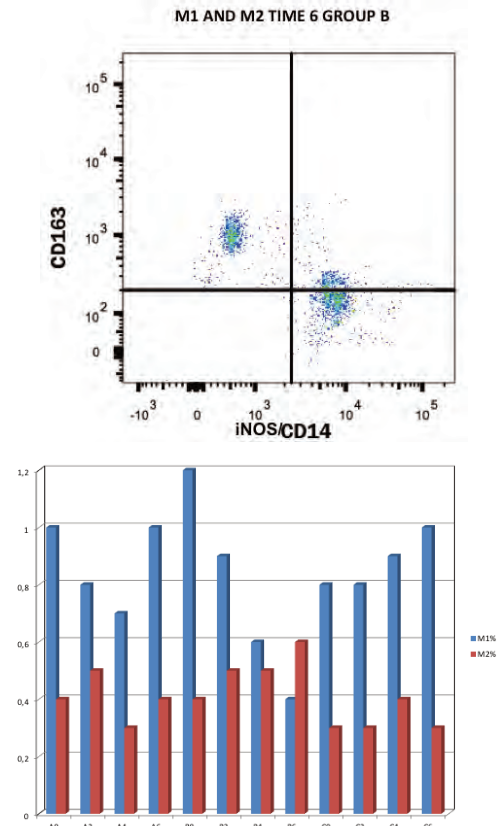
Aim: To see if sucromial iron and sodium ferriguconate support in LRMDS shows different dna metilation patterns and macrophages polarization in bone marrow.

Patients and Methods: This study is a retrospective nonrandomized study. 45 patients with low risk refractory anemia receiving support with erythropoietin 40000 UI two times/week, b12 300 mg/day and folate 15 mg/day were divided in 3 group of 15 patients. Group A received i.v. sodium ferriguconate 65 mg/day for 7 days/month, group B received sucrosomial iron tablets every other day, group C don't received iron. No significant differences among median Hb level (9.5 g/dl), median age (65 yo), sex, karyotype, sEpo, ferritin, Tsat, IPSS, transfusion need, were in the three groups. Follow-up period was 6 months. The bone marrow M1 proinflammatory macrophages (iNOS^{high}/CD163^{low}/CD14⁺) and M2 antiinflammatory (iNOS^{low}/CD163^{high}/CD14⁻) were analyzed by cytofluorimetry and dna methylation with methylation-specific PCR. Bone marrow aspiration, M1 and M2 count, dna methylation, Hb level, transfusion need, ferritin and tsat level, CRP were recorded bimonthly.

Results: All results were recorded at time 0, 2, 4 and 6 months. M1% was 1, 0,8, 0,7, 1 in group A, 1,2, 0,9, 0,6, 0,4 in group B, 0,8, 0,8, 0,9, 1 in group C. M2% was 0,4, 0,5, 0,3, 0,4 in group A, 0,4, 0,5, 0,5, 0,6 in group B, 0,3, 0,3, 0,4, 0,3 in group C. % of methylated cytosine was 6%

in group A, 3% in group B, 5% in group C. CRP (ng/ml) was 6, 8, 10, 12 in group A, 7, 5, 3, 2 in group B, 8, 6, 5, 5 in group C. Hb concentration (g/dl) was 9, 9,8, 11,5, 11,8 in group A, 9, 10,2, 11,5, 12,5 in group B, 9,5, 10, 10,5, 10,9 in group C. Transfusion need (units) was 2 in group A, 2 in group B, 2 in group C.

Conclusions: In LRMDS iron support improves erythropoietin effectiveness and reduces dna methylation. Sucrosomial iron reduces macrophages M1 polarization and CRP, improving effective hemopoiesis.



Figures.

PU21**IRON SUPPORT ENHANCES ERYTHROPOIETIN EFFECTIVENESS IN REFRACTORY ANEMIA WITH HIGH RETICULOCYTE COUNT, TRANSFERRIN SATURATION BELOW 30% AND HIGH LEVEL OF ERYPTOSIS.**

G. Giordano, A. Lucchesi, M. Magri, G. Niro, A. Camperchioli, F. Vedruccio, A. Parente, M. Paolo, L. Petrella, M. Cutuli, R. Di Marco, M. Gasperi

Regional Hospital A. Cardarelli and Molise University - Faculty of Medicine Campobasso, Irst Meldola, Italy

Introduction: Eryptosis (erythrocyte apoptosis) is an enhanced phenomenon in myelodysplastic syndromes (MDS) positively related to reticulocyte count and triggered by iron deficiency. Iron support is not recommended in MDS, also at low risk, in which iron chelation is a pivot of treatment.

Aim: Aim of this study is to see if iron support enhances erythropoietin effectiveness in refractory anemia with high reticulocyte count, transferrin saturation below 30%, regardless of ferritin level

Patients and methods: Between July 2015 and December 2018, 40 patients affected by refractory anemia with IPSS low-risk were studied. Median follow-up was 16 months (R12-28). Patients were randomized 1:1 to receive in group A (with corrected reticulocyte count <2.5%, transferrin saturation >30%) sucrosomial iron 30 mg 2 tablets orally/day + erythropoietin 40000 IU sc/t.i.w. + calcium levofolate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group B (with corrected reticulocyte count >2.5%, transferrin saturation <30%) patient received sucrosomial iron 30 mg 2 tablets orally/day + alpha erythropoietin 40000 IU sc/t.i.w. + calcium levofolate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 75 years (R70-81), M/F: 8/12. In group B median age was 72 years (R68-77), M/F: 10/10. Cytotype was normal in group A and B patients. Median level of haemoglobin was 9.5 g/dl in group A (R9-10) and 8.9 g/dl (R8.5-10) in group B. Median ferritin level was 900 ng/ml in group A (R890-1100) and 800 ng/ml (R750-950) in group B. Eryptosis was measured with cytosolic Ca²⁺, utilizing Fluo3 fluorescence, after cells staining with the Fluo-3 AM dye (Biotium, Hayward, USA). Levels of eryptosis at start of treatment were higher in group B.

Results: Group A patients increased Hb level of 1 g/dl after a median time of 6 weeks (R4-7), 11 g/dl after 3 months. Group B patients increased Hb level of 1 g/dl after a median time of 4 weeks (R3-5), 12 g/dl after 3 months. Median ferritin level was 1100 ng/ml in group A (R950-1300) and 850 ng/ml (R800-900) in group B. After treatment levels of eryptosis were higher in group A.

Conclusion: Iron support enhances erythropoietin effectiveness in refractory anemia with high reticulocyte count, transferrin saturation below 30% probably reducing level of eryptosis involved in ineffective erythropoiesis.

PU22**HIGH-DOSE MELPHALAN IS A SAFE CONDITIONING REGIMEN IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA**

D. Cangini, C. Cerchione, M.B. Giannini, V. Di Battista, F. Frabetti, E. Petracci, S. Ronconi, G. Martinelli

Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS

Background: Despite the increasing use of novel agents in multiple myeloma (MM), autologous stem cell transplantation (ASCT) is still used in fit patients > 65 years.

Aims: We report a real-life retrospective evaluation of safety of ASCT conditioned with high-dose melphalan (HD-MEL) in elderly fit MM patients.

Methods: From January 2016 to March 2019, a total of 34 consecutive MM patients underwent ASCT after a HD-MEL conditioning regimen at our institute (IRST IRCCS). A total of 25 ASCTs in patients > 65 years (Elderly group, EG) and 24 ASCTs in patients ≤ 65 years (Young group, YG) were evaluated. In EG, median age at ASCT was 69 years (r.66-73), median melphalan dose was 180 mg/mq (r.140-200) and median number of CD34+ cells re-infused was 4.76x10⁶/kg (r.3.2-7.0). In YG, median age at ASCT was 58 years (r.52-65), median melphalan dose was 200 mg/mq (r.140-200) and median number of CD34+ cells re-in-

fused was 4.31x10⁶/kg (r.3.37-8.23). Median time for absolute PMN count >500/mcl and >1000/mcl was 10 (r.9-13) and 11 (r.9-13) days in EG, respectively, and 10 (r.9-11) and 10 (r.9-11) days in YG, respectively (p 0.055 and 0.020). Median duration of G4 neutropenia was 5 days in each group and median time for absolute Plts count >20000/mcl was 12 (r.9-18) days in EG and 11 (r.9-18) days in YG, with no statistical difference. Median of transfusion requirement for red cell and platelet units was identical in each group (0 and 2, respectively). In YG, G3-4 AEs were observed in 6 (25%) patients, FUO in 10 (42%), sepsis in 1 and G3-4 mucositis in 4 (17%), with a median duration of 7 days (r.5-9). In EG, G3-4 AEs occurred in 8 (32%) patients, FUO in 5 (20%) and G3-4 mucositis in 7 (28%), with a median duration of 6 days (r.3-14). Results were not significantly different with respect to those of YG. Only 1 patient from each group developed CMV infection, and no invasive fungal infections were observed. Median duration of hospitalization was 18 days (r.16-25) in EG and 17 days in YG (r.15-38) (p=0.156). Transplant-related mortality at day +90 was 0 in evaluable patients in either group.

Conclusions: Our real-life experience comparing HD-MEL conditioning regimen in young and elderly MM patients showed no statistical difference in terms of safety. The only difference was longer engraftment time for PMN with no difference in AEs or duration of hospitalization. In conclusion, HD-MEL used as ASCT-conditioning regimen is feasible in both young and elderly MM patients.

PU23**NIVOLUMAB AS A BRIDGE TO ALLOGENEIC TRANSPLANTATION IN PATIENT WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA: EXPERIENCE OF A SINGLE CENTER**

V. Federico¹, R. Matera¹, P. Forese¹, M. Dargenio¹, S. Luponio¹, D. Carlinio¹, M.P. Fina¹, N. Di Renzo¹

¹UOC Ematologia e Trapianto di Cellule Staminali - PO "Vito Fazzi" - ASL Lecce, Italy

Allogeneic hematopoietic stem cell transplantation (alloSCT) and checkpoint inhibitors therapy are immune-based therapies that have activity in selected relapsed/refractory (R/R) hematologic malignancies. Interest has developed in combining these treatments for high-risk R/R Hodgkin's lymphoma. However, there is concern that checkpoint blockade could augment graft-versus-host disease (GvHD) and several preliminary reports suggest that toxicity, including veno-occlusive disease (VOD) of the liver occur when immune checkpoint inhibitors are used before or after alloSCT. Very few studies have evaluated the safety of checkpoint inhibitors in the pre-allogeneic setting. We report the outcomes of 5 patients R/R classical Hodgkin's-lymphoma that were treated with the anti-PD-1 antibody "nivolumab" as bridge to alloSCT. Between June 2016 and January 2019, received nivolumab 3 mg/Kg every 2 weeks as their last salvage therapy immediately before alloSCT.

Table 1.

	Total
Gender (M/F)	2/3
Median age	34 years (range 21-44)
Median of prior lines of cht	4 (range 4-5)
Median interval from last nivolumab treatment to transplant	45 days (range, 41-59)
Median nivolumab cycles	6 (range, 4-12)
Median day to neutrophil > 0.5 x 10 ⁹ /L	11 days (range 11-16)
Median day to platelet transfusion independence	17 days (range 12-23)

Two patients achieved a complete response, 2 patients received a partial response and 1 patients a stable disease. Two mismatched unrelated, 2 matched unrelated and 1 matched related donors were used. Two patients are waiting for transplantation, while 3 patients received reduced-intensity conditioning. Two out 3 were conditioned with fludarabine, thiotepa and endoxan and received bone marrow allograft while 1 with fludarabina and endoxan and received peripheral blood grafts. GvHD

prophylaxis with mycophenolate and cyclosporine was administered. All 3 patients had full hematologic recovery after alloSCT. By 32 days post-transplant, all patients achieved full donor T-cell engraftment. No graft failures was experienced. At a median follow-up of 16 months (range, 14-25), all patients continue to exhibit full donor chimerism. No patient experienced VOD of the liver or grade 3-4 GvHD. Two patient experienced grade 2 cutaneous aGvHD and responded to steroids. There were no other immune-related adverse events, or grade 3 fevers and no cGvHD. With a median follow-up of 16 months (range, 14-25) all patients remain in continuous CR. Based on our experienced, this combination seems to be feasible, not associated with higher mortality, severe GvHD, VOD or disease progression. However, additional prospective data with larger number of patients are needed to assess the exact role and toxicity of nivolumab as a bridge to alloSCT.

PU24

DIAGNOSTIC UTILITY OF SUDOSCAN FOR DETECTING BORTEZOMIB-INDUCED PAINFUL NEUROPATHY

V. Innao¹, A. Allegra¹, V. Rizzo², A. Alibrandi³, A. Mazzeo², R. Leanza¹, C. Terranova², L. Gentile², P. Girlanda², A.G. Allegra¹, A. Alonci¹, C. Musolino¹

¹Division of Hematology, Department of Human Pathology in Adulthood and Childhood "Gaetano Barresi", University of Messina; ²Department of Clinical and Experimental Medicine, University of Messina; ³Department of Economics Unit of Statistical and Mathematical Sciences, University of Messina, Italy

Objective: Bortezomib is a first-line drug in therapy of multiple myeloma. The onset of peripheral neuropathy is a dose limiting collateral effect of the drug. This neuropathy is a distal symmetric small fiber neuropathy. Nerve conduction study can be used for the diagnosis of bortezomib neuropathy, but this technique can only demonstrate alterations of the large fiber nerves. Sudoscan is a novel technique utilized to offer an evaluation of sudomotor function. The main objective of this study was to compare the sensitivity and diagnostic specificity of Sudoscan with respect the electromyographic examination after bortezomib treatment.

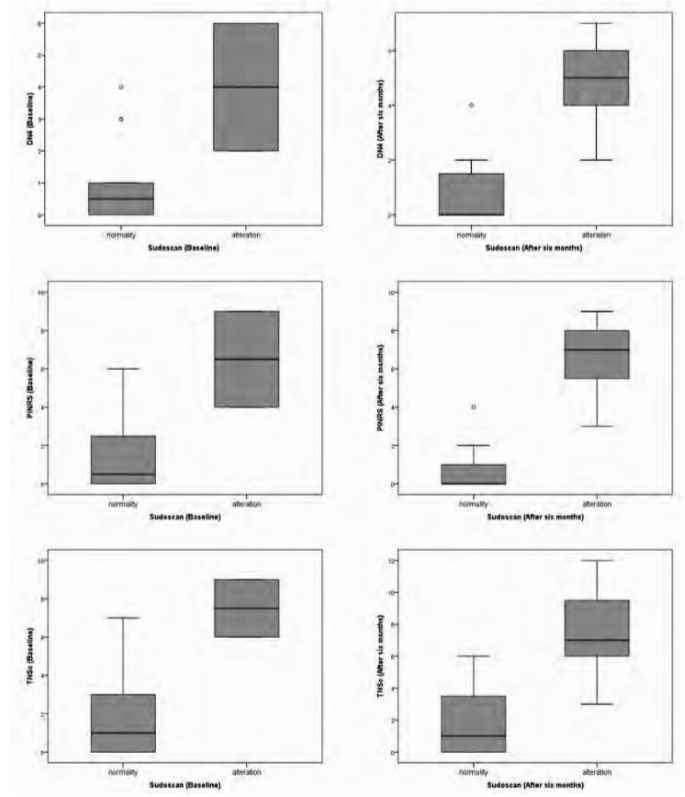


Figure 2.

PU25

ABSTRACT WITHDRAWN

PU26

BLINATUMOMAB AS A BRIDGE TO ALLOGENEIC TRASPLANT IN YOUNGER PATIENT WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). A "REAL LIFE" EXPERIENCE OF RETE EMATOLOGICA PUGLIESE (REP)

V. Federico¹, M. Dargenio¹, R. Matera¹, G. Rossi², M. Abbeante², M. D'Elia³, S. Luponio¹, N. Cascavilla², G. Specchia³, N. Di Renzo¹

¹UOC Ematologia e Trapianto di Cellule Staminali - PO "Vito Fazzi"; ²UOC Ematologia IRCCS "Casa Sollievo della Sofferenza"; ³UOC Ematologia con Trapianto - Azienda Ospedaliera -Universitaria "Policlinico", Italy

Blinatumomab bispecific T-cell engager antibody construct that redirects CD31 T cells to lyse CD191 B cells demonstrated a significantly longer overall survival vs standard of care chemotherapy in adults with relapsed/refractory acute lymphoblastic leukemia (R/R ALL). The TOWER study demonstrated that alloHSCT vs no alloHSCT after blinatumomab was associated with a 55% reduction in the risk of death (hazard ratio .45 [95% CI .24, .84]; p=.012). Here we report real life experience of "Rete Ematologica Pugliese". Between February 2017 and February 2019 five adults patients with R/R ALL Ph-orMRD-positive post induction chemotherapy receive blinatumomabas a bridge to alloHSCT (3 patients received blinatumomab were relapsed and two because were MRD-positive post induction chemotherapy). Blinatumomab was delivered as a continuous IV infusion at fixed step-wise doses in 6-week cycles: 4 weeks on (9 µg per day for days 1-7 of cycle 1 and then 28 µg per day thereafter) and 2 weeks off for each cycle. Dexamethasone premedication (20 mg IV) was required prior to each infusion and dose step to prevent cytokine-release syndrome. At any time after the first cycle, patients eligible could proceed to alloHSCT. Two patients received 2 cycles of blinatumomab, while 3 patients one cycle. Two patients obtainedMRD-negative post blinatumomab (40%) while 3 patients maintained positive-MRD after blinatumomab. After allo-transplant one additional patient obtained MRD-negativity (overall MRD negativity: 60%). No veno-occlusive dis-

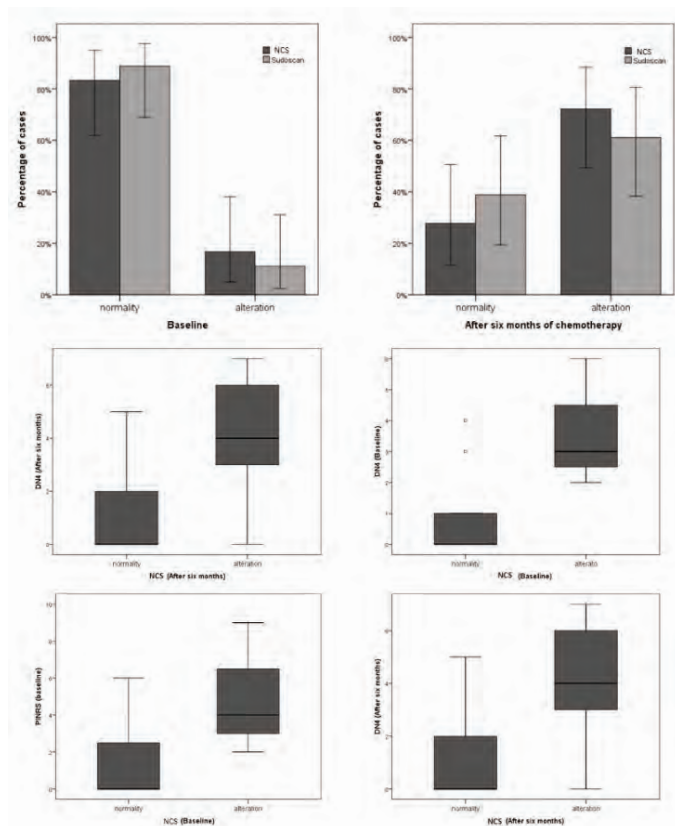


Figure 1.

ease/sinusoidal obstructive syndrome were observed. There were no other immune-related adverse events or acute grade III-IV GvHD. One patient (20%) experienced grade II cutaneous chronic GvHD. At present time four patients are alive after a median follow-up 11 months (range: 4-24 months). One patient died of relapse after 2 years. Despite the small number of cases the our real life experience confirms that transplant eligible young patients with R/R ALL Ph- or positive-MRD post induction chemotherapy may benefit from the sequential combination of blinatumomab + alloHSCT. Although in our experience MRD negativity (60%) is associated with lower relapse rate more patients need to be treated to confirm the benefit of this approach in very high-risk patients with ALL.

Table 1.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	29	24	24	24	25
Gender	Male	Male	Male	Male	Female
Date of diagnosis	01/04/2010	17/03/2015	01/12/2017	12/06/2018	03/08/2018
MRD pre blinatumomab	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE
MRD post blinatumomab	POSITIVE	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE
Source of grafts	Blood (4,7 X10 ⁶ /KG)	Blood (5,2 X10 ⁶ /KG)	Blood (8,75 X10 ⁶ /KG)	Blood (7,66 X10 ⁶ /KG)	Blood (7,08 X10 ⁶ /KG)
Day to neutrophil > 0.5 x 10 ⁹ /L	15	22	11	12	15
Day to platelets > 20 x 10 ⁹ /L	14	20	13	13	15
aGVHD	No	No	No	No	No
cGVHD	No	Si	No	No	No
VOD	No	No	No	No	No
MRD post alloHSCT	POSITIVE	NEGATIVE	NEGATIVE	POSITIVE	NEGATIVE
Relapse	Yes	No	No	Yes	No
Status	Died	Alive	Alive	Alive	Alive
OS months	24	13	11	6	4

PU27

HEMATOLOGY AND EARLY PALLIATIVE CARE: A CONSCIOUS CHOICE FOR PATIENTS AND HEALTHCARE PROFESSIONALS

M. Cruciani¹, G. Ciacci¹, D. Munteanu¹, E. Tamagnini², P. Alfieri²

¹Associazione ZeroK; ²Assistenza Domiciliare Ematologica GRADE

According to the current definition of EAPC (European Association for Palliative Care) "palliative care is the active, total care of the patients whose disease is not responsive to curative treatment. Control of pain, of other symptom, and of social, psychological and spiritual problems is paramount". Although early palliative care has already demonstrated efficacy in the management of physical, psychological and spiritual symptoms among cancer patients, interventions of simultaneous care are not yet routinely provided in Hematology wards and day services. Since patients with blood and lymphoid malignancies in advanced phases of disease generally present a significant symptom burden (fatigue, bleeding and infection risks, fever, dyspnea, pain), the role of the palliative care team is to support patients and families and to cooperate with the specialist care team in the decision-making process and in the caregiving, especially during the transition time from active treatment to the end-of-life. Nursing has a central role in the daily assistance and in critical phases: according to the model of primary nursing and relationship-based care, nurses - more than doctors - may recognize those needs that are often hidden behind non-physical symptoms (fear of death/fear of suffering) and address emerging problems to the multi-disciplinary team. One of the main unmet needs in the setting of terminally ill hematology patients is the appropriateness of the place of death: a large majority of patients still die in acute hospital beds. Any concern about the end-of-life care should be faced at the right time - earlier and simultaneously - when there is room enough for the patient to make a conscious choice regarding place of care, advance care plan-

ning, acceptance/refusal of further lines of active treatment, the balance between quality of life and life expectancy.

PU28

REAL LIFE DATA ON AZACITIDINE THERAPY FOR INTERMEDIATE-2/HIGH-RISK MDS, AML WITH MDS-RELATED CHANGES AND CMML-2: SINGLE CENTRE EXPERIENCE OF AN INTERNAL MEDICINE DEPARTMENT IN A PROVINCIAL HOSPITAL

G. Cametti¹, C. Ceretto¹, C. Paparo², L. Godio³, R. Frediani¹

¹Day Hospital SC Medicina Interna; ²Laboratorio Analisi Ospedale Maggiore ASLTO5; ³Anatomia Patologica AOU Città della Salute e della Scienza

Intermediate-2/high-risk MDS, AML with MDS-related changes and CMML-2 affect elderly patients (pts) predominantly, for whom access to central hospitals may be not simple. Clinical trials with azacitidine have shown to significantly improve survival, but real life studies have given contradictory results. 40 pts were diagnosed in our institution from march 2007 to march 2019 and treated with azacitidine: 4 MDS-EB-1, 13 MDS-EB-2, 21 AML with MDS-related changes, 2 CMML-2. 33 were male, 7 female. Median age was 77 (range 64-90). Performance status was 0 or 1. Diagnosis was established according to 2016 WHO and IPSS. Triphine biopsy/aspirate and cytogenetic testing were performed in all pts prior to treatment and repeated after every sixth cycle. Diagnosis and treatments were carried out on an outpatient regimen, limiting hospitalization to complications only. Azacitidine was administered according to the usual schedule of 75 mg/m² for 7 days every 28 and continued without dose reductions up to tolerance or progression. Response was evaluated in 26 pts who have performed at least 4 cycles and was classified according to the 2006 IWG criteria. Therapy was prematurely interrupted in 12 pts due to infection (6), intolerance (2), progression (2), cardiovascular cause (1), lost at follow-up (1). 2 pts were not evaluated for therapy started less than 4 months ago. The 26 pts evaluable for response received a median of 12 cycles (range 4-56). ORR (CR+PR+MCR+HI) was 52,6% (20 out of 38 evaluable pts); 3 pts (7,9%) had SD, 15 (39,5%) failure. Of the 26 pts treated with at least 4 cycles, 14 were transfusion dependent; 6 of them achieved transfusion independence. Of the 20 responders pts, 8 obtained CR, 5 MCR, 1 PR and 6 HI. Response to therapy was observed after a median of 6 cycles (range 3-18) and median duration of response was 7 months (range 2-30). Median follow up of the 23 responders and SD pts was 18 months (range 4-65) with median survival of 21. In the 15 failure pts, the median follow-up was 3 months (range 1-24) with median survival of 3. In conclusion therapy with azacitidine is safe and effective in elderly pts with intermediate-2/high-risk MDS, AML with MDS-related changes and CMML-2, and our real life data are comparable to those shown in the literature. Azacitidine is confirmed as a feasible therapy in an outpatient setting at the Internal Medicine Department of a provincial hospital, thus favoring access to the treatment of the older population.

PU29

ELOTUZUMAB LENALIDOMIDE AND DEXAMETHASONE IN FRAIL ELDERLY PATIENTS WITH RELAPSED MULTIPLE MYELOMA. A REAL LIFE EXPERIENCE

L. De Padua, A. Carbone, F. Saltarelli, D. Gangemi, A. andriani

UOC Ematologia Ospedale F. Spaziani, Frosinone, Italy

Association of elotuzumab(elo), lenalidomide(lena) and dexamethasone(dex),(elord) represents an useful treatment in relapsed multiple myeloma (MM). Benefit in terms of progression free survival (PFS) has been consistent in patients older than 75 years, even if few patients belonging to that advanced class of age have been enrolled in the registrative study. Here we report results of elord treatment achieved in this category of patients. From September 2017 till now we treated 3 very elderly patients with first relapsed MM. All patients had available active caregivers. They received elo at standard dose according to drug data sheet. Dose of lena was based on creatinine clearance. Dex was administered orally at dose of 20 mg during the week without elo. All patients had obtained partial response (PR) to previous treatment (bortezomib, melphalan and prednisone) with median PFS of 24 months (12-42). Median age was 81 years (81-83). Median MM frailty score was 4 (3-5). All patients had ISS stage 2. Median bone marrow infiltration was 83%(80-90). At baseline, 1 patient presented bone lesions and 2 patients moderate renal impairment. Median follow up after elord was 14,5

months(12-19). Starting dose of lena was reduced to 5 mg before 3rd cycle in all patients due to renal impairment (2 patients) or haematological toxicity (1 patient). All patients experienced 50% reduction of serum M component after a median of 3 cycles (2-5); one patient achieved very good PR (VGPR); one patient showed PR. The 3rd patient with baseline severe anemia and moderate renal impairment, achieved transfusion independence after 3 courses with a normal renal function after 13; she experienced biochemical PR with progression of costal lesion at 13th cycle needed radiation treatment. After a 19 months follow up she is still on treatment in PR. All patients improved performance status. No patients showed infusion reactions. Principal adverse events were G3-4 neutropenia (1 patient), recurring cystitis (1 patient), worsening of renal impairment (2 patients), sensitive neuropathy (1 patient). One patient with diabetes discontinued elord after 6 courses due to cardiac failure. One patient discontinued elord after 10 cycles due to extrapyramidal syndrome but after 4 months he is still in VGPR. In conclusion elderly patients are more susceptible to side effects of combination regimens; nevertheless elord with reduced dosage of lena is a feasible therapeutic option in well selected frail patients.

PU30

VITAMIN D SUPPORT IMPROVES SUCROSOMIAL IRON ABSORPTION IN VITAMIN D AND IRON DEFICIENT PATIENTS. MONOCENTRIC PROSPECTIVE RANDOMIZED STUDY.

G. Giordano, A. Lucchesi, M. Magri, G. Niro, A. Camperchioli, F. Vedruccio, A. Parente, M. Paolo, L. Petrella, M. Cutuli, R. Di Marco, M. Gasperi

Regional Hospital A. Cardarelli And Molise University - Faculty of Medicine Campobasso, Irst Meldola, Italy

Background: Vitamin d has an antiinflammatory effect inducing Th2 and Tregs response and M2 macrophage polarization and inhibits hepcidin production.

Aim: Aim of this study is to see if vitamin d support improves iron absorption in patient with sideropaenic anemia and vitamin d deficiency.

Patients and Methods: In group A 20 patients (M/F:1/2), median age 55 yo (R50-70), with vitamin d <10 ng/ml and with median Hb value 8.5 g/dl (R8-9.5), with median ferritin value 20 ng/ml (2-28) were supported with sucrosomial iron 30 mg tid for 3 months and cholecalciferol 4000 UI/day. In group A median CRP was 35 ng/ml (R20-47) and 6 patients had documented bacterial infection. In group B 20 patients (M/F:1/2), median age 60 yo (R55-68), with vitamin d <10ng/ml and with median Hb value 8.2 g/dl (R8-10), with median ferritin value 18 ng/ml (10-30) were supported with sucrosomial iron 30 mg tid for 3 months. In group B median CRP was 28 ng/ml (R20-32) and 7 patients had documented bacterial infection.

Results: In group A after 3 months patients achieved median Hb level of 12.5g/dl (R 10-13). Patients with bacterial infection achieved median Hb level of 10g/dl (R 9-11). In group B after 3 months patients achieved median Hb level of 11.5g/dl (R 9.5-12). Patients with bacterial infection achieved median Hb level of 9.5g/dl (R 9-10.5).

Conclusion: Vitamin D seems to improve sucrosomial iron absorption in patients with vitamin d deficiency, with the exception of patients with bacterial infection.

PU31

A SINGLE CENTRE EXPERIENCE OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA PATIENTS

M. Riva, L. Ammirati, A. Visentin, C. Gurrieri, A. Branca, G. Scapinello, G. Barilà, S. Imbergamo, F. Piazza, L. Trentin, R. Zambello, G. Semenzato, T. Berno

Department of Medicine, University of Padua, Hematology and Clinical Immunology Unit

Introduction: Multiple myeloma (MM) is a plasma cell malignancy that affects older adults with a median age at diagnosis of 70 years. Autologous stem cell transplantation (ASCT) is the standard of care for young newly diagnosed MM patients. The safety and efficacy of High Dose Therapy (HDT) as upfront treatment in elderly patients remain still uncertain, because elderly age is frequently associated to increased comor-

bidities and suspected increased treatment-related toxicities. In our centre we analyzed the outcome and toxicities in a homogeneous cohort of newly diagnosed elderly MM patients treated with HDT approach.

Methods: We retrospectively evaluated 24 newly diagnosed elderly MM patients, according to International Myeloma Working Group (IMWG). All of them were fit/low-risk according to the revised Myeloma Comorbidity Index (R-MCI). Patients characteristics are shown in Table 1. Melphalan 140 mg/mq conditioning regimen was administered in 14 patients (60%) and 200 mg/mq in 10 patients (40%). After ASCT we observed 5 CR (21%), 14 VGPR (58.5%), 4 PR (16.5%) and 1 death. Median time to neutrophil and platelet engraftment was 10 days (range 6-18 days). No significant difference in toxicity was found among the two groups (MEL140 vs MEL200). The non-hematological toxicities after ASCT (G3-G4) included infections in 12 patients (50%) and gastrointestinal disorders in 10 cases (41.5%). In 1 patient a cardiac toxicity (G2) was observed. The day-100 post ASCT treatment-related mortality (TRM) was 4% (1 patients died due to influenza-pneumonia). During a median follow up of 41 months, we reported secondary malignancies in 2 cases (8%) and one therapy-related myeloid neoplasm (t-MDS). The median PFS was 30 months in MEL140 group vs 53 months in MEL200 (p=0.11); no significant difference in OS was observed (p=0.3).

Conclusions: Our data, even in a small cohort of elderly and fit patients, suggest that ASCT is a safe and well-tolerated procedure. A similar profile of toxicities was detected among the two regimens (MEL140 and MEL200), but the higher dose of Melphalan (MEL200) seems to impact on the duration of response, with a significant trend in PFS. Our experience confirmed that an accurate assessment of patients performance status and comorbidities at diagnosis could represent the crucial way to develop risk-adapted treatment strategies to improve the outcome in elderly MM patients.

Table 1. Clinical characteristics

Characteristic	Patients
(N= 24)	
Age at transplant, median (range), years	67 (66-69)
Revised International Staging System at diagnosis (R-ISS), n. (%)	
Stage I	12 (50%)
Stage II	8 (33%)
Stage III	3 (12.5%)
Unknown	1 (4%)
Cytogenetic, n. (%)	
Standard-risk	11 (46%)
Intermediate-risk	4 (16%)
High-risk	3 (12.5%)
Not assessable	6 (25%)
Immunoparesis at diagnosis, n. (%)	19 (80%)
Induction therapy, n. (%)	
Bortezomib + Dexa	10 (41.5%)
Bortezomib + IMiD	11 (46%)
Bortezomib-based + infusional chemotherapy	3 (12.5%)
Response to induction, n. (%)	
CR	5 (21%)
VGPR	10 (41.5%)
PR	7 (29%)
SD	2 (8.5%)
PD	0 (0%)
High-dose therapy (HDT), n. (%)	
Mel 140 mg/mq	14 (60%)
Mel 200 mg/mq	10 (40%)
Response after ASCT, n. (%)	
CR	5 (21%)
VGPR	14 (58.5%)
PR	4 (16.5%)
Death	1 (4%)
Ig recovery: 1 year, n.	15 out of 19 with immunoparesis at diagnosis
AEs after ASCT, n. (%)	
Infection (G3-G4)	8 (50%)
Gastrointestinal disorders (G3-G4)	8 (50%)
Nervous system disorders (G2-G3)	7 (43.75)
Musculoskeletal and connective tissue disorders (G2)	2 (12.5%)
Cardiac toxicities (G2)	1 (6.25%)
Hematologic toxicity after ASCT	
Cytopenia (Hb < 10g/dL, ANC < 1.5x10 ⁹ /L, Plts < 100x10 ⁹ /L)	6 (37.5%)
MDS therapy-related	1 (6.25%)
Secondary malignancies	2 (12.5%)

PU32

RED BLOOD CELL TRANSFUSION IN AN ELDERLY PATIENT: A TAILORED APPROACH

V. La Terra¹, L. Maiorana¹, A. Saeli¹, A. Antolino², S. Cabibbo², G.O. Manenti², M. Poidomani², G. Garozzo²

¹SAMOT Ragusa Onlus, ²SIMT-UO Ematologia

For patients in palliative care the RBC transfusion is a medical treatment that needs a risk vs benefit evaluation. We present the case of a 100-year-old woman, suffering from cerebral vasculopathy with a previous history of transient ischemic attacks (TIAs), assisted by our association, SAMOT Ragusa Onlus, from March 2018. When the service was activated the patient had a Karnofsky Performance Status (KPS) of 20%, on physical examination the patient was vigilant but not very cooperative and with little time and space orientation, she presented severe sarcopenia, melena for several weeks (unknown origin), slight flap of pleural effusion on the right but not dyspnea, dry skin and dry mucous membranes. During the assistance the patient continued to suffer of recurrent episodes of melena, lasting about 15-20 days; episodes of fever of unknown origin (FUO), and additional TIAs. Consequent anemia (Hb<8g/dL) was treated with multiple RBC transfusions and for the fever a steroid therapy was used. Furthermore the patient presented edema in the hand and left foot for which it was necessary to introduce Low Molecular Weight Heparin (LMWH) with resolution of the disorder. From the beginning, the aim of the therapy was the management of the symptoms, rationalizing the therapeutic resources used, to obtain the chronicization of the clinical condition. The choice to perform RBC transfusions, evaluated by risk/benefit ratio, was guided by the presence of haemorrhage, low Hb values (<8g/dL) and symptoms such as increased fatigue and slight dyspnea. Drug therapy associated with periodic RBC transfusions has allowed a good control of the symptoms. Pharmacological treatments have been associated with a support path, guaranteed by a multidisciplinary team. The initial choice of proceeding with periodic RBC transfusions for the control of anemia and symptoms, although not devoid of risks, allowed a temporary stabilization of the clinical conditions, as a result a decline in hemoglobin values not associated with further bleeding episodes and mild anemia symptoms was observed progressively. This approach allowed agreement with the patient and with her family about the future therapeutic choices, also suggesting the choice of a therapeutic desistance. The team was thus able to plan an individualized intervention aimed at reconciling the expectations and hopes of the patient and her family members with the real clinical conditions.

PU33

MYELOID SARCOMA: AN UNUSUAL CASE PRESENTATION

M. Perrotta¹, E. Rotondo¹, D. Onofrillo², A. Sau², S. Pascuale², G. Lisi³, G. Di Paolo⁴, R. De Vito⁵, A. Spadano⁶

¹Clinica Pediatrica, Ospedale "Ss Annunziata" Chieti; ²Unità Operativa Semplificata Oncoematologia Pediatrica, Ospedale Civile di Pescara; ³Unità Operativa Complessa Chirurgia Pediatrica, Ospedale Civile di Pescara; ⁴Servizio di Ginecologia dell'infanzia e adolescenza, Ospedale Civile Pescara; ⁵Anatomia Patologica, Ospedale Pediatrico Bambino Gesù, Roma; ⁶Unità Operativa Complessa di Ematologia Clinica, Ospedale Civile di Pescara, Italy

The differential diagnosis of pelvic mass in children includes several etiologies. The most common neoplasms are rhabdomyosarcomas of the bladder, prostate in male and vagina in female, sacrococcygeal teratoma and the germ cell tumors. We have reported a case of Myeloid Sarcoma (MS) with an uncommon localization and presentation. A 9 years old girl presented vaginal muco-haematic losses; a pediatric endocrinologist was consulted on suspicion of precocious puberty. An abdominal-pelvic MRI documented a pelvic mass, markedly increased uterus size with subverted structure and swelling of the lymph nodes in the iliac-pelvic area and in the bilateral inguinal region. An incisional biopsy of the lesion and removal of a lymph node was performed. Marrow aspirates from both iliac crests and bone marrow biopsy were performed to assess the presence of any intramedullary infiltration, but it didn't show signs of acute leukemia or others neoplastic cells. Immunohistochemical and histological investigations supported the diagnosis of myeloid sarcoma according to WHO 2017. We completed the staging with total body PET CT, which documented a pathological increase in

metabolic activity of the pelvic mass. Therefore, chemotherapy was started according to the AIEOP LAM03 protocol and the patient achieved complete remission after the first cycle of chemotherapy. Myeloid sarcoma (MS) is an extramedullary mass, which consists of myeloid blasts with or without maturation, forming a tumor in which the tissue architecture is effaced. It may occur de novo or concurrently with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) or a myeloproliferative disorder. De novo (isolated) myeloid sarcoma is defined as the absence of leukemia or MDS history and the lack of current bone marrow involvement. In children, *de novo* MS is limited to case reports. The most commonly involved sites include bone, periosteum, skin, orbit, lymph nodes, gastrointestinal tract and central nervous system. Pelvic location is an uncommon location of MS. The therapeutic algorithm for MS is unclear in children. Available treatment options include systemic chemotherapy, surgical resection, radiotherapy and hematopoietic stem cell transplantation. In isolated MS, it is recommended to start AML conventional chemotherapy as soon as possible, as it is known that untreated MS will almost always progress to AML. We presented an unusual case presentation of MS both for localization and symptoms.

PU34

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION REVERTS IMMUNE DEFECTS DUE TO NEUTROPHIL SPECIFIC GRANULE DEFICIENCY IN A CHILD WITH MUTATED SMARCD2 GENE

F. Serana¹, G. Martellosio¹, M. Pinelli², L. Dotta², A. Soresina², A. Plebani², D. Tregambe¹, M. Lombardi¹, M. Micheletti¹, A. Beghin¹, A. Lanfranchi¹, E. Soncini³, R. Badolato^{*2}, F. Porta^{*3} *RB and FP share senior authorship

¹Clinical Chemistry Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia; ²Pediatric Unit and "A. Nocivelli" Institute for Molecular Medicine, University of Brescia, ASST Spedali Civili di Brescia; ³Pediatric Hematology Oncology Unit, ASST Spedali Civili di Brescia, Italy

SMARCD2 is a protein of the SWI/SNF chromatin remodelling complex which plays a role in the differentiation of myeloid cells. SMARCD2 is also involved in the expression of neutrophil specific granule proteins through the interaction with the myeloid transcription factor CEBPE. Loss of SMARCD2 function has been recently established as a cause of immune defects and dysmorphic features. Here we present the case of a 1-month old newborn who was hospitalized for growth delay, mucocutaneous candidiasis, fever, and anemia. During hospital stay, she developed ground-glass pulmonary lesions and a purulent perianal abscess (positive for ESBL+ Klebsiella pneumoniae), which were treated with systemic antibiotic and antimycotic therapy. On suspicion of primary immunodeficiency, she was transferred to a Pediatric Immunology centre, where automated differential blood count showed a fictitious neutropenia due to neutrophils incorrectly classified as monocytes. Indeed, peripheral blood smear demonstrated a normal neutrophil count associated to total lack of granules and pseudo Pelger-Huet anomaly, suggesting the diagnosis of specific granule deficiency. Bone marrow aspirate revealed marked neutrophil dysplastic features associated to prominent left-shift in maturation. X-ray skeletal imaging showed upper limb shortness and mild osteopenia. After ruling out CEBPE mutations, DNA Sanger sequencing revealed a four-base deletion in the SMARCD2 gene suggesting the need for allogeneic hematopoietic stem cell transplantation (HSCT). HLA compatibility study in the family found an HLA-identical brother not carrying the specific mutation. HSCT was performed at the age of 6 months, after Busulfan, Thiothepa, and Fludarabine conditioning. Despite prophylaxis with ATG and Cyclosporine, she developed grade-1 cutaneous GVHD responsive to steroid therapy. Neutrophil and platelet engraftment (respectively at 18 and 29 days after transplantation) were demonstrated, with a 100% chimerism in PMN and 96.2% in PBL. Perineal lesions fully recovered, and the patient was discharged in good health 38 days after transplantation. Mutation of SMARCD2 is a very recently defined rare condition associated with myelodysplasia, severe infective complications, syndromic features. Our study suggests that allogeneic HSCT can provide a safe and efficient haematological cure of specific granule deficiency due to SMARCD2 mutations.

PU35**DOSE-ADJUSTED EPOCH-R: A RETROSPECTIVE STUDY IN THE TREATMENT OF AGGRESSIVE LYMPHOMAS**

M. Anichini, S. Kovalchuk, B. Puccini, I. Romano, C. Mazzoni, E. Galli, A. Bosi

SOD Ematologia, Azienda Ospedaliero Universitaria Careggi, Firenze, Italy

L'obiettivo di questo studio è valutare l'efficacia e la tollerabilità del regime immunochemioterapico dose-adjusted EPOCH-R (etoposide, prednisone, vincristina, ciclofosfamide, doxorubicina, rituximab) in pazienti con linfomi aggressivi, tra cui Grey Zone Lymphoma con caratteristiche intermedie tra linfoma diffuso a grandi cellule B e linfoma hodgkin classico, linfoma diffuso a grandi cellule B double-expressor e linfoma primitivo mediastinico in stadio avanzato. I dati clinici sono per ora esigui e il trattamento ideale è ancora incerto. Sono stati selezionati 21 pazienti trattati presso l'Ematologia di Firenze dal 2015 al 2018: 7 erano GZL, 8 erano PMBCL, 6 erano DE. L'età mediana dei pazienti alla diagnosi era di 39 anni, 11 erano maschi e 10 erano femmine. Il 67% dei pazienti presentava uno stadio avanzato di malattia (IIIA-IVB). Il 29% presentava sintomi B, il 57% aveva massa bulky. Il 47% dei pazienti aveva un IPI score maggiore o uguale a 2. Il 19% non ha ricevuto alcuna dose-escalation, il 14% ha raggiunto il primo livello di dose escalation, il 33% il secondo, il 23% il terzo, il 9% il quarto. Il 76% e il 23% avevano elevati livelli di LDH e beta2microglobulina. L'ORR era del 76% (61% RC, 14% RP), il resto è andato incontro a progressione (23%). Considerando gli 8 casi PMBCL in stadio avanzato, 6 sono andati in remissione completa senza radioterapia, 1 ha avuto remissione parziale di malattia, con la radioterapia ha ottenuto remissione completa, 1 è andato incontro a progressione di malattia e recidiva a livello encefalico e successivo exitus (12%). L'EFS era del 87%, l'OS dell'88%. Riguardo ai casi di GZL, 4 pazienti hanno raggiunto remissione completa e parziale di malattia, 3 hanno avuto progressione di malattia e recidiva (42%); con trattamento di salvataggio 2 hanno ottenuto risposta completa, 1 è deceduto. L'EFS era del 57%, l'OS dell'86%. Valutando i DE, 5 hanno ottenuto remissione completa, 1 è andato incontro a progressione di malattia e recidiva (16%). L'EFS era del 83%, l'OS del 100%. Tutti i pazienti hanno avuto manifestazioni correlate alla chemioterapia tra cui tossicità epatica grado IV (4%), neurotossicità da vincristina (17%), neutropenia e anemia di grado >3 (66% e 33%). Il regime DA-EPOCH-R scelto per pazienti con linfomi aggressivi dà un alto tasso di risposta, una remissione duratura e una tossicità accettabile per quanto concerne i casi PMBCL e DE. Si è osservato una minore efficacia nei GZL in termini di RC (28%).

PU36**ABO BONE MARROW TYPE INCOMPATIBILITY: EXPERIENCE WITH SEDIMENTATION AND AUTOMATIC SEPARATION METHOD FOR RBC**

F. Culurgioni, R. Floris, C. Musiu, M.G. Orofino, A. Piroddi, G. La Nasa

Hematology and Stem Cell Transplantation Unit, Ospedale Oncologico "A. Businco", Azienda Ospedaliera Brotzu, Cagliari, Italy

The ABO blood group incompatibility in donor and recipient is often correlated with various immunohematological complications. Many scientific evidence show as the ABO incompatibility impacts on overall survival, event-free survival, transplant-related mortality, graft-versus-host disease, and time to neutrophil and platelet engraftment. To identify at the best processes in relation to the type, quantity of material and processing start times to obtain the best recovery of nucleated and CD 34/kg cells in allogenic transplant from bone marrow stem cell source. The single-transplant center retrospective study, included 17 patients with hematological malignancies who underwent a first allogeneic hematopoietic cell transplantation between 2015 and 2019 all patients receiving bone marrow blood as stem cell source and were included in this evaluation. The ABO incompatibility was evaluated in respect to type of donor, pediatric or adult, MUD or familiar donor, quantity of BM from deerytrocytation, time of working up, type of sedimentation medium. Post NC/Kg cell recovery and post-separation CD34 cell recovery were also evaluated. For ABO manipulation, 3 were performed with Voluven medium, 2 performed with Vonten medium, 11 performed with Haesteril medium. Donor stem cell sources were 23,52% pediatric and 76,41% adults, anyway the 58,82% was donor

from MUD source and 41,1% from family allogenic. We have performed processing with minimin 267 ml and max 2167 ml of bone marrow addicted with separation medium. In 52,94% of donors stem cell the processing was performed before 12 hours from the explant, while in the remaining 47,05% after 12 hours from explant. The average of the nucleated cells / kg pre-processing was 5.85% with a cellular recovery of the red blood cells after separation of the 77.63%. For the CD34 recovery evaluation; post processing were 88,41%. These results show that ABO incompatibility after rbc depletions does not seem to influence these parameters in patients undergoing allogeneic stem cell transplantation. There are no significant differences with the different sedimentation in media used, Only the use of the cell separator, while maintaining a high recovery rate of CD34 cells (87.3%) leads to a loss of nucleated cells/kg (68%). In the sedimentation methods evaluation and availability for small to medium laboratories, show excellent recoveries of nucleated cells and good yields in the recovery of post-processing CD34 cells.

PU37**TFR IN A YOUNG PHILADELPHIA-POSITIVE CML PATIENT UNDERGOING TREATMENT WITH INTERFERON-A (IFN)**

S. Ceglie, A.R. Scortechini, S. Rupoli, G. Micucci, E. Morsia, K.B. Garvey, S. Mancini, A. Olivieri

Clinica di Ematologia, Università Politecnica delle Marche, Italy

In the 80s, INF was considered as a step forward in the treatment of CML and its use was the standard of care for patients unfit for bone marrow transplantation. Today, BCR-ABL tyrosine kinase inhibitors (TKIs), such as Imatinib, induce remarkable responses in CML patients and have become the mainstay of CML therapy. However, TKIs are not toxicity-free, which plays a significant role in treatment adherence and can ultimately affect treatment outcomes. We report the case of a 21-year-old man who was diagnosed in our Center in 1996 with CML in chronic phase, with initial signs of activation. He was admitted with neutrophilic leukocytosis (WBC 26.7x10⁹/L, ANC 82%) and Ph chromosome was positive in all metaphases analyzed by chromosome banding analysis on bone marrow sample. Molecular analysis was not available in our center at that time. According to guidelines at that time, the patient was treated with Interferon-A (INF) and low doses of Aracytin. No match was found in HLA-typing from his brother. Treatment was initially well tolerated, with a good partial cytogenetic response at 6 months (Ph+ 75%) and a complete cytogenetic response (CCyR) at 24 months despite the interruption of Aracytin for intolerance. Once having availability of molecular analysis for qualitative bcr-abl fusion transcripts, we tested our patient in 2000 and he resulted positive for bcr-abl p210. Complete molecular response (CR) was achieved in 2005 and we continued the treatment until 2010, when it was interrupted due to psychotic symptoms. Despite the discontinuation, the patient maintained a molecular and hematological CR and CCyR at serial evaluations every 4 months (last in February 2019). Complete hematologic responses were observed in 80% of CML patients treated with IFN and in 7-10% of them complete cytogenetic responses (CCyR) were obtained; CCyR achieved with IFN and maintained with or without Imatinib or any other therapy significantly correlates with long term survival in CML patients who mostly have MR4.0 (Malagola *et al.*, 2014). According to these well-established findings and to our case report (which is one of several), we think that the use of single agent Interferon-A or in combination should be reconsidered in TKI-intolerant patients and in unfit for bone marrow transplantation patients.

PU38

FEASIBILITY AND SAFETY OF DIRECT-ACTING ANTIVIRAL AGENTS TREATMENT IN ACTIVE HCV INFECTION DURING ALLOGENEIC STEM CELL TRANSPLANTATION

L. Pochintesta¹, E. Fronti², A. Arcari¹, L. Prezioso³, S. Bassi¹, E. Trabacchi¹, C.F. Moroni¹, P. Bernuzzi¹, I. Manfra³, V. Valenti¹, A. Lazzaro¹, C. Bosi¹, S. Guidotti⁴, D. Sacchini², A. Rossi⁴, D. Vallisa¹

¹Haematology Unit and Bone Marrow Transplant Centre, "Guglielmo da Saliceto" Hospital, Piacenza; ²Infectious Diseases Unit, "Guglielmo da Saliceto" Hospital, Piacenza; ³Hematology and BMT Unit, University of Parma; ⁴Immunohaematology Unit, "Guglielmo da Saliceto" Hospital, Piacenza, Italy

We report two cases of simultaneous administration of DAAs therapy and conditioning during allogeneic transplant in patients with exacerbation of chronic hepatitis C virus infection. Case 1: a 69-year-old RAEB-2 man (IPSS:2,rIPSS:5) with a chronic HCV infection (genotype 1b). A liver biopsy showed mild fibrosis (Ishak score: grade 7/18 and stage 2/6). He underwent 10 courses of Azacytidine for 7/28 days. On May 2015 a re-evaluation showed an increase of blasts to 30%, so an unrelated allogeneic HSCT was planned.

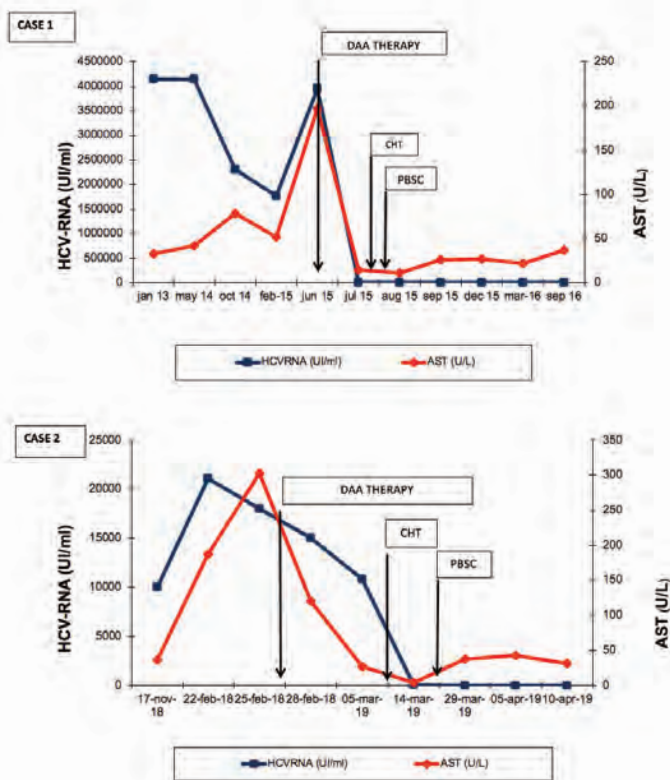


Figure 1. Two cases of integration of DAA therapy and conditioning during allogeneic transplant in the treatment course of HCV positive MDS (case 1) and MM patient (case 2).

During pre-HSCT assessment ALT raised to 332 U/L and HCV-RNA reached 3.949.000 UI/ml. From 22nd June, he started Sofosbuvir 400 mg/day, Da-clatasvir 60 mg/day and Ribavirin 1.000 mg/day for 12 weeks. Suddenly ALT level normalized and on day 14 HCV-RNA was undetectable. On 27th July he underwent to RIC-HSCT with Tiohepa (5 mg/kg/day -4), Fludarabine (50 mg/m²/day -3, -2,-1) and Busulfan (3.2 mg/kg/day -3, -2). The GVHD prophylaxis consisted of ATG 5mg/kg/day for 5 days, cyclosporine and methotrexate. On 4th August he received PBSC graft (CD34+ 5.62x10⁶/kg). The neutrophils engraftment was on day +12 and platelets on +13, no grade III-IV toxicities occurred. Bone marrow evaluation at +30 days showed CR, with full donor T cell chimerism. Today the patient is in CR, with normal liver tests and undetectable HCV-RNA. Case 2: a 50-years-old IgG -Multiple Myeloma ISS2, woman, diagnosed on 2009, affected by hepatitis C, genotype 1a. She achieved sCR after 4-VTD courses, 2-ABMT followed

by 2-VTD consolidation courses on 2013. From June 2017 she had two relapses with a refractory disease so a TCR/CD19 cells depleted haploidentical transplant was planned. Pre-HSCT laboratory tests revealed: serum ALT 391 U/L, HCV RNA 21.060.175 UI/L. An elastography showed a 3.65 kPa hepatic stiffness. On 26 Feb 2019 she started Sofosbuvir and velpatasvir 400/100 mg/die. Within 4 weeks with DAAs ALT levels normalized and HCV RNA became undetectable. In the same time, she started conditioning with Tiohepa (5 mg/kg/day -5, -4), Fludarabine (50 mg/m²/day from -6 to -2), Treosulfan (12 mg/m²/day -9, -8, -7), ATG (6 mg/kg/day for 5). On 19 March she received PBSC graft (CD34+ 10.2x10⁶/kg). Full engraftment was on +11 day without complications. On April she showed normal liver test, HCV-RNA remained undetectable. In our experience it was possible to perform an allogeneic HSCT using concurrently conditioning and DAAs therapy without delay of engraftment, toxic effects and VOD.

PU39

A CASE OF MYELOID SARCOMA (MS) INVOLVING KIDNEYS

F. Guidotti, A. Gardellini, M. Zancanella, F. Alberio, V. Saccà, M. Feltri, L. Ambrosiani, M. Turrini

Division of Hematology, Valduce Hospital, Como, Italy, Department of Pathology, Valduce Hospital, Como, Italy

Background: MSs are rare extramedullary haematological tumors which generally occur during the natural course of AML or CML. Rarely, their appearance precedes peripheral blood and bone marrow manifestation of disease. Herein we report a case of MS involving collecting system of both kidneys in a patient affected by myeloproliferative neoplasm (MPN).

Case Report: In October 2016, a 72-year old man with multiple comorbidities, following the findings of splenomegaly and thrombocytosis, was diagnosed with JAK2 V617F mutated MPN. He started therapy with hydroxyurea and periodic follow up. In February 2017, he was admitted to hospital because of fever and worsening of renal function. Laboratory test showed normal WBC and platelet count and moderate anaemia. An abdomen US revealed left ureteronephrosis with the presence of a suspected mass at the pyeloureteral joint; spleen diameter was 22 cm. CT scan confirmed a hyperdense lesion which wrapped the left caliceal cavities. A similar finding, though less evident, was described at the right kidney. Imaging at the end of antibiotic therapy was unmodified. In the following days, we observed a rapid increase of LDH and appearance of leukocytosis with blast forms in peripheral blood. Therefore, we performed bone marrow evaluation and a US-guided needle biopsy of the left renal mass. Both the histologies were consistent with localization of acute monocytic leukaemia. As the patient was not eligible for intensive chemotherapy, low dose cytarabine was started. However general conditions rapidly worsened with acute renal failure and subsequent death.

Discussion: Common sites of involvement by MS are skin, bone, soft tissue, lymph nodes, reproductive or digestive organs and central nervous system. To our knowledge, only five cases of renal MS have been described in literature: they radiologically appear as soft tissue masses on plain CT and as well-defined homogeneously enhanced masses on enhanced CT. In our patient, MS and its related obstructive nephropathy represented the first sign of the acute evolution of a known chronic haematological malignancy, preceding by some weeks the appearance of leukocytosis. In conclusion, in patients with a history of myeloid neoplasm, even in the absence of other evidence of active disease this hypothesis should be considered in the differential diagnosis of collecting system tumors.

PU40

RARE MDS/MPN OVERLAP: WHEN JAK2 V617F MEETS DEL(5Q) MUTATION

S. Rupoli¹, E. Morsia², G. Goteri^{2,3}, D. Capelli¹, A.R. Scortechini¹, A. Poloni^{1,2}, G. Micucci², K.B. Garvey², S. Ceglie², A. Fiorentini², M. Angelini⁴, S. Mancini⁵, G. Discepoli⁶, N. Viola⁷, A. Olivieri^{1,2}

¹Clinica di Ematologia Ospedali Riuniti di Ancona; ²Università Politecnica delle Marche; ³Anatomia Patologica Ospedali Riuniti di Ancona; ⁴Laboratorio di Biologia Molecolare Ospedale Mazzoni di Ascoli Piceno; ⁵Laboratorio di Biologia Molecolare Ospedali Riuniti di Ancona; ⁶Laboratorio di Citogenetica Ospedali Riuniti di Ancona; ⁷Laboratorio di Citofluorimetria Ospedali Riuniti di Ancona, Italy

A 55-year-old female was referred to another hospital for macrocytic anemia, mild leukocytosis and thrombocytosis, increased LDH and mild hepatomegaly. The bone marrow (BM) on smear showed dyserythropoiesis with 5-6% blast cells and dysplastic megakaryocytes. No ring sideroblasts were evidenced. BM trephine biopsy showed grade 2 fibrosis and increased cellularity with expansion of all three lineages, with dysplastic and proliferative changes and increased blasts. Conventional cytogenetic and FISH testing showed the presence of del(5q)(q13q33) in 15% of metaphases. JAK2 V617F mutation was detected by PCR. The patient was stable with lenalidomide 10 mg daily every 28 days for 24 months, when for the anemia lenalidomide was stopped and a treatment with ESA, oral prednisone and packed red cell transfusions was started. In few months the patient suffered for a deteriorating picture and was referred to us for increased splenomegaly, severe anemia, thrombocytopenia, eosinophilia and peripheral blastosis (22%) showing a basophilic differentiation at flow cytometry. BM trephine biopsy confirmed blastic transformation consistent with a diagnosis of Acute Basophilic Leukaemia. A complex karyotype was detected with two related clones; the first one: 45, XX, del(5)(q13 q33), del(7)(q22q32), t(7;17)(p12;q21)-17[2]/44; the second one: 44, XX that had also t(4;16)(q12;p11) and -16 [21]. Molecular analysis showed mutated JAK2 V617F (42.29% by RQ-PCR and 27% by NGS), mutated TP53, exon 8 (48% by NGS) and absence of mutation in all the other genes investigated (NPM1, FLT3/ITD, FL3/TKD, cKIT, FIP1L1/PDGFR, SF3B1, MPL, CALR, DNMT3A, GATA2, TET2, ASXL1, BRAF, CBL, IDH1, IDH2, KRAS, NRAS, PTPN 11, RUNX1, SRFS2). A small MDS subset is known to show concomitant del(5q) and JAK2 mutation, together with BM proliferative features and thrombocytosis. Up to now, the WHO recommends considering cases like these not among MDS/MPN-unclassifiable, but among MDS with isolated del(5q). However, the percentage of blasts in our case is too high, and for that, at the best of our knowledge, we consider it as MDS with excess of blasts and fibrosis. In the recent literature review on such cases by Bürki et al., only few showed eosinophilia, BM fibrosis and rapid blastic progression like ours. This small subset of cases should be further investigated to answer to some clinical questions regarding the efficacy of lenalidomide alone or in combination with Ruxolitinib.

PU41

EFFICACY AND SAFETY OF RITUXIMAB, BENDAMUSTINE AND DEXAMETHASONE AS INDUCTION THERAPY IN FRAIL ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA

L. Pezzullo, A. Bruno, G. Cassiodoro, R. Bianco, G. Villani, R. Rosamilio, M. Martorelli, R. Fontana, R. Guariglia, B. Serio, C. Selleri

Hematology, Department of Medicine and Surgery, Salerno University, Italy

The median age of patients with diffuse large B-cell lymphoma (DLBCL) is 70 years old with approximately 50% of the DLBCL patients older than 65 years and 15% older than 80 years. Given that the population continues to age, the management of older DLBCL patients with comorbidity and age-related organ dysfunction is currently widely debated. We compared in a cohort of very frail older DLBC, not eligible for anthracycline-based therapy, activity and safety of Bendamustine (70 mg/m²) in association with rituximab (375 mg/m²) and Dexamethasone (20 mg from day 1-4) (RD-Benda 70) administered every 3 weeks for 4 courses (RD-Benda 70x4) followed every 3 weeks by Rituximab consolidation for other 4 doses versus a palliative therapy consisting in courses of oral cyclophosphamide (200 mg/day for 7 days) administered every 4 weeks plus dexamethasone (20 mg/day for 4 days) every 2

weeks (C-D). From January 2016 to December 2018, have enrolled in the study 32 (15 F; 17 M) very frail DLBCL patients, according to the Comprehensive Geriatric Assessment, with a median age of 83 years (range 70-98): 12 pts (7F, 5M, median age 85 years, range 73-95) received palliative therapy, 20 pts (9F, 11M, median age 81 years, range 66-88) were treated with RD-Benda. In RD-Benda group, after a mean follow-up of 12 months (range 1-37), the overall response rate was 93% in 15 evaluable patients, with 86% of complete remission (CR n=13) and 14% of partial response (n=2), while only 1 patient experienced non-response. During the first 6 months from diagnosis 8 pts died: 2 for disease progression and 6 for causes unrelated to lymphoma: opportunistic pulmonary infections and cytomegalovirus (CMV) reactivation. Due to the high incidence of CMV reactivation (50%) in pts not receiving CMV prophylaxis, the last 5 pts received valgancyclovir prophylaxis (900 mg/day 3 times a week) and not one of them experienced CMV reactivation. Currently, in this RD-Benda group the estimated median disease-free survival is 33 months and the median OS is 12 months (40% OS at 36 months). In the C-D group, no patient achieved CR and 6/12 (50%) are alive at time of analysis with a median overall survival (OS) of 3 months (range 1-7 months). Our data provide evidence that RD-Benda regimen, although it requires mandatory CMV prophylaxis, is an effective and safe option as first-line therapy for the treatment of elderly frail DLBCL patients. Further larger trials are needed to confirm these preliminary data.

PU42

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): THE ROLE OF FLOW CYTOMETRY IN COUNTING AND IN EVALUATION OF CD34+ HEMATOPOIETIC STEM CELL (HSCS) VIABILITY

R. Nuccorini, S.P. Pascale, D. Stabile, A. Amendola, D. Vertone, M. Cimminiello, N. Filardi, A. Matturro, M. Pizzuti

U.O. Ematologia AOR San Carlo di Potenza, Italy

Background: Autologous Stem Cell Transplantation(ASCT) is gold standard for patients (pts) with Multiple Myeloma (MM). Flow Cytometry (FC) plays an important role in clinical decisions since it acts like a "bridge" between haematology and transfusion medicine. In fact FC is present in different moments during transplantation: 1. CD34+cells monitoring, 2. CD34+cells collection, 3.qualitative stem cells assessment by viability (before conditioning therapy and at the time of infusion),4.evaluation of immunological recovery.

Methods: We studied CD34+ cell enumeration and viability using a reference FC procedure employing STEM-KIT Beckman coulter and modified International Society of Hematotherapy and Graft Engineering (ISHAGE). For viability evaluation we used a cut-off of 20% according to JACIE procedures.

Patients: 27 adults with MM, with median age of 63 years (range:35-74) who received a first line therapy according VTD or Cy-BOR (11vs16). 24 achieved ≥PR, 3 were NR, underwent a salvage therapy (2KRD, 1RAD).

Results: All pts were mobilized collecting a median 7,95x10⁶ CD34+cells/Kg (range:4-21) in a median number of 1 aphereses (range1-2). Viability pre-conditioning therapy was evaluated in 25 pt with a median value of 47% (13-81). All pts underwent ASCT. 7 pts performed a double ASCT according to clinical decision (total trasplantation procedures: 34). Viability at the time of infusion was evaluated in all 34 procedures with a median value of 57% (27-85). Engraftment was achieved at day 11 for neutrophils (range: 9-14) and at day 15 for platelets (range 10-21). With a median follow up of 21 months (range: 6-43) all pts were alive and we observed 4 progressive disease. Stratifying pts according to therapeutic regimen (VTDvsCyBOR) and age (<60 vs >60 years) we not observed significant differences in terms of viability, CD34+cells count and engraftment.

Conclusions: From data analysis stratified by age, we not observed significant differences, and, as reported in literature, the younger pts collected more CD34+cells than the older ones (median: 9,25 vs 5,6). This suggests that even in older pts, HSCs maintain their characteristics and are able to mobilize and reconstitute the marrow in the same time frame as young pts. Further studies will be needed to improve the standardization of the method for assessing viability and to clarify which is the cut-off to use to define the quality of the apheresis, and to integrate the laboratory information into clinical practice.

PU43**PHOTOPHERESIS OFF-LINE WITH TECHNIQUE MACOPHARMA: EVALUATIONS OF TECHNICAL PARAMETERS IN ADULTS AND PEDIATRIC PATIENTS.**

F. Culurgioni, R. Floris, C. Musiu, M.G. Orofino, A. Piroddi, G. La Nasa

Hematology and Stem Cell Transplantation Unit adult and pediatric, Cancer Hospital "A. Businco", Azienda Ospedaliera Brotzu

Extracorporeal photopheresis (ECP) is a therapy for graft vs host disease (GVHD) after allo transplant therapy and in Cutaneous T-cell Lymphoma as lymphoproliferative disorder characterized by skin involvement due to accumulation of t-cells. The common types are mycosis fungoides (MF) and Sèzary Syndrome (SS). We analyzed a group of 6 unselected patients with: 1 mycosis fungoides (MF), 1 Sèzary Syndrome (SS), 1 Anemia Fanconi allotransplant with cGVHD, 1 NHL allo transplant with GVHD, 1 MDS-HR allotransplant with GVHD IV and one LAM allotransplant with GVHD IV, only one was pediatric patient. All patients, have been observed in our institution from 01/01/2018 to 15/04/2019; the evaluation is initiated by the use of the Macogenic 2 off-line equipment, from to start 3 were male and 3 female; median age at follow-up 42. In this work we tried to correlate known specific parameter as: treatment cycles, irradiation joules, initial hematocrit, final hematocrit, bag dilution, infusion volume, with other innovative ones: irradiation times and validation of product uniformity curves within the eva bag, all these findings from the Macogenic off-line equipment. From april 2018 to today, the apheretic collections were carried out with the Kobe spectra and Optia cellular separator. All therapists using photopheresis treatments have benefited patients, with a minimum of two treatments and a maximum of 13 treatments. The phototherapy scheme included two continuous sessions, a week of rest, after the first benefits, two consecutive treatments once a month. All apheretic samples were irradiated by 2,001 Joule in 10 minutes, all were taken by dilution with physiological solution from an average starting hematocrit of 3.91 HT to 1.87 HT at final volume of 300 ml. All products released for infusion to the patient were microbiologically negative and all critical parameters were controlled. The infusion time in all were performed in 30 minutes. Presence of air bubbles in 83% of the validation curves evaluations were detected, due to the presence of non-irradiated micro-zones and requiring a corrective action. Acceptability targets have been set, as no more than 5 unirradiated micro-areas were to be accepted. The SOPs has been corrected with greater care in handling the EVA bags after the insertion of the 3 ml of the drug 8-MOP metoxalene and complete air leakage. ECP is a widely recommended treatment modality as second-line treatment, particularly in steroid-refractory GVHD.

PU44**FAVORABLE EFFICACY OF DECITABINE IN ACUTE MYELOID LEUKEMIA DESPITE A STABLE BONE MARROW BLASTOSIS: A CASE REPORT**

G. Guaragna, E. Rinaldi, A. Spina, M. Girasoli, M.R. Coppi, M.C. Brocca, D. Pastore

UOC Ematologia - Ospedale "A. Perrino", Bari, Italy

Secondary acute myeloid leukemia (AML) refers to the development of AML after myelodysplastic syndromes (MDS) and/or with myelodysplastic related changes (defined as the presence of 50% or more dysplastic cells in at least two cell lines) or with MDS-related cytogenetic abnormality. The hypomethylating agent decitabine (DAC) is currently approved for the treatment of AML. We report a case report of a patient referred to our institution with secondary AML (s-AML) with myelodysplastic related changes. The bone marrow biopsy showed 40% of blasts and the cytogenetic analysis showed trisomy 8. At the time of diagnosis the patient had a severe anemia with red blood cell transfusion dependency of about 3 RBC units/month, a severe neutropenia and a moderate thrombocytopenia. He started treatment with decitabine iv at 20 mg/mq for 5 days in 28-day cycles and antibiotic prophylaxis with quinolone (7 days for each cycle). After 4th cycle the bone marrow blastosis was 20%, after 8th cycle the patient obtained a decreased transfusion dependency (about 1 RBC unit/month) and after 13th cycle he achieved transfusion independence although the bone marrow biopsy showed stable blastosis (about 20%). At the end of 16th cycle he died due to intestinal perforation. The DAC treatment has

been well tolerated and the patient never needed hospitalization. In this case report DAC demonstrated a favorable efficacy improving quality of life and survival (overall survival 18 months) and safety profile, despite a stable bone marrow blastosis.

PU45**MULTIPLE PAINFUL WAXING AND WANING LYMPHADENOPATHY DURING TREATMENT WITH BLINATUMOMAB**

E. Rotondo¹, M. Perrotta¹, A. Sau², S. Pascale², A. Spadano³, D. Onofrillo²

¹Clinica Pediatrica, Ospedale "Ss Annunziata" Chieti; ²Unità Operativa Semplice Oncoematologia Pediatrica, Ospedale Civile di Pescara; ³Unità Operativa Complessa di Ematologia Clinica, Ospedale Civile di Pescara, Italy

Blinatumomab, a bispecific monoclonal anti CD-19/CD3 antibody, was approved for use in patient > 1 years old with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL). We reported a case of multiple painful waxing and waning lymphadenopathy in a young male patient during treatment with blinatumomab. In May 2014 the 18 years old patient was admitted to the Hematology Unit with diagnosis of Acute B Lymphoblastic Leukemia. He was treated according to the GIMEMA 1308 protocol for standard risk until the conclusion of the therapy in May 2016. Twenty months after the end of the therapy, the patient experienced an isolated bone marrow relapse. Two cycles of fludarabine, high-dose cytarabine and mitoxantrone were performed. Due to the evidence of minimal residual disease (MRD) positivity, Blinatumomab therapy was started. During the second cycle of blinatumomab treatment, high fever with shivering and multiple painful lymphadenopathy occurred. The young patient presented waxing and waning lymphadenopathy involving the neck, axillae and inguinal region. Ultrasound showed reactive lymph nodes, the treatment with Blinatumomab was temporarily interrupted and antibiotic and anti-inflammatory therapy was started on suspicion of reactive lymphadenopathy. Due to the persistence of waxing and waning painful lymphadenopathy a biopsy was performed. A inguinal lymph node biopsy revealed extramedullary and CD19 positive relapse of leukemia. Bone marrow disease assessment showed 2% of CD19-positive blasts. Treatment with Inotumuzab was therefore started as bridge to bone marrow transplantation. Blinatumomab is effective and safe in treating relapsed/refractory ALL or NHL in meta-analysis. The common adverse effects included pyrexia, headache, neutropenia, infections. Clinicians should maintain a high level suspicion for the evolution of extramedullary leukemia. Our case shows a multiple waxing and waning lymphadenopathy during blinatumomab therapy with extramedullary CD19-positive relapse of disease. Extramedullary and CD19-negative disease are common during blinatumomab failure in refractory/relapsed ALL; a few cases of extramedullary and CD19 positive relapse of disease are described.

PU46**EFFICACY OF RITUXIMAB CONSOLIDATION AND MAINTENANCE IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WITH GRADE 2-3 DEAVILLE SCORE POST-INDUCTION THERAPY OR IN SECOND COMPLETE REMISSION**

L. Pezzullo, R. Rosamilio, G. Cassiodoro, R. Guariglia, C. Martorelli, R. Fontana, R. Bianco, A. Carobene, A. Bruno, L. Marino, G. Villani, B. Serio, C. Selleri

Division of Hematology, Department of Medicine and Surgery, University of Salerno, Italy

The addition of rituximab to induction chemotherapy has greatly improved the outcome of patients with diffuse large B-cell lymphoma (DLBCL). However, relapse prevention with anti-CD20 antibody maintenance therapy is not indicated for DLBCL or other aggressive lymphomas. In contrast to the established role of rituximab maintenance therapy for advanced follicular lymphoma with a high tumor burden, it remains controversial whether rituximab confers beneficial effects on aggressive lymphoma when used as maintenance therapy. Recent prospective trials based on response adapted therapy strategies using FDG-PET in patients with aggressive non Hodgkin lymphoma have yielded conflicting results. The aim of our study was to evaluate the role of maintenance with rituximab in DLBCL patients (excluding double or triple HIT) with a PET

Deauville score of 2-3 after induction therapy or in complete remission (CR) after a second-line therapy. From 2012 to 2018 we treated 120 DLBCL patients with R-CHOP or R-CHOP-like regimens. Of this cohort, 23 patients (19%), 11 male and 12 female with a median age of 64 years (range 38-78 years) were treated with weekly rituximab consolidation (4 doses of 375 mg/m²) followed by rituximab maintenance (375 mg/m²) every 2 months for 24 months or until relapse or until unacceptable toxicity. Fourteen (61%) out of 23 DLBCL patients had at the end of the induction a PET response with a Deauville score of 2-3, the remaining 9 DLBCL patients (39%) had obtained a CR after a second-line treatment (only one after autologous transplantation). After a median follow-up of 46 months from diagnosis, 16 patients (70%) are in CR with a negative PET, while 7 (30%) relapsed (4 still alive and 3 dead for disease progression). Noteworthy, only 20% of DLBCL patients (3/15) relapsed when treated with rituximab consolidation and maintenance after first-line induction, while 50% (4/8) of patients relapsed when treated after second-line therapy. The progression free survival and overall survival in DLBCL patients with low-grade (2-3) Deauville score after first-line induction or in second complete remission, treated with rituximab consolidation and maintenance was 37 and 46 months. Our retrospective data seem to document the efficacy of rituximab consolidation and maintenance therapy in diffuse large B-cell lymphoma (DLBCL), with PET of score 2-3 Deauville post first-line induction or in second complete remission. Large-scale prospective cohort studies are needed to validate the preliminary data of our observations.

PU47

RESPONSE TO IBRUTINIB OF AN AGGRESSIVE IG-A LYMPHOPLASMACYTIC LYMPHOMA CARRYING THE MYD88 L265P GENE MUTATION

F.M. Quaglia, G.M. Rigolin, E. Saccenti, M. Negri, M. Dabusti, M. Ciccone, A. Urso, M. Laudisi, A. Cuneo

Section of Hematology Department of Medicine Azienda Ospedaliera Universitaria Integrata University of Verona, Section of Hematology Department of Medical Sciences Azienda Ospedaliero-Universitaria S. Anna University of Ferrara, Department of Morphology Surgery and Experimental Medicine University of Ferrara, Italy

Lymphoplasmacytic lymphoma (LPL) is characterized by the proliferation of B lymphocytes with varying degrees of plasmacytic differentiation involving bone marrow (BM), lymph nodes or spleen. Waldenstrom macroglobulinemia (WM) is a subset of LPL in which the malignant clone produces an IgM paraprotein. LPL patients with IgA/IgG paraprotein account for less than 5% of LPLs. MYD88 mutation triggers survival through BTK activation in WM, a disease responding to ibrutinib, whereas non-IgM LPL has not been extensively investigated at the molecular level. In 2014 a 66 year-old woman presented with symptomatic anemia (Hb 9 g/dl), with IgAk monoclonal spike (1.6 g/dl) (Figure 1) and an otherwise unremarkable serum chemistry profile.

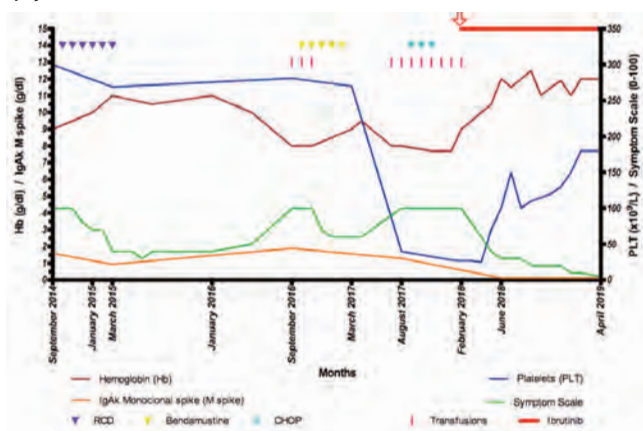


Figure 1. Diagram illustrating modifications in hemoglobin and IgAk monoclonal spike (left Y axis), platelets and symptoms (right Y axis) by treatment in our patient. Symptom Scale: 0–100-point scale, based on patient's reported symptoms. Higher scores indicate more severe symptoms. RCD: Rituximab-Cyclophosphamide-Dexamethasone; CHOP: Cyclophosphamide-Doxorubicin-Vincristine-Prednisone. Red arrow: initiation of ibrutinib.

A BM biopsy showed an 80% infiltrate by lymphocytes and lymphoplasmacytoid cells. A CT scan documented neither adenopathy nor splenomegaly. Diagnosis of IgA-secreting LPL was made. The patient was treated with RCD with minor response (Figure 1). Eighteen months later she presented with progressive disease (Hb 8 g/dl, IgAk monoclonal spike 1.9 g/dl). After 5 cycles of bendamustine, the BM aspirate showed 90% lymphoid cells. Adenopathies, splenomegaly and ascitis were noted on a CT scan. After CHOP (3 cycles) our patient developed thrombocytopenia ($30 \times 10^9/L$), transfusion-dependent anemia (Hb 7.7 g/dl) and clinical deterioration (Figure 1). We performed genetic studies with a targeted NGS approach detecting mutations in 20 genes frequently mutated in CLL (ATM, BIRC3, BRAF, CDKN2A, PTEN, CDH2, DDX3X, FBXW7, KIT, KLHL6, KRAS, MYD88, NOTCH1, NRAS, PIK3CA, POT1, SF3B1, TP53, XPO1, ZMYM3). The MYD88 L265P mutation was identified. Given the identification of MYD88 L265P in the peripheral blood, ibrutinib appeared a reasonable option. In February 2018 our patient started ibrutinib 420 mg/die (Figure 1). Hb and PLT improved from day +35 (Hb 10-12 g/dl, PLT $> 100 \times 10^9/L$). In July 2018 no ascitis and 50% reduction of adenopathies and spleen were shown on a CT scan. In April 2019 the patient is still on full dose ibrutinib with transfusion independence and good performance status. To the best of our knowledge this is the first case of response to ibrutinib in an aggressive IgA LPL with MYD88 mutation.

PU48

TREATMENT FREE REMISSION IN PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: A CASE REPORT

M.B. Greve, C. Alati, G. Praticò, B. Martino

Grande Ospedale Metropolitano di Reggio Calabria, Italy

A 72-year-old male patient with pain in the left hypochondrium was referred to the Department of Hematology. Upon admission, the results of the emergency blood routine tests were as follows: White blood cell (WBC) count $11.5 \times 10^9/L$, hemoglobin concentration 12 g/l and platelet count $25 \times 10^9/L$; the blast proportion was 90% and a t(9, 22), trisomy of chromosome 2 karyotype. Negative lumbar puncture. Submitted in the GIMEMA LAL 1205 protocol, good responder to the steroid prephase. Start dasatinib day +1 and treatment was discontinued to day +9 for intestinal perforation. The patient was treated with surgery. Patient re-start oral dasatinib monotherapy (70 mg qd) for 22 days as induction therapy and he obtained a complete remission and negative MRD, P190 0.38. The patient continued to receive oral dasatinib dose escalation until 140 mg/qd and he obtained complete remission in day +43, +57, +84, respectively P 190 0.59, 0.03, 0.005. After seven years in which the patient maintained complete haematological and molecular remission, he reduced dasatinib to 100 mg qd for dyspnea without pleural effusion. Three years after hospitalization in internal medicine for iatrogenic pleural effusion and deterioration of clinical conditions. The patient decides to stop treatment with Dasatinib. After 2 years from the voluntary drop-out, it maintains complete hematology and molecular response.

PU49

SYNCHRONOUS MULTIPLE PRIMARY CANCERS (SMPC) IN PATIENTS WITH NON HODGKIN LYMPHOMA (NHL)

F. Guidotti, M. Zancanella, A. Gardellini, F. Alberio, V. Saccà, M. Feltri, L. Ambrosiani, M. Turrini

Division of Hematology, Valduce Hospital, Como, Italy; Department of Pathology, Valduce Hospital, Como, Italy

Background: SMPC are defined as two or more primary tumors occurring within 6 months of each other. Until now, only few cases have been reported concerning hematological malignancies with SMPC. According to the literature, prognosis can be determined independently as a function of the stage of each cancer as SMPC doesn't appear to constitute a risk factor, despite surely complicating patient management. As we received 4 SMPC patients in a relatively short time, it reminded us to carefully consider the possibility of such condition. Case reports: Here we report 4 NHL patients with a synchronous malignancy: two of them had concomitant multiple myeloma (MM) while two had lung cancer; one of them interestingly showed both tumors in

the same lung lesion which is an extremely rare condition. P#1 was diagnosed with IA FL and a staging osteomedullary biopsy revealed plasma cells infiltration consistent with MM. As FL didn't require treatment, therapy for MM was started. In P#2, during diagnosis of IVA DLBCL, a rapid increase in M protein was observed leading to diagnosis of MM; precarious conditions with several comorbidities impaired adequate treatment, CTX at intermediate dose was administered in order to control both malignancies but the patient rapidly died. P#3 was referred to our center with a diagnosis of DLBCL with gastric localization; CT scan revealed a lung lesion infiltrating diaphragm whose histology was consistent with squamocellular carcinoma. Because of severe symptoms due to gastric involvement, chemotherapy (R-CHOP) was soon initiated and radiotherapy on the lung nodule performed between 1° and 2° cycle. P#4 was diagnosed with stage IVB MCL; CT scan revealed a lung nodule and needle biopsy was consistent with adenocarcinoma; he underwent therapeutic lung resection and histology showed coexistence of adenocarcinoma and mantle cell lymphoma. He then started chemotherapy for MCL. **Conclusions:** Although an increased long-term risk for developing secondary neoplasms has been well demonstrated among NHL survivors, a histologic diagnosis of 2 synchronous malignancies is rare, even more if their coexistence is demonstrated at the same site. Management of SMPC may be difficult, requiring multidisciplinary collaboration with sequential or sometimes joint treatment. There are no general guidelines and the approach should be individualized taking into consideration the different biological behaviors between cancers and patient's characteristics.

Table 1.

	Gender	Age (yr)	One primary cancer		Another primary cancer		Outcome
			Diagnosis	Treatment	Diagnosis	Treatment	
#1	M	65	Follicular NHL	-	Multiple Myeloma	VTD + ASCT	FL: CR MM: PD
#2	F	84	DLBCL	Palliative (I-CTX)	Multiple Myeloma	Palliative (I-CTX)	dead
#3	M	61	DLBCL	R-CHOP	Lung Cancer	Radiotherapy	DLBCL: CR Lung: SD
#4	M	72	Mantle cell lymphoma	R-Bendamustine	Lung cancer	Surgery	ongoing

PU50

AZACITIDINE IN A PATIENT WITH MYELODYSPLASTIC SYNDROME AND MAMMARY CANCER: A CASE REPORT

G. Guaragna, E. Rinaldi, M. Girasoli, A. Spina, D. Pastore

UOC Ematologia - Ospedale "A. Perrino", Bari, Italy

Azacitidine (AZA) is a hypomethylating agent approved for the treatment of myelodysplastic syndromes with Intermediate-2 or High IPSS risk. We report the case of a 75 years old man with a Myelodysplastic Syndrome (normal karyotype) and a previous diagnosis of mammary cancer treated with surgery and hormone therapy. At presentation, the patient had some little lungs' nodules and an increase of CA15.3. At diagnosis he was transfusion dependent (Hb < 8,5 g/dl), with neutropenia (1400/ μ l) and thrombocytopenia (75000/ μ l). We started rHuEPO but the patient lost response after an initial increase of Hb value. So we associate AZA 75 mg/mq for 7 consecutive days every 28 days. When the Hb and granulocytes count increased to normal value, he started treatment with Exemestane for the increasing Ca15.3 value. After 4 years and 21 cycles with AZA the patient has a Hb value > 10 g/dl, a normal count of granulocytes and a mild thrombocytopenia. The Ca 15.3 value is stable, the lungs' nodules are the same in number and dimensions and there is no evidence of progression of mammary carcinoma. In this case report AZA demonstrates a favorable efficacy and safety profile in a patient with coexistent mammary cancer in treatment.

PU51

EFFICACY OF PROTON BEAM THERAPY IN DIFFUSE LARGE B CELL LYMPHOMA WITH MEDIASTINAL BULKY INVOLVEMENT

F. Saltarelli¹, F. Dionisi², R. Righetto², L. DePadua¹, G. Tomei¹, A. Andriani¹

¹UOC Ematologia Ospedale "F. Spaziani" Frosinone; ²Unità Protonterapia APSS Trento, Italy

Radiotherapy (RT) is currently part of consolidative treatment for Non Hodgkin Lymphoma with bulky disease. Patients undergoing RT experience late toxicities due to radiation dose to organs at risk such as heart, coronary vessels, esophagus, thyroid and lungs. The risk of radiation-induced secondary cancer and respiratory failure is also not negligible for long-term survivors. Due to its unique physical properties of a finite range in tissue and zero dose beyond the end of their path, Proton therapy (PT) can reduce toxicity to uninvolved tissues tailoring the dose to the target compared to conventional photon RT. Limited data exist demonstrating the clinical benefit of proton radiotherapy for Non Hodgkin Lymphoma patients. We evaluated consolidative PT following standard chemo-immunotherapy in a patient with Diffuse Large B-Cell Non Hodgkin Lymphoma (DLBCL) with mediastinal bulky disease. A 39 year-old man was diagnosed with DLBCL NOS stage III with mediastinal bulky in September 2017. He received RCHOP for six courses plus two additional Rituximab somministrations. Pre RT evaluation at the end of first line chemo-immunotherapy showed complete response (CR) with PET/CT scan score 2 according to Deauville 5 point lymphoma scale. In order to complete treatment trying to minimize radiation to organs at risk and related late toxicities, he was referred to Proton Therapy Centre in Trento (Italy). Active scanning proton therapy using single Field Optimization technique was used. Patient's setup consisted of deep inspiration breath hold (80% maximum inhalation volume) with the use of Active Breath Coordinator device (Elekta™, Sweden). Treatment volumes and doses were based on International Lymphoma Radiation Oncology Group (ILROG) guidelines. The patient received 36 Gy Rbe (1,8 Gy per fraction) to the pre-chemotherapy tumor volume and 40 Gy Rbe (2 Gy per fraction) on the residual tumor volume using simultaneous integrated boost (SIB) technique. Treatment with PT was completed in May 2018; it was well tolerated with no \geq G2 acute toxicities. No late severe toxicities were observed at 10 months follow-up. A PET/CT scan after 3 months showed treatment CR. PT represents the most advanced radiation therapy currently available. The potential of PT to reduce acute and late sequelae in comparison with conventional RT should be considered into multidisciplinary Lymphoma boards and included into a risk adapted treatment strategy for lymphoma patients eligible to receive consolidative RT.

PU52

TREATMENT WITH BELINOSTAT IN RELAPSED MYCOSIS FUNGOIDES PATIENT

G. De Santis, R.M. Miccolis, C. Buquicchio, V. Carluccio, C. Germano, M. Leo, G. Lerario, S. Mallano, M.T. Santeramo, M.S. Santoro, C. Plati, G. Tarantini

UOC di Ematologia con Trapianto, Barletta, Italy

Belinostat is a pan-histone deacetylase inhibitor with antitumour and anti-angiogenic properties. Mycosis fungoides (MF) and its leukemic variant, Sézary syndrome (SS), are malignancies of skin-homing T cells that comprise the majority of cutaneous T cell lymphomas (CTCL). Female patient of 45 years diagnosed in October 2008 with Mycosis Fungoides stage T1N0M0B0 on biopsy cutaneous lesion of the thoracic region. Treatment: Phototherapy and local RT obtaining a Complete Remission. In February 2011, disease recurred with a patchy skin lesion at the thoracic region for which 6 cycles of mono chemotherapy with Gemcitabine were performed obtaining a Partial Response (September 2011). In October 2011 a plaque lesion appeared on the right foot (tibiotarsal joint and hallux). Treatment with Gemcitabine-Bexarotene was started. Because of further progression of lesions (ulcerations) polychemotherapy CHOP regimen was performed obtaining a Partial Response (February 2012). She subsequently received photoapheresis and TSEB therapy. In July 2013 the appearance of cutaneous lesions. Treatment: Liposomal pegylated doxorubicin 4 infusions, Radiotherapy and Bexarotene-PUVA therapy.

In July 2016, the patient comes to our observation. The physical examination shows papula lesions in the buttocks, left thigh, perineum and left groin. Skin biopsy: Mycosis Fungoide granulomatous; osteomedullary biopsy: free of infiltration; absence of peripheral blood involvement; no adenomegalies and organomegalies: stage T2bN0M0B0. Therapy: CHOEP 6 cycles obtaining a Complete Response (November 2016). January 2017: appearance of new lesions Progression Disease. Treatment with Brentuximab-Vedotin (Adcetris): complete resolution after 6th cycle. The patient performed a total of 15 cycles when patchy skin lesions appeared in the same sites. (December 2017). Treatment: 4 cycles of Bendamustine obtaining Partial Remission. May 2018 PBSC autotransplantation. June 2018 Complete Remission. July 2018 appearance of high plaque and hardened lesion, pruritic right scapular region: stage T1N0M0B0. Treatment with Belinostat was started (Beleodaq 1000 mg/m²): 1630 mg (BSA 1,63m²) administered via IV once daily on days 1-5 of a 21 day cycle. After the 3rd cycle lesion was hypopigmented spot, after the 6rd cycle the lesion clearer, plain and no more pruritic. The patient hasn't experienced any toxicity. The patient will continue the treatment considering the good response on the lesion and good tolerability.

PU53

SYNERGIC ACTION OF IBRUTINIB, ORAL CYCLOPHOSPHAMIDE AND DESAMETASONE IN THE TREATMENT OF A MULTI-RELAPSED MANTLE-CELLS LYMPHOMA: A CLINICAL CASE

L. Pezzullo, R. Rosamilio, G. Cassiodoro, A. Bruno, R. Bianco, G. Villani, M. Martorelli, R. Fontana, R. Guariglia, B. Serio, C. Selleri
Hematology, Department of Medicine and Surgery, Salerno University, Italy

Mantle cell lymphoma (MCL) is an incurable type of B-cell lymphoma generally characterized by relapse, despite a good response to conventional chemotherapy. Ibrutinib is indicated for the treatment of adult patients with relapsed or refractory MCL, with an overall response rate of approximately 70% (CR: 20%). Numerous papers have shown a better response if ibrutinib is associated with other chemotherapy cycles. We associated ibrutinib, in a case of multi-relapse mantle cell lymphoma, which initially did not respond to this drug, with oral cyclophosphamide and dexamethasone, with a surprising result.

Case Report: a 62 year-old female patient, in 2001, at the age of 44, was diagnosed with MCL and was treated with chemotherapy according to the CEOP scheme for 6 cycles obtaining a complete remission (CR). The response lasted less than a year, so she went to second line therapy with ESHAP scheme for 5 courses, rituximab for 4 courses followed by autologous peripheral stem cell transplantation conditioned with BEAM chemotherapy and further 6 courses of rituximab, obtaining again a CR. In 2015 the patient presented with a 16x9 cm solid mass infiltrating the left biceps femoris muscle, site of MCL relapse at biopsy. Revaluated the patient as IV A stadium, third line of chemotherapy was performed in according to scheme R-BAC 500 for 5 cycles with achievement a CR again. After 2 years from the end of treatment, a further relapse of MCL at left leg was documented and the patient was treated with R-DHAOX for 6 cycles, obtaining CR again. After 7 months a CT/PET and a FNAB showed a recurrence in left biceps femoris muscle. The patient started treatment with ibrutinib alone at a dose of 560 mg/die (fifth line of therapy) but at revaluation after 1 month therapy the muscle mass getting worse at ecography. For the containment of disease, the patient start oral cyclophosphamide 200 mg/day for 5 days and dexamethasone 8 mg/day for 4 days and were associated with ibrutinib at a dosage of 420 mg/day. No toxicity is been documented and interestingly after one month of combination therapy, the muscle femoris mass is no longer documentable on the NMR. Now the patient is at + 4 month, by start combination therapy, in CR. In this clinical case, the synergistic effect of Ibrutinib with cyclophosphamide and dexamethasone is evident and surprising. Moreover, this therapy did not give any toxicity and was extremely well tolerated. It would be interesting to start such combination therapy in patients with multi-treated and fragile mantle-cells or aggressive non-Hodgkin lymphoma.

Main Program

ANTIBODY-BASED IMMUNOTHERAPY

P.L. Zinzani

Institute of Hematology "Seràgnoli" University of Bologna, Bologna Italy

In recent years, many new antibodies, either naked or armed with toxin or radionuclides have been investigated to enhance the field of active agents directed against already identified ("old") targets. This was particularly the case for the antigens CD19 and CD20 but also some other ones. The success of these developments has been variable and underlines the need to better characterize the mode of action of these molecules. Combinations studies of these new agents with other molecules, in particular those with an immunomodulatory potential, are also currently developed with some promising results.

The introduction of rituximab more than 20 years ago has profoundly renewed the field of lymphoma treatment.¹ With the proof of concept that targeting a cell surface molecule expressed on lymphoma cell surface will improve patient's outcome, other monoclonal antibodies against other antigens were developed, with—so far—limited success when used as single agents.² More interestingly, new anti CD20 antibodies were also developed, and several ways to enhance the therapeutic activity of this class of drugs were explored that may eventually apply to other monoclonal antibodies. Another path actively growing is represented by antibody drug conjugates (ADCs) or immunoconjugates, directed against several old or new targets.³ Finally, the ligation of PD-1 with PD-L1 activates a critical immune checkpoint leading to T cell dysfunction, exhaustion, and tolerance. Anti-PD-1 or anti-PD-L1 monoclonal antibodies can reverse the immune checkpoint, releasing the brake on T cell responses.

NEW ANTI CD20 ANTIBODIES

The demonstrated activity of rituximab and its use the treatment of most B cell malignancies has prompted the development of rituximab biosimilars (recently approved in many countries) but also of other anti CD20 antibodies targeting different CD20 epitopes or engineered to increase complement dependent cytotoxicity (CDC), antibody dependent cellular cytotoxicity (ADCC), or direct cell killing. While many anti CD20 clones were developed at the preclinical level and some investigated in early phase studies, only a few are still currently in the clinic.

Obinutuzumab, a type 2 glyco engineered anti CD20, has shown some clinical activity in most lymphoma subtypes, but only studies performed in patients with FL demonstrated sufficient benefit to provide health authorities approval. In a randomized study comparing bendamustine single agent versus bendamustine combined with obinutuzumab and followed by obinutuzumab maintenance in patients who were assessed as refractory to rituximab, the experimental arm resulted in significant progression free survival (PFS) and overall survival (OS) benefits.⁴ The GALLIUM phase 3 study⁵ demonstrated a significant improvement of PFS in combination with chemotherapy in untreated patients with FL, while no benefit was observed in the same study for patients with marginal zone lymphoma. Finally, ublituximab is another glyco engineered anti CD20 antibody developed in CLL, but only early results were presented for patients with lymphoma, either as single agent or in combinations with different drugs, and no randomized studies are currently evaluating ublituximab against other anti CD20 antibodies in lymphoma.

CD19: THE RENEWAL OF AN OLD TARGET

Studies in the late 80s were exploring naked or blocked ricin conjugated anti CD19 antibodies, an antigen that is nowadays on the main stage with CD19 directed chimeric antigen receptor (CAR) T cells. However, an anti CD19 antibody with an Fc glyco engineered portion (MOR208), resulting in enhanced ADCC and antibody dependent cellular phagocytosis (ADCP), is also under development. This antibody appears to also exert in vitro some direct activity on the B cell receptor signaling that induces direct cytotoxicity. A single agent phase 2 study was performed in 92 patients with R/R DLBCL, FL and mantle cell lymphoma, with response rates ranging from 26% (DLBCL) to 29% (FL), and a few patients experiencing a prolonged response.⁶ The main toxicities appear to be infusion related reactions and neutropenia (each 12% all grades and 9% grades 3 and 4 for neutropenia). Two registration programs are under development for this drug: a randomized study comparing MOR208 associated with bendamustine versus rituximab bendamustine in R/R DLBCL (NCT02763319) and a combination of MOR208 with lenalidomide (NCT02399085) in a similar population. Preliminary results of the latest study indicated an overall response rate (ORR) of 58% with 33% of complete responses (CRs) in 72 patients (median age 72 years and median number of prior lines of therapy of two), and final results of the study are expected in the next months.

cities appear to be infusion related reactions and neutropenia (each 12% all grades and 9% grades 3 and 4 for neutropenia). Two registration programs are under development for this drug: a randomized study comparing MOR208 associated with bendamustine versus rituximab bendamustine in R/R DLBCL (NCT02763319) and a combination of MOR208 with lenalidomide (NCT02399085) in a similar population. Preliminary results of the latest study indicated an overall response rate (ORR) of 58% with 33% of complete responses (CRs) in 72 patients (median age 72 years and median number of prior lines of therapy of two), and final results of the study are expected in the next months.

CD37 TARGETED WITH A NEW RADIOLABELED ANTIBODY

CD37 is a tetraspanin expressed in most B cell and T cell malignancies, and several compounds directed against this antigen were investigated in the last decade, such as otlertuzumab (NCT00614042) or BI 836826 (NCT01403948), as well as some ADCs targeting this antigen. Recently, a radio immunoconjugate was developed consisting of the murine monoclonal antibody HH1 (against human CD37) coupled with the emitting isotope Lutetium 177 ($t_{1/2} = 6.7$ days) chelated to DOTA (a chemical linker). Based on results obtained in phase 1/2 studies,⁷ a registration clinical trial is currently being performed in patients with FL having received two lines of therapy and refractory to rituximab (NCT01796171).

ARMED ANTIBODIES: ESTABLISHED TARGETS AND EMERGING ONES

Several ADCs have been now approved in Hodgkin and CD30+ T cell lymphoma (brentuximab vedotin), in leukemia (gemtuzumab and inotuzumab ozogamycin) and in solid tumors (trastuzumab emtansine and Ado trastuzumab emtansine in breast cancer). But many new agents are being developed, with technological modifications aiming to improve three key parameters involved in ADC activity: the antibody itself, to enhance target selectivity and effective internalization after its binding; the linker stability, to avoid premature release of the poison; and the warhead to increase the cytotoxic potency.

Recently, moxetumomab pseudotox, an immunotoxin composed of a single chain variable fragment (scFv) directed against CD22 and fused to a truncated *Pseudomonas* exotoxin (PE38) was approved for patients with R/R hairy cell leukemia. In a phase 2 study with 80 patients, the ORR and CR rates were respectively of 75% and 41%, with durable responses in patients achieving minimal residual disease negativity.⁸

Polatuzumab vedotin is an IgG1 directed against CD79b coupled with the antimetabolic agent monomethyl auristatin (MMAE) currently actively developed in B cell lymphoma. Response rates as single agent in patients with R/R disease, at the optimal drug dosing (2.4 mg/kg), were observed in 14/25 patients with DLBCL and 7/15 patients with FL, respectively.⁹ Toxicities were essentially consisting of grades 3 and 4 neutropenia and neuropathy. A randomized phase 2 in relapse/refractory patients assessing its association with rituximab bendamustine (B R) showed discordant results according to lymphoma histology: while polatuzumab did not appear to add activity to the B R regimen for patients with FL, significantly improved response rates (40% versus 15%), PFS (median 6.2 months versus 2 months) and OS (11.8 months versus 4.7 months) were observed in the DLBCL group¹⁰ for patients receiving the polatuzumab B R combination. The Polarix study (NCT03274492) currently investigates the use of polatuzumab vedotin in combination with R CHP (R CHOP omitting vincristine) against R CHOP in the first line setting of patients with DLBCL.

Pyrobenzodiazepines (PBDs) represent a new class of antibiotic antimetabolic agents binding as dimers the DNA minor groove, active at picomolar concentrations and insensitive to MDR1+ efflux mechanisms, that appear particularly promising in preclinical studies.³ Loncastuximab tesirine (ADCT 402) targets CD19, and this ADC appears more effective in animal models than CD19 directed ADC using tubulins binders (maytansinoid and auristatin). And a bystander activity was also observed in preclinical models. A large phase 1/2 study (NCT02669017) was performed, and preliminary results in R/R DLBCL patients showed 53/132 (40%) responders, including 29 patients (22%) achieving a CR. A pivotal study in DLBCL is being currently conducted (NCT03589469).

CHECKPOINT INHIBITORS

Classical Hodgkin lymphoma (cHL) is very sensitive to PD1/PL1 blockade due to genetic alterations in 9p24.1 leading to the high expression of PDL1. Although majority of NHLs have a much lower sensitivity to PD1/PDL1 blockade, a few subtypes such as primary CNS lymphoma, primary testicular lymphoma, primary mediastinal lymphoma harbor 9p24.1 alterations making them vulnerable to PD1 blockade. EBV-associated lymphomas have a virally mediated increased expression of PDL1 making them sensitive to PD1 blockade.

Program cell death protein 1 (PD-1) is an important immune checkpoint receptor expressed on activated T cells.¹¹ PD-1 binds with its ligand (PD-L1 or PD-L2) on tumor cells, and the tumor microenvironment promoting tolerance for tumor evasion which leads to tumoral growth.¹² The dependence of PD-1 signaling suggests an area of vulnerability to checkpoint blockade which could restore the antitumoral immunity. In relapsed or refractory (R/R) classical Hodgkin lymphoma (cHL), the FDA has approved nivolumab and pembrolizumab due to high response rates in this disease. Pembrolizumab has also been approved in relapsed or refractory primary mediastinal B cell lymphoma. In lymphoma, multiple PD1 or PDL1 inhibitors are being studied in clinical trials.

Classical Hodgkin Lymphoma

In 23 heavily pretreated relapsed/refractory (R/R) cHL patients, a phase I trial of nivolumab (PD-1 inhibitor) resulted in an overall response rate (ORR) of 87% and complete response (CR) rate of 17%.¹³ Durable responses were seen with 35% of patients having a response at 1.5 years (13). Similar results were seen with the phase I trial with pembrolizumab (PD-L1 inhibitor) which showed a ORR of 65% and CR rate of 16% in 31 heavily treated R/R cHL patients (14); median PFS was 11.4 months.¹⁴

In the phase 2, CHECKMATE 205 study for nivolumab in 243 R/R cHL relapsed after autologous stem cell transplant (ASCT), ORR remained high at 69% and CR was 16%.¹⁵ Median PFS was 12–18 months (15). Similar results were seen in the phase 2 trial of pembrolizumab (KEYNOTE 087) of 210 patients with R/R cHL with ORR of 69% and CR rates of 22%, even in patients with primary refractory disease.^{16,17} Immune-related adverse events were similar to those observed in solid malignancies with most common reactions being diarrhea, rash, pruritis, infusion reaction, fatigue, thyroid disease with only 4–6% of patients discontinuing for toxicity.^{13–15} Due to high response rates in heavily pretreated patients and low toxicity profile, both nivolumab and pembrolizumab were granted accelerated FDA approval for the treatment of R/R cHL.

High response rates are correlated with increased PD-L1 expression. In the CHECKMATE 205 study, all 45 patients with tumor samples had 9p24.1 alterations with either copy gain or amplification. PD-L1/PD-L2 amplification has been associated with higher response to nivolumab and pembrolizumab.¹⁵

Given the high efficacy and low toxicity profile of checkpoint inhibitors, numerous trials using PD-1/PD-L1 inhibitors in combination with other therapies are underway to increase the cure rates and minimize toxicity for frontline therapy and achieve more durable responses for patients with R/R cHL.

In the frontline setting, there are several studies incorporating nivolumab in combination with AVD (doxorubicin, vinblastine, dacarbazine) (NCT03004833), AVD±B (doxorubicin, vinblastine, dacarbazine ± bleomycin depending on age) (NCT03033914), or A-AVD (brentuximab, doxorubicin, vinblastine, dacarbazine) (NCT03233347) in early or advanced stage cHL. Pembrolizumab in combination with AVD is also being investigated in newly diagnosed cHL (NCT03226249). In the elderly population with coexisting comorbidities unable to tolerate the standard of care multiagent chemotherapy, nivolumab is being used in combination with vinblastine (NCT03580408) or brentuximab (NCT02758717) to determine if a less toxic regimen can yield promising results. Pembrolizumab monotherapy is also being investigated in the frontline setting for those unable to tolerate standard ABVD (NCT03331731).

Since checkpoint blockade monotherapy has been so successful in the R/R setting, there is increased interest in combining checkpoint inhibitors with conventional chemotherapy, monoclonal antibodies or other immunomodulatory agents. Nivolumab and brentuximab vedotin (monoclonal antibody drug conjugate to CD30) resulted in an encoura-

ging ORR of 82% and an impressive CR of 61% in a Phase 1/2 trial in 62 R/R cHL in first salvage.¹⁸ Of the 62 patients, 66% (41 patients) directly proceeded to ASCT after nivolumab/brentuximab treatment while another 13 patients proceeded to additional salvage prior to ASCT.

Finally, the tolerability and safety of checkpoint blockade has also been evaluated in cHL who underwent relapse after an allogeneic stem cell transplant (allo-SCT). A retrospective review of 20 patients showed that nivolumab induced an ORR of 95% and CR of 42%.¹⁹ One year progression-free survival (PFS) was 58% and OS of 79%. However, 6 patients (30%) developed GVHD of which 2 died as a result of GVHD. Thus, while response rates are high, nivolumab should be used in caution in the post-alloSCT setting.

Diffuse Large B-Cell Lymphoma

Several subtypes of diffuse large B cell lymphoma (DLBCL) have been shown to have aberrations of 9p24.1 including primary mediastinal B cell lymphoma (PMBCL), gray zone lymphoma (GZL) which shares overlapping clinical features of DLBCL and cHL, primary CNS lymphoma (PCNSL) and primary testicular lymphoma (PTL), and result in high PD-L1 and PD-L2 expression.

KEYNOTE-013 was a phase 1b trial evaluating pembrolizumab in the treatment of heavily treated patients with R/R PMBCL (20). Seventeen patients were evaluable for efficacy. The ORR was 41% (with a CR rate of 81% had decreases in target lesions. Neither median duration of response or median OS was reached at follow-up of 11 months.²⁰ Two patients (12%) remained on study for the maximum 2 years and remained in remission suggesting that in a select population, PD-1 inhibition offers a durable response. Similar results were noted on the phase II study of pembrolizumab in R/R PMBCL, leading to FDA approval of pembrolizumab in patients with PMBCL who have relapsed after 2 or more prior therapies.

Recently, Zinzani *et al.*²¹ reported a phase I/II (CHECKMATE436) evaluating the combination of nivolumab and brentuximab vedotin in the treatment of heavily treated with R/R PMBCL. Thirty patients were evaluable for efficacy. The ORR was 70% with a 43% complete metabolic response rate. Median duration of response, median PFS, and median OS have not been reached.

There are promising outcomes with PD-1 inhibition in GZL. In one study, 61% of GZL tumors were found to harbor 9p24.1 alterations.²² In a small case series of patients with R/R GZL, all of whom had 9p24.1 alterations, treatment with either nivolumab or pembrolizumab resulted in CRs in all patients.²³ A Phase 2 clinical trial conducted by the NCI is currently underway to evaluate the ORR of pembrolizumab for GZL and other rare, aggressive NHL (NCT03255018).

PCNSL and PTL are distinct subtypes of large cell B cell lymphoma that arise in sanctuary, extranodal sites. One study demonstrated that PCNSL and PTL have a defining genetic signature distinct from other large B cell lymphoma and frequently contain 9p24.1/PD-L1/PD-L2 copy number alterations and translocations, allowing for tumoral escape of immune surveillance.²⁴ In a case series of patients who had r/r PCNSL or PTL with CNS relapse, nivolumab was used after failure of multiple standard options.²⁵ All patients had clinical and radiographic responses with PD-1 blockade and durable remissions were seen. Current phase 2 trials evaluating the role of either nivolumab or pembrolizumab in R/R PCNSL and R/R PTL are underway (NCT02857426, NCT03558750, NCT02779101, NCT03255018).

In a phase I trial of nivolumab in R/R DLBCL, the ORR was 36% (4/11) with CR of 18% (2/11), but remissions were less than 3 months in half of the responders.²⁶ For the 2 patients with CR, the duration of response was widely varied ranging from 6 to 77 weeks. Only two patients had low-level polysomy of PD-L1/PD-L2 and had responses of PR and SD (stable disease). The rest of the cases were negative for PD-L1 and PD-L2 via immunohistochemistry (IHC). The phase 2 trial of nivolumab in patients with DLBCL (CHECKMATE 139, NCT02038933) has completed accrual and results are expected later this year. Hopefully, the phase II trial will also shed light on 9p24.1 alterations and expression of PD-1 ligands in DLBCL and whether these biomarkers can predict response to anti-PD-1 therapy.

Follicular Lymphoma

The expression of PD1 in follicular lymphoma (FL) is an area of active study. In FL, PD-1 is expressed on tumor-infiltrating lymphocytes (TIL)

and the tumor microenvironment (TME) but not in the tumor cells.²⁷ Diffuse staining of PD-1+ FL cells were associated with shorter time to transformation and the presence of PD-1+ T cells were an independent predictor of transformation in FL.²⁸ Given this, interest in checkpoint blockade was generated in patients with R/R follicular lymphoma. While nivolumab single agent produced a modest ORR of 40% and CR of 10% in ten FL patients, the duration of response was long and ongoing in all responding patients: ongoing responses at 27–32 weeks in PR patients and an ongoing response of almost 82 weeks in the patient who achieved CR.²⁶

In a phase II trial of 30 relapsed FL patients, rituximab and pidilizumab resulted in ORR of 66% and CR of 52% which is higher than the historical ORR and CR of 40 and 11%, respectively in patients retreated with rituximab.²⁹ Median PFS was 18.8 months and not reached for the 19 responders. Toxicities were low with no grade 3 AE. Similar results have been seen in other studies combining monoclonal CD20 and PD-1/PD-L1 inhibition. ORR was 57% in a Phase Ib trial of 27 relapsed FL patients treated with atezolizumab and obinutuzumab.³⁰ Rituximab and pembrolizumab in a phase II study of 27 relapsed FL patients resulted in ORR of 80% and CR of 60%.³¹ Lenalidomide has been combined with the PD1 or PDL1 inhibition with the hope to augment immune response against follicular lymphoma. One such study, which combined obinutuzumab with lenalidomide and atezolizumab (NCT02631577) has completed accrual in r/r FL and results are expected soon.

T-cell Lymphoma

PD1 expression has generally been found to be low or absent in malignant T cells in patients with T cell lymphomas in most circumstances. Lymphocytes in the microenvironment also do not express high levels of PD1.³² A notable exception to low PD-1 expression is seen in angioimmunoblastic T cell lymphoma (AITL), which has shown both PD-1 as well as PD-L1 expression on neoplastic cells.³³ Lesokhin et al. conducted a phase 1 trial treating relapsed/refractory hematologic malignancies with nivolumab.²⁶ Nivolumab was dosed at both 1 and 3 mg/kg every 2 weeks. Pertinent to this review, there were 13 patients with mycosis fungoides (MF), 3 patients with peripheral T cell lymphomas (PTCL), and 5 patients with “other” T cell lymphomas. There were no deep responses seen among the patients with peripheral T cell lymphoma, but 2 of the 5 patients (40%) had a PR with a median PFS of 14 weeks. Of the MF patient's, the response rate was 15% (2/13), and 9 of the 13 patients (69%) had stable disease. Median PFS for the MF group was 10 weeks. A phase 2 study in a group of 24 patients with MF that use of pembrolizumab resulted in an overall response rate of 38% (9/24). There was one complete remission, and 8 partial responses.³⁴ The selective efficacy of anti-PD-1 blockade in MF patients potentially coincides with the increased PDL-1 levels seen on the malignant cells in this disease. Along similar lines, PD-1 blockade has shown efficacy with both nivolumab and pembrolizumab in the treatment of NK/T cell lymphoma. Li *et al.* found responses in 4/7 relapsed patients treated with pembrolizumab (2 CR, 2 PR, ORR 57%).³⁵ Kwong et al. showed success with pembrolizumab in NK/T cell lymphoma patients that had failed L-asparaginase treatment. Seven patients were treated, and all showed some form of response: 5 patients (71%) had CR which were durable, with 2 (29%) achieving deep responses with molecular remissions; 2 patients (29%) achieved PR.³⁶

Further investigations are needed to draw any definitive conclusions, but subsets with early data showing promise include MF, AITL, and NK/T cell lymphoma, as well as some limited efficacy and PTCL.

References

- Salles G, Barrett M, Foà R, et al. Rituximab in B-cell hematologic malignancies: a review of 20 years of clinical experience. *Adv Ther.* 2017; 34(10): 2232-2237.
- Carter PJ, Lazar GA. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat Rev Drug Discov.* 2018; 17(3): 197- 223.
- Beck A, Goetsch L, Dumontet C, Corvaia N. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat Rev Drug Discov.* 2017; 16(5): 315-337.
- Cheson BD, Chua N, Mayer J, et al. Overall survival benefit in patients with rituximab-refractory indolent non-Hodgkin lymphoma who received obinutuzumab plus bendamustine induction and obinutuzumab maintenance in the GADOLIN study. *J Clin Oncol.* 2018; 36(22): 2259- 2266.

- Marcus R, Davies A, Ando K, et al. Obinutuzumab for the first-line treatment of follicular lymphoma. *N Engl J Med.* 2017; 377(14): 1331- 1344.
- Jurczak W, Zinzani PL, Gaidano G, et al. Phase II: a study of the CD19 antibody MOR208 in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma. *Ann Oncol.* 2018; 29(5): 1266- 1272.
- Kolstad A, Madsbu U, Stokke C, et al. A higher amount of Lilotomab pre-dosing increases the antibody-adjusted Auc and has a protective effect against myelosuppression of lutetium (177Lu)-lilotomab satetraxetan in indolent NHL patients. *Haematologica.* 2017; 102: 468- 469.
- Kreitman RJ, Dearden C, Zinzani PL, et al. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. *Leukemia.* 2018; 32(8): 1768- 1777.
- Palanca-Wessels MC, Czuczman M, Salles G, et al. Safety and activity of the anti-CD79B antibody-drug conjugate polatuzumab vedotin in relapsed or refractory B-cell non-Hodgkin lymphoma and chronic lymphocytic leukaemia: a phase 1 study. *Lancet Oncol.* 2015; 16(6): 704- 715.
- Sehn L, Kamdar M, Herrera AF, et al. Randomized phase 2 trial of polatuzumab vedotin (pola) with bendamustine and rituximab (BR) in relapsed/refractory (r/r) FL and DLBCL. *J Clin Oncol.* 36(15 suppl): 7507- 7507
- Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* 1992;11(11):3887-3895.
- Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol.* 2001;2(3):261-268.
- Ansell SM, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med.* 2015;372(4):311-199.
- Armand P, Shipp MA, Ribrag V, Michot JM, Zinzani PL, Kuruvilla J, et al. Programmed death-1 blockade with pembrolizumab in patients with classical Hodgkin lymphoma after brentuximab vedotin failure. *J Clin Oncol.* 2016;34(31):3733-3739.
- Younes A, Santoro A, Shipp M, Zinzani PL, Timmerman JM, Ansell S, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol.* 2016;17(9):1283-1294.
- Chen R, et al. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic Hodgkin lymphoma. *J Clin Oncol.* 2017;35(19):2125-2132
- Zinzani PL, et al. Pembrolizumab monotherapy in patients with primary refractory classical Hodgkin lymphoma : subgroup analysis of the phase 2 KEYNOTE-087 study. *Hematol Oncol.* 2017;35(52):136-137.
- Herrera AF, Moskowitz AJ, Bartlett NL, Vose JM, Ramchandren R, Feldman TA, et al. Interim results of brentuximab vedotin in combination with nivolumab in patients with relapsed or refractory Hodgkin lymphoma. *Blood.* 2018;131(11):1183-1194.
- Herbaux C, Gauthier J, Brice P, Druze E, Ysebaert L, Doyen H, et al. Efficacy and tolerability of nivolumab after allogeneic transplantation for relapsed Hodgkin lymphoma. *Blood.* 2017;129(18):2471-2478.
- Zinzani PL, Ribrag V, Moskowitz CH, Michot JM, Kuruvilla J, Balakumaran A, et al. Safety and tolerability of pembrolizumab in patients with relapsed/refractory primary mediastinal large B-cell lymphoma. *Blood.* 2017;130(3):267-270.
- Zinzani PL, Santoro A, Gritti G, Brice P, Barr PM, Kuruvilla J, et al. Nivolumab combined with brentuximab vedotin for relapsed/refractory primary mediastinal large B-cell lymphoma: efficacy and safety from the Phase II Checkmate 436 study. *J Clin Oncol.* 2019; August 9;JCO1901492. doi: 10.1200/JCO.19.01492. [Epub ahead of print]
- Eberle FC, Salaverria I, Steidl C, Summers TA, Pittaluga S, Neriah SB, et al. Gray zone lymphoma: chromosomal aberrations with immunophenotypic and clinical correlations. *Mod Pathol.* 2011;24(12):1586-1597.
- Melani C, Major A, Schowinsky J, Roschewski M, Pittaluga S, Jaffe ES, et al. PD-1 blockade in mediastinal gray-zone lymphoma. *N Engl J Med.* 2017; 377(1): 89-91.
- Chapuy B, Roemer MGM, Stewart C, Tan Y, Abo RP, Zhang L, et al. Targetable genetic features of primary testicular and primary central nervous system lymphomas. *Blood.* 2016;127(7):869-881.
- Nayak L, Iwamoto FM, LaCasce A, Mukundan S, Roemer MGM, Chapuy B, et al. PD-1 blockade with nivolumab in relapsed/refractory primary central nervous system and testicular lymphoma. *Blood.* 2017;129(23):3071-3073.
- Lesokhin AM, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol.* 2016;34(23):2698-2704.
- Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. *Clin Cancer Res.* 2011;17(13):4232-4244.
- Smeltzer JP, Jones JM, Ziesmer SC, Grote DM, Xiu B, Ristow KM, et al. Pattern of CD14+ follicular dendritic cells and PD1+ T cells independently predicts time to transformation in follicular lymphoma. *Clin Cancer Res.* 2014;20(11):2862-2872
- Westin JR, Chu F, Zhang M, Fayad LE, Kwak LW, Fowler N, et al. Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. *Lancet Oncol.* 2014;15(1):69-77.
- Palomba ML, Till BG, Park SI, Morschhauser F, Cartron G, Marks R, et al. A phase IB study evaluating the safety and clinical activity of atezolizumab combined with obinutuzumab in patients with relapsed or refractory non-Hodgkin lymphoma (NHL). *Hematol Oncol.* 2017;35:137-138.
- Nastoupil LJ, Westin JR, Fowler NH, Fanale MA, Samaniego F, Oki Y, et al. Response rates with pembrolizumab in combination with rituximab in patients with relapsed follicular lymphoma: interim results of an open-label, phase II study. *J Clin Oncol.* 2017;35:7516-7519.
- Wang Y, Wu L, Tian C, Zhang Y. PD-1-PD-L1 immune-checkpoint blockade in malignant lymphomas. *Ann Hematol.* 2018;97(2):229-237.
- Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death-1 (PD-1)

- is a marker of germinal center-associated T cells and angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol.* 2006;30(7):802–810.
34. Khodadoust M et al. Pembrolizumab for treatment of relapsed/refractory mycosis fungoides and Sezary syndrome: clinical efficacy in a multicenter phase 2 study. *Blood.* 2016.
35. Li X, Cheng Y, Zhang M, Yan J, Li L, Fu X, et al. Activity of pembrolizumab in relapsed/refractory NK/T-cell lymphoma. *J Hematol Oncol.* 2018;11(1):15.
36. Kwong YL, Chan TSY, Tan D, Kim SJ, Poon LM, Mow B, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing l-asparaginase. *Blood.* 2017;129(17):2437–242.

GENETIC-BASED RISK STRATIFICATION IN CHRONIC LYMPHOCYTIC LEUKEMIA: IS IT STILL USEFUL?

A. Cuneo, M. Cavallari, F. Cavazzini, M. Ciccone, M. Dabusti, P. Tomasi, F. Rotondo, A. Urso, F.M. Quaglia, M. Laudisi, A. Betulla, L. Tognolo, A. Bardi, E. Saccenti, E. Volta, A. Melandri, M. Rossini, A. Spina, E. Tammiso, G.M. Rigolin

Hematology Section, Department of Medical Sciences, University of Ferrara, Italy; Azienda Ospedaliero-Universitaria, Arcispedale S. Anna, Ferrara, Italy

Introduction

Chronic lymphocytic leukemia (CLL) displays a variable clinical behavior, with many patients living for years without symptoms and other patients requiring early therapeutic intervention attaining short lasting responses to chemoimmunotherapy (CIT), with a 0.5% incidence of transformation into Richter's syndrome per year of observation.^{1,2}

Before the introduction of mechanism-based treatment targeting the B-cell receptor and BCL2, such as ibrutinib, idelalisib, and venetoclax, a plethora of prognostic factors were identified and discussed in excellent reports.³ Many of these prognostic factors are represented by biomarkers that reflect specific pathogenetic steps of CLL, as shown in Figure 1. Among disease-related biomarkers, genetic features have had an increasingly important role over the last 10 years and the mutational landscape of CLL was recently reviewed.⁴

In this analysis we considered that i) prognostic markers usefully inform the clinician on the time to first treatment (TTFT) and overall survival (OS), whereas "predictive markers" may guide the selection of the most appropriate treatment, ii) the availability of effective first-line regimens and salvage treatment make prognostication and prediction of response to therapy an important exercise in clinical practice^{4,5} iii) novel treatments with approved drugs have largely replaced CIT in high risk patients and in the relapsed/refractory setting,¹ v) the introduction of novel effective agents may prolong survival of CLL patients and might change the epidemiology of Richter's syndrome (RS).²

We therefore focused our attention on the role of genetic testing in the management of the newly diagnosed patient, in treatment planning and in the prediction of the risk of RS.

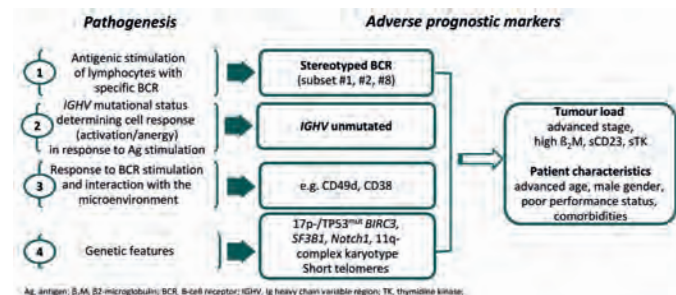


Figure 1. Pathogenic steps and corresponding prognostic markers in CLL. BCR stimulation by autoantigens, external antigens or by neo-epitopes generated during the apoptotic process cause variable cell responses depending on the reactivity of the BCR structure (e.g. stereotyped receptors) and on the *IGHV* mutational status. Cell activation occurs within a complex interaction in the microenvironment. Cell divisions, oligoclonal expansion and genetic instability cause the emergence of clones with primary and secondary molecular-cytogenetic lesions, resulting into a heterogeneous clinical behavior.

Genetic markers, TTFT and OS

A new diagnosis of CLL has a negative impact on quality of life⁶ and a recent analysis showed that 14% and 12% of CLL patients experienced moderate-severe depression and anxiety symptoms, respectively.⁷ Therefore, the identification of prognostic factors predicting TTFT, a surrogate marker of OS, may assist the clinician in reassuring the patients with low-risk disease and to plan adequate follow-up in those with more aggressive biologic features.

A prospective study of 710 patients revealed a median TTFT of 110.2 months (95% CI, 95.9–124.6).⁸ In a real world analysis of 335 untreated CLL patients diagnosed between 2006 and 2016 at a single center with a >90% capture of incident cases, the median TTFT was >10 years.⁹ However, variation in TTFT exists, depending on clinicobiological features,¹⁰ including genetic markers, i.e. 17p- or *TP53* mutation, here referred to as *TP53* disruption, 11q- and *IGHV* mutational status. Interestingly, ultra-stable CLL patients as defined by the absence of disease progression for over 10 years were shown to be characterized by favorable immunogenetics. i.e. mutated *IGHV* (*M-IGHV*) gene, absence of 11q-/17p- and by the absence of known unfavorable driver mutations, copy number aberrations and novel recurrent genetic lesions.¹¹

In a large multicentre study, *TP53* disruption, trisomy 12 and stereotyped subset #2 membership were equivalently associated with the shortest TTFT among 1224 stage-A patients with *M-IGHV* CLL (treatment probability at 5 and 10 years of 40% and 55%, respectively), whereas among 676 stage A *IGHV*-unmutated (*U-IGHV*), CLL adverse prognosticators included *TP53* disruption, del(11q) and/or *SF3B1* mutations (5- and 10-year treatment probability of 78% and 98%, respectively).¹²

The international prognostic index for patients with CLL identified 5 independent prognostic factors and assigned weighted risk scores to each factor: 17p13 deletion/*TP53* mutation (score 4), *U-IGHV* (score 2), serum 2-microglobulin concentration >3.5 mg/L (score 2), stage Binet B–C or Rai I–IV (score 1) and age>65 years (score 1). The patients with low risk disease (i.e. score 0-1) accounted for approximately 50% of the entire cohort and had a TTFT exceeding 10 years in watch-and-wait patients enrolled in a trial and in 2 independent cohorts (13); the patients with intermediate-risk disease (i.e. score 2-3) showed a median TTFT of approximately 36-48 months, whereas the remaining patients with high-risk disease (score 4-6) or very high-risk disease (score 7-10) required early treatment intervention.

CLL nowadays has a prolonged life expectancy in the majority of cases. The median OS was 95.3 months [95% CI 89.7–98.5] in a large study including 3472 treatment naive patients, the vast majority of whom had an indication for treatment.¹⁵ The CLL-IPI score predicts for a 79% survival probability at 10 years in low-risk patients as compared with a 10-year survival probability of 39.2%, 21.9% and 3.5% in intermediate, high, and very high-risk patients, respectively.

Noteworthy, the IPI score can be used in clinical practice with the understanding that the prediction accuracy for CLL prediction models are approximately 0.7 as assessed by receiver operating characteristic curve accuracy (C statistic), which means in practical terms that the model predicts outcome correctly in approximately two-third of individuals.¹⁴

Genetic markers and response to CIT

Nowadays CLL prognostication can be used in clinical practice as a continuum,¹⁴ as exemplified in Figure 2, where the expected TTFT and PFS in patients with high-risk and low-risk CLL treated with CIT is reported, showing that improvement is necessary in patients with unfavorable genetic features.

***TP53* disruption** Given the disappointing results obtained with CIT¹ it is mandatory to perform genetic testing in experienced laboratories to spare unnecessary toxicity to the patients with *TP53* disruption, irrespective of age and fitness status. The advantage of ibrutinib in the older patients with 17p- was documented in a phase-3 trial, showing that the median PFS was not reached [95% CI 14.7–NE] at a median follow-up of 31.3 months in the ibrutinib-obinutuzumab group as compared with 11.3 months [9.5–15.3] in the chlorambucil-obinutuzumab group.¹⁵ Likewise, a PFS advantage of ibrutinib (plus rituximab) vs bendamustine and rituximab was documented.¹⁶ The fixed-duration combination venetoclax and rituximab was associated with longer PFS as compared with the standard chlorambucil and obinutuzumab regimen in patients with comorbidity and *TP53* disruption (CLL14 GCLLSG trial).¹⁷

BIRC3 mutations *BIRC3* mutations showed an independent association with an increased risk of progression (HR 2.8 [95% confidence interval 1.4-5.6]) in multivariate analysis adjusted for *TP53* disruption and *IGHV* mutation status in a retrospective cohort of 287 CLL treated with first-line fludarabine, cyclophosphamide and rituximab (FCR). Though these data require validation in prospective studies, *BIRC3* mutations may represent a new useful molecular predictor of poor response to fludarabine-containing regimens.¹⁸

NOTCH1 The adjunct of rituximab or ofatumumab to the classical chemotherapy backbones (i.e. chlorambucil or fludarabine plus cyclophosphamide) did not result in the expected increase in PFS in patients with *NOTCH1* mutations, which may represent a possible biomarker of resistance to these anti-CD20 monoclonal antibodies.² Interestingly the outcome of CLL patients treated with obinutuzumab combined to chlorambucil was improved independent of *NOTCH1* mutation. The mechanism underlying the anti-CD20 refractoriness associated with *NOTCH1* mutations remains obscure and because these observations represent post-hoc analyses, prospective studies are warranted to validate these observations.

Complex karyotype (CKT) In the CLL14 trial,¹⁹ evidence was provided that the presence of a CKT in 15% of the patients enrolled in the CIT arm predicted for an inferior outcome, with a median PFS of 19 months vs not reached (NR) in patients without CKT (HR 2.790 [95% CI 1.631-4.772], p<0.001) and for a shorter OS (HR 3.736 [95% CI 1.357-10.287], p=0.006). These data confirm previous findings in a fraction of elderly patients who were submitted to conventional karyotyping in the CLL11 trial.²⁰ Overall, there is robust evidence that having a CKT as defined by the presence of 3 or more clonal aberrations in metaphase cells predicts for an inferior outcome in CLL treated with CIT.²¹ Interestingly, the hypothesis was generated in two independent studies that among patients with CKT, those with unbalanced chromosome translocation and those with 5 or more aberrations may represent a very high risk subset.^{22,23}

U-IGHV gene The *U-IGHV* gene poses the patients at higher risk or progression as compared with the *M-IGHV* gene when using CIT regimens.¹ Direct comparisons in clinical trials documented a clear PFS advantage of novel mechanism-based treatment over CIT in the *U-IGHV* subset.^{15,16,17,24} To the contrary, no PFS difference between new drugs and CIT was detected in the *M-IGHV* subset of CLL. It is worth noting that the trials using ibrutinib compared fixed-duration treatment (CIT) and continuous treatment (ibrutinib). The time to second objective disease progression (PFS2) was not assessed in these trials and it is noteworthy that the European medicine agency (EMA) recommended PFS2 as a meaningful endpoint to account for the possibilities that continuous treatment may negatively affect OS despite improving PFS by increasing long-term toxicity, or altering the tumor population or microenvironment to induce drug resistance or evolution of an aggressive clone.²⁵ Ibrutinib-Rituximab proved superior in terms of OS to the classical fludarabine, cyclophosphamide and rituximab (FCR) regimen.²⁴ However, few deaths occurred (4/354 vs 10/175) in this analysis and the minimal number of changes of experimental outcomes from events to non-events sufficient to shift the statistical threshold p value was low in this study,²⁶ with a fragility index=4.

In the first salvage setting BR may still represent an option for a limited number of patients preferring second line treatment of short-duration, provided that they show a favorable genetic profile, have a limited disease and have had a long duration of response to first line CIT.²⁷

Genetic markers and response to mechanism-based treatment

Prognostic markers

BCR and BCL2 inhibitors revolutionized the treatment of patients with R/R CLL and with high-risk disease subsets requiring first line treatment. In this paragraph we took into considerations only approved drugs with a consolidated role in clinical practice, i.e. ibrutinib, idelalisib and venetoclax.

The *U-IGHV* configuration, as well as mutations of *NOTCH1*, *SF3B1* and *BIRC3* were not predictive of an inferior PFS in patients with R/R CLL treated with ibrutinib (28). Likewise, the *U-IGHV* had no impact on the clinical outcome in patients treated with venetoclax plus rituximab (29) or idelalisib plus rituximab (30). Thus, these efficacious drugs largely overcame the negative prognostic power of these genetic markers (Table 1).

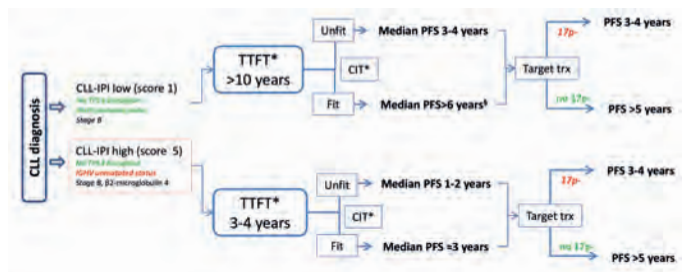


Figure 2. CLL prognostication as a continuum. The natural history and predicted PFS based on the assessment of prognostic factors in patients treated with first-line chemoimmunotherapy is reported, showing that there is need for improvement in high risk disease. Genetic markers with unfavorable or favorable prognostic significance are in red and green character, respectively. *Data on median TTF were drawn from the International CLL-IPI working group (Lancet Oncol. 2016; 17:779-90). Median PFS was drawn from the CLL11 and iLLUMINATE trials (Goede V. N Engl J Med 2014; 370:1101-10, Moreno C. Lancet Oncol 2019; 20:43-56) using chlorambucil and obinutuzumab in unfit patients and from the CLL8 and CLL10 trials (Fischer K. Blood 2016; 127:208-15; Eichhorst B. Lancet Oncol 2016; 17:928-42) using FCR or BR in fit patients. Data on median PFS with target treatment were drawn from the trials with the longest follow-up so far reported, i.e. Resonate and Murano (Byrd JC. Blood 2019; 133:2031-2042; Seymour J. N Engl J Med 2018; 378:1107-20) using ibrutinib or venetoclax plus rituximab. §: Approximately 2 out of 3 fit patients treated with FCR are free from progression at a median follow-up of 5.9 years (Fischer K. Blood 2016).

Table 1. Genetic markers with an independent negative prognostic effect or with established predictive value in CLL.

Chemoimmunotherapy (CIT)					
Parameter	Prognostic		Predictive		comments
	TTF	OS			
del(17p)/TP53 ^{MUT}	Yes	Yes	Yes	Yes	CIT regimens are not efficacious [see text]
IGHV unmutated	Yes	Yes	Yes	Yes	All CIT regimens produce an inferior outcome as compared with M-IGHV [see text]
del(11q)	Yes	Yes	Yes	Yes	Inferior outcome with chemotherapy (Oscier DG et al, Blood 2013; 121: 468-75); not significant with CIT
NOTCH1 ^{MUT}	No	Yes	Yes	Yes	Inferior outcome with chemotherapy (Oscier DG et al, Blood 2013; 121: 468-75); not significant with CIT; may predict for lack of efficacy of rituximab [see text]
SF3B1 ^{MUT}	No	Yes	No	No	Inferior outcome with chemotherapy, not significant with CIT (Oscier DG et al, Blood 2013; 121: 468-75)
BIRC3 ^{MUT}	Unkno wn	Yes	Yes	Yes	Inferior outcome with CIT, requires validation in prospective trials [see text]
Shortened telomeres	Unkno wn	Yes*	Yes*	Yes*	Inferior outcome with CIT (*as a continuous variable); detection method requires validation [Ibarra] BMC et al. Leukemia 2019 Mar 25 - Epub ahead of print]
Complex karyotype	Yes	Yes	Yes	Yes	Inferior outcome in patients with comorbidities treated with CIT [see text]
Stereotyped receptors	Yes	Yes	No	No	Subset #1, #2 and #8 show an aggressive course with CIT [Stamatopoulos K et al. Leukemia 2017; 31:282-91]; subset #8 presents high risk of transformation [see text].
Novel agents					
Parameter	Prognostic		Predictive		Comments
	PFS	OS			
del(17p)/TP53 ^{MUT}	Yes*	Yes*	No	No	Worse PFS with del(17p) with ibrutinib [see text], worse PFS and OS with venetoclax and rituximab in the CLL14 trial [*published in an abstract form at EMA 2019, see text]
del(11q)	No	No	No	No	[Byrd JC et al. Blood 2019;133:2031-42; O'Brien S et al. Blood 2018; 131:1910-1919.] No impact with venetoclax nor idelalisib [Sharman J. J Clin Oncol 2019; 37:3391-1402; Anderson MA. Blood 2017; 129:3362-3370]
IGHV unmutated	No	No	No	No	No impact with new drugs [see text]
NOTCH1 ^{MUT}	No	No	No	No	No impact with ibrutinib [see text]
SF3B1 ^{MUT}	No	No	No	No	No impact with ibrutinib [see text]
BIRC3 ^{MUT}	No	No	No	No	No impact with ibrutinib [see text]
Complex karyotype	Controversial	Controversial	No	No	Shorter PFS and OS in some studies with ibrutinib [Thompson et al. Cancer 2015; 121:3612-21] and venetoclax [Anderson MA. Blood 2017; 129:3362-3370], no difference in others [Byrd JC et al. Blood 2019; 133:2031-2042; Al-Sawaf Hemasphere 2019;3:51 p.4 5106]. No difference with idelalisib and rituximab [Kreuzer KA et al. Leukemia 2019 Aug 19 - Epub ahead of print]
BTK/PLCY mutations	No	No	Yes	Yes	Not present at baseline. Anticipate failure to ibrutinib [see text]
BCL2 mutations	No	No	Yes	Yes	Not present at baseline. Anticipate failure to Venetoclax [see text58]

Green: negative prognostic significance at multivariable analysis in at least 1 prospective clinical trial; Orange: negative prognostic significance at multivariable analysis in observational retrospective studies or conflicting data in different trials; Red: No documented negative prognostic/predictive significance

In the R/R setting, 17p- was associated with a statistically non-significant trend for shorter PFS in patients on ibrutinib, with a median PFS of 40.1 months vs NR at a median follow-up of 44 months.²⁸ At variance, in a phase-1b/2 trial (31) the median PFS and OS were significantly shorter (26 and 57 months, respectively) in patients with del (17p) than in other cytogenetic groups and 17p- was the only genetic factor predicting for a shorter OFS and OS at multivariable analysis. Heterogeneity of patient population enrolled in the different trials may account for the observed difference. The 17p- anomaly was not associated with an inferior outcome with venetoclax and rituximab (23.8-month median follow-up);²⁹ however, *TP53* disruption was associated with a higher probability to develop progression in 130 responder patients who underwent per-protocol cessation of venetoclax-rituximab at month 24.³² No PFS difference was observed in patients with or without 17p- treated with idelalisib and rituximab (18-month median follow-up).³⁰ As described in detail elsewhere,²¹ CKT was associated with shorter PFS and OS at univariate analysis in phase 1b/2 studies using ibrutinib.^{31,33} However, these findings were not confirmed in the Resonate trial²⁸ where CKT was associated with a 40.8 months median PFS vs NR in patients without CKT (Hazard ratio 1.292 with 95% CI 0.770-2.168)

Venetoclax proved to be less effective in patients with CKT in a phase 2 study including heavily pre-treated patients³⁴ whereas no PFS difference was reported with idelalisib and rituximab in patients with or without CKT.³⁵ Thus, additional prospective studies and longer follow-up are required to unequivocally establish the prognostic/predictive value of *TP53* disruption and CKT with these new drugs.

Interestingly, prognostic factors were evaluated in a pooled cohort of 2475 previously treated patients enrolled in phase-3 trials using CIT, ibrutinib, idelalisib, and venetoclax.³⁶ Genetic markers, i.e. 17p-, *ATM* mutations and *U-IGHV* were included among 28 candidate clinicobiologic parameters. The final model based on multivariable analysis identified B M, LDH, hemoglobin and time from initiation of last therapy as the strongest prognostic factors. Although CKT and mutations of *TP53*, *NOTCH1*, *SF3B1*, *BIRC3* were not assessed, this study provides the clinician with a useful prognostic model that can be easily ascertained.

In the first line setting limited information on the prognostic significance of *TP53* disruption is presently available. In the venetoclax plus obinutuzumab arm of the CLL14 trial, patients with 17p- had a shorter PFS than patients without 17p- ($p=0.001$) (17), whereas no PFS difference was noted in patients with or without CKT.¹⁹

Predicting CLL progression on ibrutinib and venetoclax

In the targeted treatment era, the emergence of mutations conferring resistance to a specific molecule may represent an important predictor of disease progression, as they are detectable prior to clinical relapse and may dictate switch to other targeted interventions.¹

Two seminal papers^{37,38} showed that the development of BTK Cys481 mutations or *PLCG2* gain-of function mutations that activate phospholipase Cg 2 are responsible for the emergence of resistant clones under ibrutinib. These mutations were not identified before treatment in any patient and accounted for approximately 80% of CLL progressions under ibrutinib. The early detection of small clones harboring these mutations predicted for CLL progression after a median of 9 months.³⁸

A Gly101Val mutation in *BCL2* was identified in 7 out of 15 patients with CLL progression on treatment with venetoclax.³⁹ The mutation was not present in pre-treatment specimens and was detectable before clinical relapse through sensitive allele-specific digital-droplet PCR. Thus, even though venetoclax resistance is a heterogeneous phenomenon, the detection of the Gly101Val mutation could potentially serve as an early biomarker of impending disease progression.

Predicting transformation into RS

Predictive biomarkers include the *NOTCH1* mutations, *TP53* disruption and the usage of BCR subset #8,² defined by the expression of stereotyped IGHV4-39 B-cell receptor, characterized by excessive antigen reactivity leading to significant cell activation.⁴⁰

Among CLL treated with oral agents targeting BCR or *BCL2*, a CKT in CLL lymphocytes was found in 8 of 17 patients with RS (47%) (34). Likewise, Rogers *et al.*⁴¹ found a CKT in 28/42 (67%) patients who subsequently developed RT and Miller *et al.*⁴² found an association between

near-tetraploidy (4 copies of most chromosomes within a cell) with CKT, showing that 6/9 patients with this peculiar cytogenetic pattern developed RS. In this study, near-tetraploidy and CKT represented independent predictors of ibrutinib discontinuation due to RS.

Conclusion and perspectives

The presence of *TP53* disruption mandates the use of new drugs. Though additional data are required to reach an unequivocal evidence-based consensus on the optimal treatment in CLL without *TP53* disruption, drugs targeting BCR or *BCL2* may represent the preferred first-line option in the *U-IGHV* subset and, possibly, in patients with *BIRC3* mutations or CKT.

Prognostic biomarkers may also play an essential role to define the eligibility in trials exploring the efficacy of early treatment in high-risk patients, whereas predictive markers can be exploited to define risk-based treatment strategies using new drugs upfront or a combination thereof.

Nowadays, the treating physician has the opportunity to choose among different drugs and treatment strategies, including finite-duration and continuous treatment.⁴³ Therefore, coordinated efforts are required to strengthen the role of predictive biomarkers in clinical practice. The following considerations may be pertinent

While surrogate endpoints are important to show the efficacy of treatment, OS remains the most significant outcome measure,⁴⁴ especially when comparing continuous and fixed-duration treatment. Thus, PFS2 should be assessed in trials, as recommended by EMA.²⁵ Would *U-IGHV* hold its negative predictive significance in trials comparing CIT and continuous treatment and using PFS2 as an endpoint?

In the absence of direct comparisons between new agents, the identification of markers predicting the duration of response with ibrutinib, idelalisib and venetoclax is a largely unmet clinical need.

The impact of "high-CKT", as defined by the presence of 5 or more aberrations or unbalanced translocations should be further explored. As conventional cytogenetic analysis is cumbersome, surrogate markers of karyotype/genome complexity should be investigated.

Dynamic predictive markers (i.e. mutations conferring resistance to ibrutinib and venetoclax) represent potentially useful tools and their impact on treatment decision need to be further investigated.

Considering that CLL is predominantly a disease of the elderly and the increasing cost of new drugs (45), the validation of predictive biomarkers represents a necessary step to allow clinicians to design a sustainable treatment strategy, giving the patients the highest probability of achieving a durable response with a real benefit in terms of quality of life. It is therefore anticipated that predictive biomarkers will have an increasingly important role to foster translation of trial results into reality.

References

- Hallek M. Chronic lymphocytic leukemia: 2019 update on diagnosis, risk stratification and treatment. *Am J Hematol*. 2019 Jul 30. doi: 10.1002/ajh.25595
- Rossi D, Spina V, Gaidano G. Biology and treatment of Richter syndrome. *Blood*. 2018;131(25):2761-2772.
- Eichhorst B, Hallek M. Prognostication of chronic lymphocytic leukemia in the era of new agents. *Hematology Am Soc Hematol Educ Program*. 2016(1):149-155.
- Gaidano G, Rossi D. The mutational landscape of chronic lymphocytic leukemia and its impact on prognosis and treatment. *Hematology Am Soc Hematol Educ Program*. 2017 Dec 8;2017(1):329-337. doi: 10.1182/asheducation-2017.1.329
- Bosch F, Dalla-Favera R. Chronic lymphocytic leukaemia: from genetics to treatment. *Nat Rev Clin Oncol*. 2019 Jul 5. [Epub ahead of print]
- Shanafelt TD, Kay NE. Comprehensive management of the CLL patient: a holistic approach. *Hematology Am Soc Hematol Educ Program*. 2007;324-31.
- Robbertz AS, Weiss DM, Awan FT *et al.* Identifying risk factors for depression and anxiety symptoms in patients with chronic lymphocytic leukemia. *Support Care Cancer*. 2019 Jul 22. [Epub ahead of print]
- Hochstetter MA, Busch R, Eichhorst B *et al.* Early, risk-adapted treatment with fludarabine in Binet stage A chronic lymphocytic leukemia patients: results of the CLL1 trial of the German CLL study group. *Leukemia*. 2017;31(12):2833-2837.
- Rigolin GM, Cavallari M, Quaglia FM *et al.* In CLL, comorbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI. *Blood*. 2017;129(26):3495-3498.
- Martinelli S, Cuneo A, Formigaro I *et al.* Identifying High-Risk Chronic Lymphocytic Leukemia: A Pathogenesis-Oriented Appraisal of Prognostic and Predictive Factors in Patients Treated with Chemotherapy with or without Immunotherapy. *Mediterr J Hematol Infect Dis*. 2016;8(1):e2016047
- Raponi S, Del Giudice I, Marinelli M *et al.* Genetic landscape of ultra-stable chronic lymphocytic leukemia patients. *Ann Oncol*. 2018;29(4):966-972.
- Baliakas P, Moysiadis T, Hadzidimitriou A *et al.* Tailored approaches grounded on

- immunogenetic features for refined prognostication in chronic lymphocytic leukemia. *Haematologica*. 2019; 104(2):360-369.
13. International CLL-IP1 working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IP1): a meta-analysis of individual patient data. *Lancet Oncol*. 2016;17(6):779-790. doi: 10.1016/S1470-2045(16)30029-8
 14. Montserrat E, Gale RP. Predicting the outcome of patients with chronic lymphocytic leukemia: Progress and uncertainty. *Cancer*. 2019 Aug 5. [Epub ahead of print]
 15. Moreno C, Greil R, Demirkan F et al. Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (ILLUMINATE): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol*. 2019;20(1):43-56. -
 16. Woyach JA, Ruppert AS, Heerema NA et al. Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL. *N Engl J Med*. 2018;379(26):2517-2528.
 17. Tausch E, Bahló J, Robrecht S et al. Genetic markers and outcome in the CLL14 trial of the GCLLSG comparing frontline obinutuzumab plus chlorambucil or venetoclax in patients with comorbidity. *Hemasphere* 2019; 3:S1 p.4 S105
 18. Diop F, Moia R, Favini C, Spaccarotella E et al. Biological and clinical implications of BIRC3 mutations in chronic lymphocytic leukemia. *Haematologica*. 2019 Aug 1. [Epub ahead of print]
 19. Al-Sawaf O, Lilienweiss E, Bahló J et al. High efficacy of venetoclax plus obinutuzumab in patients with complex karyotype (CKT) and chronic lymphocytic leukemia (CLL): a prospective analysis from the CLL14 trial. *Hemasphere*. 2019; 3:S1 p.4 S106
 20. Herling CD, Klaumünzer M, Rocha CK et al. Complex karyotypes and KRAS and POT1 mutations impact outcome in CLL after chlorambucil-based chemotherapy or chemoimmunotherapy. *Blood* 2016; 128:395-404
 21. Cavallari M, Cavazzini F, Bardi A et al. Biological significance and prognostic/predictive impact of complex karyotype in chronic lymphocytic leukemia. *Oncotarget*. 2018;9(76):34398-34412.
 22. Rigolin GM, Saccenti E, Guardalben E et al. In chronic lymphocytic leukaemia with complex karyotype, major structural abnormalities identify a subset of patients with inferior outcome and distinct biological characteristics. *Br J Haematol*. 2018;181(2):229-233.
 23. Baliakas P, Jeromin S, Iskas M et al. Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. *Blood*. 2019;133(11):1205-1216.
 24. Shanafelt TD, Wang XV, Kay NE et al. Ibrutinib-Rituximab or Chemoimmunotherapy for Chronic Lymphocytic Leukemia. *N Engl J Med*. 2019;381(5):432-443.
 25. European Medicine Agency. Guideline on the evaluation of anticancer medicinal products in man (13 December 2012). https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-evaluation-anticancer-medicinal-products-man-revision-4_en.pdf
 26. Del Paggio JC, Tannock IF. The fragility of phase 3 trials supporting FDA-approved anticancer medicines: a retrospective analysis. *Lancet Oncol* 2019; 20: 1065-9
 27. Cuneo A, Follows G, Rigolin GM et al. Efficacy of bendamustine and rituximab as first salvage treatment in chronic lymphocytic leukemia and indirect comparison with ibrutinib: a GIMEMA, ERIC and UK CLL FORUM study. *Haematologica*. 2018; 103: 1209-1217.
 28. Byrd JC, Hillmen P, O'Brien S et al. Long-term follow-up of the RESONATE phase 3 trial of ibrutinib vs ofatumumab. *Blood*. 2019;133(19):2031-2042.
 29. Seymour JF, Kipps TJ, Eichhorst B et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *N Engl J Med*. 2018;378(12):1107-1120.
 30. Sharman JP, Coutre SE, Furman RR et al. Final Results of a Randomized, Phase III Study of Rituximab With or Without Idelalisib Followed by Open-Label Idelalisib in Patients With Relapsed Chronic Lymphocytic Leukemia. *J Clin Oncol*. 2019;37(16):1391-1402.
 31. O'Brien S, Furman RR, Coutre S et al. Single-agent ibrutinib in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. *Blood* 2018; 131:1910-1919.
 32. Kater AP, Seymour JF, Hillmen P et al. Fixed Duration of Venetoclax-Rituximab in Relapsed/Refractory Chronic Lymphocytic Leukemia Eradicates Minimal Residual Disease and Prolongs Survival: Post-Treatment Follow-Up of the MURANO Phase III Study. *J Clin Oncol* 2019; 37(4):269-277.
 33. Thompson PA, O'Brien SM, Wierda WG et al. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. *Cancer*. 2015;121(20):3612-21.
 34. Anderson MA, Tam C, Lew TE et al. Clinicopathological features and outcomes of progression of CLL on the BCL2 inhibitor venetoclax. *Blood*. 2017; 129:3362-3370
 35. Kreuzer KA, Furman RR, Stilgenbauer S et al. The impact of complex karyotype on the overall survival of patients with relapsed chronic lymphocytic leukemia treated with idelalisib plus rituximab. *Leukemia*. 2019 Aug 19. [Epub ahead of print]
 36. Soumerai JD, Ni A, Darif M et al. Prognostic risk score for patients with relapsed or refractory chronic lymphocytic leukaemia treated with targeted therapies or chemoimmunotherapy: a retrospective, pooled cohort study with external validations. *Lancet Haematol*. 2019;6(7):e366-e374.
 37. Ahn IE, Underbayev C, Albitar A et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. *Blood*. 2017;129(11):1469-1479.
 38. Woyach JA, Ruppert AS, Guinn D et al. BTKC481S-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. *J Clin Oncol*. 2017 May 1;35(13):1437-1443. doi: 10.1200/JCO.2016.70.2282
 39. Blombery P, Anderson MA, Gong JN et al. Acquisition of the Recurrent Gly101Val Mutation in BCL2 Confers Resistance to Venetoclax in Patients with Progressive Chronic Lymphocytic Leukemia. *Cancer Discov*. 2019 (3):342-353.
 40. Gounari M, Ntoufa S, Apollonio B et al. Excessive antigen reactivity may underlie the clinical aggressiveness of chronic lymphocytic leukemia stereotyped subset #8. *Blood*. 2015;125(23):3580-7.
 41. Rogers KA, Huang Y, Ruppert AS et al. A single-institution retrospective cohort study of first-line R-EPOCH chemoimmunotherapy for Richter syndrome demonstrating complex chronic lymphocytic leukaemia karyotype as an adverse prognostic factor. *Br J Haematol*. 2018; 180:259-266.
 42. Miller CR, Ruppert AS, Heerema NA et al. Near-tetraploidy is associated with Richter transformation in chronic lymphocytic leukemia patients receiving ibrutinib. *Blood Adv*. 2017; 1:1584-1588.
 43. Cuneo A, Foà R. Relapsed/Refractory Chronic Lymphocytic Leukemia: Chemoimmunotherapy, Treatment until Progression with Mechanism-Driven Agents or Finite-Duration Therapy? *Mediterr J Hematol Infect Dis*. 2019;11(1):e2019024.
 44. Kumar S, Rajkumar SV. Surrogate endpoints in randomised controlled trials: a reality check. *Lancet*. 2019;394(10195):281-283.
 45. Chen Q, Jain N, Ayer T et al. Economic Burden of Chronic Lymphocytic Leukemia in the Era of Oral Targeted Therapies in the United States. *J Clin Oncol* 2016. 35:166-174.

FIRST LINE THERAPY: IS THERE STILL A ROLE FOR CHEMOTHERAPY?

A. Tedeschi, G. Zamprognà, M. Montillo

ASST Grande Ospedale Metropolitano Niguarda, Niguarda Cancer Center, Milano, Italy

The natural history of Chronic Lymphocytic Leukemia (CLL) is highly heterogeneous. Up to one third of patients may never require treatment and the disease will not affect survival, however most patients will develop a symptomatic, active disease and treatment should be initiated.

Clinical observation is considered the standard of care for the management of early Binet stage A CLL and this independently from disease prognostic features. Several studies have addressed the value, in treatment-naïve Binet stage A patients with high risk prognostic features, of an early intervention with fludarabine or chlorambucil or fludarabine cyclophosphamide and rituximab (FCR) in prolonging progression free survival and overall survival. Although chemotherapy based treatment led to a better progression free survival (PFS), it failed to show a survival benefit and was characterized by increased cytopenia and severe infections. A randomized trial, CLL12, comparing ibrutinib versus placebo is ongoing to verify whether the introduction of a new targeted therapy may change the approach in patients with high risk prognostic features although asymptomatic not in need of therapy according to IWCLL guidelines. The primary objective of the study was met as event free survival showed to be significantly longer for patients randomized to receive ibrutinib. Surprisingly, although typical side effects of ibrutinib were recorded, there were not significant differences in the rate of grade 3-4 adverse events, infections and serious adverse events in the two arm groups. Furthermore, similar rates of infections were observed. The study has not yet shown an OS benefit so that in clinical practice the observational approach should not be modified. Treatment should be considered in patients showing B symptoms, cytopenia or progressive and significant lymphadenopathy and organomegaly according to the guidelines of the International Workshop on CLL (IWCLL).

Therapy before target agents approval, “early days”

While traditionally treated with chemoimmunotherapy, the therapeutic options for CLL have expanded after the approval of three new oral inhibitors. Therefore, with a variety of reasonable options, choice in first-line has become more challenging.

Before the availability of the inhibitors, patients characteristics as age, fitness and comorbidities, were the only factors predictive of treatment tolerability that could affect treatment decision. Several trials established FCR as the standard of care treatment, demonstrating for the first time an overall survival improvement in the CLL8 study. However only 10% of the study patients were ≥ 70 years, ECOG PS was ≤ 1 and patients presented normal renal function. Even with these restrictive criteria 76% of patients experienced grade 3-4 adverse events, 25% grade 3-4 infections and a high rate of myelosuppression. The CLL10 study comparing FCR versus bendamustine rituximab (BR), clearly demonstrated FCR superiority. Although in physically fit patients older than 65 years BR showed to be better tolerated (71% of patients experienced grade 4 adverse effects resulting in life-threatening consequences and/or hospitalization compared with 41% in the BR group) with no significant difference in PFS. Based on these results for a fit CLL population FCR has been considered the reference standard first line

treatment in patients aged <65 years and BR for patients aged ≥ 65 years. In unfit patients, with significant comorbidities, FCR or BR are associated with an excess of toxicity. In this patient group with a Comorbidity Illness Rating Scale (CIRS) score > 6 the combination of chlorambucil with obinutuzumab in the CLL11 trial provided a survival advantage over chlorambucil alone. Notably obinutuzumab chlorambucil demonstrated also clinically meaningful improvement compared to rituximab chlorambucil, median PFS 28.9 versus 15.7 months, and also provided an improvement in OS, median not reached versus 73.1 months. Similarly a PFS advantage of ofatumumab plus chlorambucil over chlorambucil was observed in the Complement-1 study in patients unsuitable for fludarabine based treatment. Therefore chlorambucil with the combination of an anti CD20 antibody was considered a good option for frontline treatment of CLL in patients too frail to tolerate high intensity chemoimmunotherapy.

Therapy after target agents approval in del(17p)/TP53 mutated patients

Patients with del(17p) or TP53 mutation have shown very poor outcome with chemotherapy based regimens, even with the most intensive FCR regimen overall survival resulted to be only 33.1 months.

In contrast to chemoimmunotherapy, novel target agents do not exert their anti leukemic activity through genotoxic mechanism and are therefore active irrespectively of TP53 dysfunction. Consistently phase 2/3 trials with the BCR inhibitors, ibrutinib and idelalisib, and BCL2 inhibitor, venetoclax, demonstrated better responses and more favourable survival outcomes in a high proportion of patients harboring TP53 aberrations. There are not many studies addressing the role of the inhibitors in first line treatment. Ibrutinib has shown activity in patients with del(17p) or TP53 mutation, even relapsed/refractory, with estimated 24-month PFS rates of 63 to 82%. In the first-line setting, a single-center experience reported 5-year PFS of 74% in 34 previously untreated patients which is substantially better than historical PFS outcomes with first-line chemoimmunotherapy.

There are no trials addressing the role of idelalisib specifically in del(17p) patients. In a phase III study comparing idelalisib-rituximab versus rituximab plus placebo in R/R patients, PFS was significantly longer in patients treated with idelalisib (not reached versus 5.5 months) and the outcome was similar when patients were stratified according to the presence or absence of del(17p) deletion or TP53 mutation. Venetoclax in 158 pretreated del(17p) patients led to an objective response of 77% with a complete response (CR) of 20%, a PFS of 54% at 24 months, and an OS of 73%.

Based on these data ibrutinib is considered the treatment of choice in patients carrying del(17p) or TP53 mutation, for patients clinically ineligible for a BTKi based treatment, idelalisib or venetoclax may be considered as alternative first line treatment options.

Therapy after ibrutinib first line approval, “nowadays”

Of the three targeted therapies (ibrutinib, idelalisib, venetoclax) available at present, only ibrutinib has received broad approval by both EMA and FDA as first line treatment even in non del(17p)/TP53 mutated patients. The approval was based on the results of the phase III Resonate-2 trial addressed to non del(17p) patients 65 years or older randomized to receive chlorambucil or ibrutinib. The BTKi was significantly superior in terms of PFS and OS at the first analysis independent of age stratification (≥ or <75 years). The favourable outcome was maintained after a longer follow-up with a 5 year PFS of 70% versus 12%. Only 6% of patients showed a disease progression while on ibrutinib. Importantly, OS benefit was maintained with ibrutinib despite the crossover demonstrating that in the setting of an elderly unfit population, it is important to achieve immediate disease control. Patients with del(17p) were excluded from the study, in contrast to chemoimmunotherapy, among patients treated with ibrutinib, a similar PFS was observed when patients were stratified according to the high risk prognostic features, del(11q) and IGHV unmutated status. This is consistent with the unmutated IGHV enhanced signalling through BCR.

Chlorambucil monotherapy was considered the standard treatment for elderly unfit patients at the time of Resonate-2 trial design, but this is no longer true since the CLL11 trial demonstrated the superiority of chlorambucil obinutuzumab. The Illuminate trial was conducted to compare the recommended regimen from international guidelines, obinutuzumab chlorambucil, versus obinutuzumab ibrutinib. The trial

confirmed the superiority in terms of PFS of the BTKi and most importantly, confirmed the results of previous reports of the role of ibrutinib in patients with high-risk genomic features (IGHV unmutated, del(17p), del(11q) identifying a poor outcome population with chemoimmunotherapy. Furthermore it should be considered that in both studies time to next treatment was in favour of ibrutinib when compared to chemotherapy based treatment. Notably, the significant PFS benefit was also seen in patients with bulky disease. The overall safety profile with single-agent ibrutinib appeared favorable to chlorambucil-obinutuzumab in a time-matched analysis.

Although cross-trial analysis may have inherent limitations a PFS benefit was also observed when comparing the population treated with single-agent ibrutinib in the Resonate-2 trial with patients receiving the standard chlorambucil-obinutuzumab of the Illuminate study excluding those with del(17p).

Ibrutinib was well tolerated in the Resonate-2 trial with limited bone marrow suppression and manageable gastrointestinal symptoms. Long-term follow-up showed that adverse events (AEs) with ibrutinib reduce over time. Most grade 3 or higher AEs were seen in the first 12 months of treatment, consistently, discontinuations and dose reductions were most often needed in the first years. Overall after 5 years of follow-up 21% of patients discontinued ibrutinib due to AEs and 58% of patients are still continuing the BTKi demonstrating the general tolerability of the drug.

Hypertension which is considered an AE of special interest with ibrutinib is well managed. Atrial fibrillation (AF) is common with ibrutinib treatment (10.4%) with a higher incidence compared to the control arms in randomized studies. Treatment discontinuation due to AF is rare overall suggesting that appropriate management and a favourable benefit-risk profile allow patients to continue treatment. The rate of major hemorrhagic episodes was low and decreasing over time.

The matter of ibrutinib tolerability in the elderly unfit population has been analyzed in a posthoc pooled analysis of 3 phase III randomized trials. Patients ≥75 years treated with the BTKi showed not only a benefit for PFS but also a trend to a better OS. Notably, the favourable outcome was maintained in patients with a past medical history of cardiac disorder, tachyarrhythmia, hypertension, infection, or bleeding. No less important, treatment with the BTKi provides a rapid improvement of anemia and disease symptoms which are fundamental in the elderly population. Although there may be a general concern to administer an indefinite continuous treatment with ibrutinib versus a fixed duration chemoimmunotherapy, the favourable outcomes and tolerability in the elderly unfit support the central role of ibrutinib treatment not only in patients with high risk prognostic features.

The role of chemoimmunotherapy has also greatly diminished after the introduction of ibrutinib even in the fit and younger population and has modified the landscape of predictive factors at the time of treatment requirement. In fact the Alliance study and the ECOG study clearly demonstrated the advantage of ibrutinib based treatment over BR or FCR. The Alliance study was addressed to patients over 65 years eligible for bendamustine at 90 mg/sqm treatment. Patients were randomized to receive BR versus ibrutinib monotherapy or in combination with rituximab. Ibrutinib based treatment was superior to chemoimmunotherapy in terms of PFS in the whole population (at 2 years 88-87% with ibrutinib containing regimen versus 74% with BR). Importantly the significant difference was recorded only in the unmutated population underlining the importance of mutational status in decision making. Furthermore PFS survival was longer with the ibrutinib-containing regimens than with bendamustine plus rituximab in all cytogenetic factor-related subgroups, with a greater difference among patients with del(17p). The importance of the mutational status as a predictive factor in first line has also been emphasized by the results of the ECOG study randomizing younger fit patients to receive FCR versus ibrutinib rituximab. Ibrutinib rituximab resulted in a PFS and importantly OS advantage and again no difference in PFS was observed between the two arms in mutated IGHV. These results confirm the role of FCR in the treatment of younger fit patients and it is now established that patients who derive the most long term benefit from first line FCR are those with unmutated IGHV without high risk features at FISH analysis (no del(17p), no del 11q). At the MD Anderson Cancer Center PFS after FCR in mutated IGHV patients resulted approximately 55% at 10 years. Similarly Italian and German studies showed the same outcome plateau of PFS curves suggesting even a possible cure in some patients.

Future directions

Near Future

An important aspect of BTK inhibitors treatment is that it should be administered continuously as with these agents minimal residual disease responses cannot be achieved. This translates in an indefinite duration of treatment with arising issues regarding compliance and costs. This is more significant in the younger population who may remain on treatment for decades.

Unlike BTKi venetoclax induces deep molecular remissions at the same time providing the opportunity of a chemo free regimen with fixed treatment duration.

In the CLL-14 study comparing fixed duration venetoclax obinutuzumab versus chlorambucil obinutuzumab in patients with comorbidities, PFS at 24 months was significantly higher in the venetoclax-obinutuzumab group than in the chlorambucil-obinutuzumab one: 88.2% versus 64.1%. This benefit was also observed in patients with TP53 deletion, mutation, or both, and in patients with unmutated IGHV. Patients treated with the bcl-2 inhibitor reached a high rate of minimal residual disease (MRD) negativity in peripheral blood and bone marrow (75.5% versus 35.2% and 56.9% versus 17.1%, respectively). Considering the high efficacy of venetoclax obinutuzumab combination with a safe toxic profile FDA approved this regimen in first line treatment.

Next Future

To mitigate BTKi specific toxicity new generation BTKi with less off-target effects compared to ibrutinib are under development. Preliminary data on acalabrutinib and zanubrutinib even in first line showed favourable efficacy and safety.

Considering that preclinical investigations have indicated potential synergistic interaction of the combination of venetoclax and ibrutinib, studies with fixed dose regimens are ongoing. In a phase 2 study addressed to high-risk and older patients with at least one high risk feature (del(17p), mutated TP53, del11q, unmutated IGHV, or ≥65 years) ibrutinib and venetoclax were planned for 24 cycles. After 12 cycles of combined treatment, 88% of the patients had complete remission or complete remission with incomplete count recovery, and 61% had remission with undetectable minimal residual disease. Responses were noted in older adults and across all high-risk subgroups. Importantly the adverse-event profile was similar to what has been reported with ibrutinib and venetoclax.

In the future studies will rely on MRD detection to address treatment discontinuation in order to avoid continuous therapy that may translate in an excess of toxicity and resistance development.

Conclusions

Despite the introduction of new targeted agents, treatment should be reserved only to patients showing B symptoms, cytopenia or progressive and significant lymphadenopathy and organomegaly according to the guidelines of the International Workshop on CLL (IWCLL).

Patients carrying del(17p) or TP53 mutation should receive ibrutinib in first line when clinically feasible independently of patients age. Idealisib in combination with rituximab may be an option although toxicity may limit its use. Venetoclax should also be considered in patients unsuitable for BCRi treatment.

For fit patients aged ≤ 65 years non del(17p)/TP53 mut considering that in Italy Ibrutinib is not reimbursed in this setting, FCR treatment should be considered the treatment of choice. If ibrutinib were reimbursable the choice between FCR or the BTKi would depend on mutational status and FISH features. FCR is still a reasonable option for patients with IGHV mutated and without high risk FISH features as del(11q). FCR would not be recommended in unmutated IGHV patients. The risk of secondary MDS/AML should be taken into consideration.

Treatment choice depends on age, comorbidities that may limit chemoimmunotherapy tolerability favoring ibrutinib treatment in elderly patients. Moreover ibrutinib should be the treatment of choice in patients with unmutated IGHV, del(11q)del and bulky disease.

Table 1. Chemoimmunotherapy for CLL.

Study	Pts characteristics **	Regimen*	N/ppts	ORR***	CR***	Follow-up
Hallek et al, 2010	TN Age ≥ 18 CRS ≤ 6	FC vs FCR	817	80 vs 50	22 vs 44	PFS 25.5 vs 47 % at 5 yrs OS 78.7 vs 67 % at 5 yrs
Fischer et al, 2016	TN, Age ≥ 18 CRS score ≤ 6, CrCl ≥ 70 ml/min, del(17p) excluded	FCR vs BR	564	95 vs 96	40.7 vs 31.5	PFS 53.2 vs 41.7 at 37.1 mo OS 90.6 vs 92.2 at 36 mo
Goede et al, 2014	TN, Age ≥ 18	CHL vs CHL-R	781	31.4 vs 85.7	0 vs 7.1	PFS 38.9 CHL-Obi vs 15.7 CHL-R at 59.4mo; 11.1 CHL at 62.5 mo
Goede et al, EHA 2018	CRS >6, CrCl < 70 ml/min	CHL-Obi vs CHL-Obi ¹		77.3 vs 22.8		OS n.s. CHL-Obi vs 73.1 CHL-R at 59.4 mo; 56.7 at 60.5 mo
Hilmen et al, 2015	TN, Age ≥ 18, not eligible to FCR	CHL vs CHL-Ofa	447	59 vs 92	1 vs 14	Median PFS 13.1 mo vs 22.4 mo OS 87 vs 89 at 2 yrs

* TN= treatment naïve, R/R= relapse/refractory. ** ORR= overall response rate, CR= complete response, PFS= progression free survival, OS= overall survival. *** FC= fludarabine, cyclophosphamide; FCR= fludarabine, cyclophosphamide, rituximab; R= rituximab; BR= bendamustine, rituximab; CHL= chlorambucil; Ofa= ofatumumab; CHL-Obi= Chlorambucil, Obinotuzumab.

Table 2. Randomized trials in CLL.

Study	Pts characteristics**	Regimen *	N/ppts	ORR***	CR***	PFS***	OS***
Burger et al, 2015	TN 5-70 yrs or 65-80 yrs eligible for FCR, FCDG 0-2, del(17p) excluded	Ibrutinib vs CHL	269	92% at 60 mo vs 35 at 18-6 mo	30 vs 2	70 vs 12 estimated at 60 mo	88 vs 58 estimated at 60 mo
Mitreva et al, 2019	TN ≥65 yrs or TN < 65 yrs with CR5-6, CrCl<70 ml/min, del(17p), TP53 mut	CHL-Obi vs Ofa-Ibrutinib	229	73 vs 88	8 vs 19	19 vs NA at 11.1 mo	85 vs 88 at 30 mo
Woyach et al, 2018	TN ≥ 65 yrs FCDG 0-2	BR vs Ibrutinib-R vs Ibrutinib	547	81 vs 98 vs 93	28 vs 14 vs 7	18 vs 58 vs 87 estimated at 7 yrs	95 vs 94 vs 90 estimated at 7 yrs
Shanafelt et al, 2018	FN <70 yrs FCDG 0-2 del(17p) excluded	Ibrutinib-R vs FCR	529	95.8 vs 81.5	17.2 vs 30.2	85.4 vs 72.9 at 3 yrs	98.8 vs 91.5 at 3 yrs
Fischer et al, 2019	TN CRS ≤ 6 CrCl < 70 ml/min	Venetoclax- Opi vs CHL-Obi	492	84.7 vs 73.8	49.5 vs 28.1	88.2 vs 84.1 at 28 mo	91.8 vs 88.3 at 28 mo
Jain et al, 2019	At least one of: TN, del(17p), TP53mut, IGHV unmutated, ≥ 65 yrs.	Ibrutinib+ Venetoclax	80	74	74	58 at 12 mo	99 at 12 mo

* TN= treatment naïve, R/R= relapse/refractory. ** ORR= overall response rate, CR= complete response, PFS= progression free survival, OS= overall survival. *** FCR= fludarabine, cyclophosphamide, rituximab; CHL= chlorambucil; R= rituximab; BR= bendamustine, rituximab, CHL-OBI = Obinotuzumab-Chlorambucil.

References

Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet*. 2010;376(9747):1164-1174.
Eichhorst B, Fink AM, Busch R, et al. Frontline Chemoimmunotherapy with Fludarabine (F), Cyclophosphamide (C), and Rituximab (R) (FCR) Shows Superior Efficacy in Comparison to Bendamustine (B) and Rituximab (BR) in Previously Untreated and Physically Fit Patients (pts) with Advanced Chronic Lymphocytic Leukemia (CLL): Final Analysis of an International, Randomized Study of the German CLL

- Study Group (GCLLSG) (CLL10 Study). *Blood*. 2014; 124:19.
- Goede V, Fischer K, Busch R, et al. Obinutuzumab plus Chlorambucil in Patients with CLL and Coexisting Conditions. *N Engl J Med*. 2014; 370:1101-1110.
- Goede V, Fischer K, Dyer, MJ et al. Overall survival benefit of obinutuzumab over rituximab when combined with chlorambucil in patients with chronic lymphocytic leukemia and comorbidities: final survival analysis of the CLL11 study. *EHA Learning Center*. 2018;15: 215923.
- Hillmen P, Robak T, Janssens A, et al. Chlorambucil plus ofatumumab versus chlorambucil alone in previously untreated patients with chronic lymphocytic leukaemia (COMPLEMENT 1): a randomised, multicentre, open-label phase 3 trial. *The Lancet*. 2015; 385(9980), 1873-1883.
- Fisher K, Al-Sawaf O, Bahlo J, et al. Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. *N Engl J Med*. 2019; 380:2225-2236.
- Jain N, Keating M, Thompson P, et al. Ibrutinib and Venetoclax for First-Line Treatment of CLL. *N Engl J Med*. 2019; 380:2095-2103.
- Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med*. 2014;371:213-223.
- Burger JA, Tedeschi A, Barr PM, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med*. 2015;373:2425-2437.
- Tedeschi A, Burger JA, Barr PM, et al. Five-year follow up of patients receiving ibrutinib for first-line treatment of chronic lymphocytic leukemia: S107. *HemaSphere*. 2019; 3:5-6.
- Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. *N Engl J Med*. 2018;378:1107-1120.
- Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-Rituximab or Chemoimmunotherapy for Chronic Lymphocytic Leukemia. *N Engl J Med*. 2019; 381:432-443.
- Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib Alone or in Combination with Rituximab Produces Superior Progression-Free Survival (PFS) Compared with Bendamustine Plus Rituximab in Untreated Older Patients with Chronic Lymphocytic Leukemia (CLL): Results of Alliance North American Intergroup Study A041202. *Blood*. 2018;132:6.

DIRECT ORAL ANTICOAGULANTS AND CANCER-ASSOCIATED THROMBOSIS

V. De Stefano, E. Rossi

Institute of Hematology, Catholic University, Fondazione Policlinico A. Gemelli IRCCS, Rome, Italy

Introduction

Venous thromboembolism (VTE) is a significant cause of both morbidity and mortality in patients with cancer.¹⁻⁴ Active cancer accounts for 20% of the overall incidence of VTE,⁵ and the annual rate of VTE in patients with cancer is 0.5% compared to 0.1% in the general population.⁶ The risk for VTE and recurrent VTE is highest among patients with hematologic malignancies, lung cancer, gastrointestinal cancer (stomach/colon), pancreatic cancer, kidney cancer, and bone cancer, patients with myelodysplastic disorder, and patients with metastases [2,7]. In cancer patients the risk of VTE is amplified in the presence of risk factors such as older age, platelet count $\geq 350 \times 10^9/L$, hemoglobin < 100 g/L or use of red cell growth factors, leukocyte count $\geq 11 \times 10^9/L$, BMI > 35 kg/m², inherited thrombophilia, chemotherapeutic agents, and hormonal therapies.^{2,6,8} Risk predictions models include the Ottawa score for recurrent cancer-associated VTE and the Khorana score for chemotherapy-associated VTE.^{9,10}

Cancer patients with VTE are at increased risk for both bleeding and VTE recurrence.¹¹ According to the results of the CLOT and CATCH trials, low molecular weight heparin (LMWH) for at least 3-6 months is recommended over vitamin K-antagonists (VKA) in cancer-associated VTE, suggesting to treat indefinitely patients with active malignancy and ongoing treatment.^{12,13}

Direct oral anticoagulants (DOACs) for treatment of VTE in cancer patients

DOACs dabigatran, rivaroxaban, apixaban, and edoxaban are approved for the treatment of acute VTE, and the combined six phase-3 trials have included > 1500 patients with active cancer. Subgroup analyses of these patients, either pooled or separately reported, suggest that DOACs could be a safe and efficacious alternative to VKA therapy for the treatment of cancer-associated VTE. Of 771 patients with cancer enrolled in the Hokusai-VTE trial, 378 were assigned to edoxaban and 393 to warfarin. Recurrent VTE occurred in 4% of the patients given edoxaban and in 7% of the patients given warfarin (hazard ratio [HR] 0.53, 95%CI 0.28-1.00, $p=0.0007$). Clinically relevant bleeding (major or non-major) occurred in 12% of the patients who received edoxaban and in 19% of the patients who received warfarin; the HR for clinically relevant bleeding was 0.4, 95%CI 0.45-0.92, $p=0.017$. Therefore, edoxaban was found non-inferior to warfarin for the treatment of cancer

patients with VTE, and with less clinically relevant bleeding.¹⁴ The only additional data on DOAC use in cancer patients come from pooled analyses. In one analysis, 514 patients with active cancer who were treated with a DOAC were compared to 459 treated with a VKA. The pooled incidence rate of recurrent VTE during DOAC therapy was 4.1% compared to 6.1% with VKAs (HR 0.66, 95% CI 0.38-1.2). The rate of major and clinically-relevant non-major bleeding was similar in both groups (15% vs 16%, RR 0.94, 95% CI 0.7-1.3).¹⁵ A pooled analysis examining only dabigatran therapy found similar results.¹⁶

Specific data from three direct head-to-head comparisons of DOACs with LMWHs are currently available (Table 1).

Table 1. Summary of major trials comparing DOACs with LMWH. Six-month study outcomes are reported for all trials. VTE, venous thromboembolism.

Trial	VTE recurrence (%)	Major bleeding (%)	Clinically relevant non-major bleeding (%)	Mortality (%)
Hokusai VTE Cancer [17]	7.9 vs 11.3	6.9 vs 4.0	14.6 vs 11.1	89.5 vs 36.6
Edoxaban vs Dalteparin	Edoxaban was non-inferior to dalteparin in combined outcome of VTE recurrence or major bleeding. The rate of major bleeding occurred more often in the edoxaban group ($p=0.04$). Rates of non-major bleeding and mortality were not significantly different.			
SELECT-D [18]	4 vs 11	6 vs 4	13 vs 4	25 vs 30
Rivaroxaban vs Dalteparin	Rivaroxaban had a significantly lower rate of VTE recurrence (hazard ratio 0.43, 95% CI 0.19-0.99). Rates of major bleeding and mortality were not significantly different; the rate of non-major bleeding was significantly higher in the rivaroxaban arm (hazard ratio 3.76, 95% CI 1.63-8.69).			
ADAM VTE [19]	3.4 vs 14.1	0.0 vs 2.1	6.2 vs 4.2	15.9 vs 10.6
Apixaban vs Dalteparin	Significant reduction in VTE recurrence with apixaban (hazard ratio 0.21, 95%CI 0.09-0.47, $p=0.0182$). No significant difference in bleeding rates or mortality			

Hokusai VTE Cancer trial

This study randomized 1,050 cancer patients with proximal deep vein thrombosis (DVT), acute symptomatic pulmonary embolism (PE), or incidental PE to 5 days of LMWH followed by either oral edoxaban (60 mg once daily) or subcutaneous dalteparin (200 IU/kg once daily for 1 month, then 150 IU/kg once daily) for a period of 6-12 months [17]. The study utilized a composite primary outcome of recurrent VTE or major bleeding. Edoxaban was non-inferior to dalteparin (12.8% vs 13.5%, $p=0.006$ for non-inferiority). VTE recurrence was not significantly different between edoxaban and dalteparin (7.9% vs. 11.3%, $p=0.09$). Major bleeding occurred more often in the edoxaban group compared with dalteparin (6.9% vs 4.0%, $p=0.04$). Clinically relevant non-major bleeding (CRNMB) was also higher in the edoxaban arm (14.6%) compared with the dalteparin arm (11.1%), but this difference did not reach statistical significance. Overall survival was similar between the two groups.

SELECT-D trial

This study compared rivaroxaban (15 mg twice daily for 3 weeks, then 20 mg daily for a total of 6 months) with dalteparin (200 IU/kg daily for 1 month, then 150 IU/kg daily for 5 months) in 406 patients with active cancer and PE (symptomatic or incidental) or proximal DVT [18]. The primary outcome was VTE recurrence. Rivaroxaban had a lower rate of VTE recurrence than dalteparin (4% vs 11%, HR 0.43, 95% CI 0.19-0.99). Rates of major bleeding were not significantly different (4% vs. 6%, HR 1.83, 95% CI 0.68-4.96). However, CRNMB rates were significantly higher in the rivaroxaban arm (13% vs. 4%, HR 3.74, 95% CI 1.63-8.69). Overall survival was similar between the two groups.

ADAM VTE trial

The ADAM VTE trial is the first study comparing a DOAC to LMWH for treatment of cancer-associated VTE that had major bleeding

as its primary outcome, with VTE recurrence and a composite of major bleeding plus CRNMB as secondary outcomes. This study was published in abstract form in 2018.¹⁹ This trial compared apixaban (10 mg twice daily followed by 5 mg twice daily) with dalteparin (200 IU/kg daily for 1 month followed by 150 IU/kg once daily) for 6 months in 300 patients with cancer-associated VTE. Apixaban was associated with significantly lower VTE recurrence compared with dalteparin (3.4% vs 14.1%, HR 0.21, 95% CI 0.09–0.47, $p=0.0182$). Major bleeding was not significantly different between the apixaban and dalteparin arms (0.0% vs 2.1%, $p=0.99$), nor was the combined major bleeding and CRNMB rate (6.2% vs. 6.3%, HR 0.91, 95% CI 0.41–1.94, $p=0.88$). Overall survival was similar between the two groups.

Unlike the pivotal trials comparing DOACs to VKAs in the general population, these trials had strict inclusion criteria for patients with active cancer. The results of these three studies suggest that DOACs are at least non-inferior to LMWH for preventing VTE recurrence in the cancer population. This conclusion has been corroborated by recent meta-analyses.^{20,21} In a cohort study on 1698 cases of gynecological cancers, 107 (6.3%) cases had VTE; 54 cases were treated with DOACs and 53 with VKA. Three patients on VKA (5.7%) developed recurrent VTE, and one patient on DOACs (1.9%) showed clinically relevant bleeding. DOACs were non-inferior to VKA with respect to the composite outcome, including recurrent VTE and relevant bleeding (hazard ratio 0.31, 95% CI 0.03–3.12, $p=0.363$).²²

However, both Hokusai VTE Cancer and SELECT-D, as well as the meta-analyses, observed increased bleeding events, including numerous gastrointestinal bleeds, in the DOAC arms. Many of the bleeding events occurred in patients with esophageal and gastric cancers.^{17,18}

Ongoing randomized trials examining DOACs for cancer-associated thrombosis will help to clarify many outstanding questions. These trials include the Caravaggio study (NCT03045406), comparing apixaban with dalteparin; the CANVAS trial (NCT02744092), comparing DOAC therapy (rivaroxaban, apixaban, edoxaban, or dabigatran, by investigator's choice) with LMWH with or without a transition to warfarin; the CONKO-0211 (NCT02583191) and CASTA-DIVA (NCT02746185) studies, comparing rivaroxaban with LMWH; and the single-arm CAP study (NCT02581176), investigating the use of apixaban as treatment for cancer-associated thrombosis. Information obtained from these trials will further inform clinicians regarding the efficacy and safety profile of DOACs as treatment of cancer-associated VTE.

Direct oral anticoagulants (DOACs) for primary prevention of VTE in cancer patients.

Recent clinical trials (summarized in Table 2) have examined the utility of DOACs, in conjunction with clinical prediction scores, for primary thromboprophylaxis in high-risk cancer patients.

Table 2. Summary of published trials for primary thromboprophylaxis in high-risk cancer comparing DOACs with LMWH. VTE, venous thromboembolism.

Trial	VTE recurrence (%)	Major bleeding (%)	Clinically relevant non-major bleeding (%)	Mortality (%)
AVERT [23]	4.2 vs 10.2	3.5 vs 1.8	7.3 vs 5.5	12.2 vs 9.8
Apixaban vs placebo	Apixaban significantly reduced the rate of VTE (hazard ratio 0.41, 95%CI 0.26-0.65). Major bleeding was higher in the intention-to-treat analysis (hazard ratio 2.0, 95%CI 1.01-3.95). There was no difference in non-major bleeding.			
CASSINI [24]	2.60 vs 6.41	1.38 vs 0.99	2.72 vs 1.38	20.0 vs 23.8
Rivaroxaban vs placebo	Rivaroxaban significantly reduced the rate of VTE (hazard ratio 0.40, 95%CI 0.20-0.80). Lower rate of VTE recurrence (hazard ratio 0.43, 95% CI 0.19-0.99). Rates of major bleeding and non-major bleeding were not significantly different.			

AVERT trial

The AVERT trial²³ is a randomized, placebo-controlled, double-blind clinical trial comparing 6 months of apixaban (2.5 mg twice daily) with placebo in 574 patients with a Khorana score ≥ 2 . Patients with condi-

tions predicting an increased risk of significant bleeding, hepatic dysfunction, renal insufficiency, and thrombocytopenia were excluded. The primary efficacy outcome of VTE occurrence rate was lower in the apixaban group compared with the placebo group (4.2% vs. 10.2%, HR 0.41, 95% CI 0.26–0.65, $p<0.001$, number needed to treat of 17). Major bleeding was higher in the apixaban group compared with the placebo group (3.5% vs 1.8%, HR 2.00, 95% CI 1.01–3.95, $p=0.046$) in the intention-to-treat analysis, although analysis limited to the treatment period (median duration 157 days vs. 155 days) showed that the bleeding rate was not significantly higher (2.1% vs 1.1%, HR 1.89, 95% CI 0.39 to 9.24, number needed to harm of 100). There was no difference in non-major bleeding or mortality. No fatal bleeds or bleeds into critical organs occurred.

Cassini trial

The CASSINI trial²⁴ is a randomized, placebo-controlled, double-blind clinical trial comparing 6 months of rivaroxaban (10 mg once daily) with placebo in 841 adult ambulatory patients with a Khorana score ≥ 2 . Notably, during the screening period of this trial, compression limb ultrasound was performed, and patients with evidence of DVT on ultrasound examination were excluded. During the on-treatment period, rivaroxaban significantly reduced the primary endpoint of VTE or VTE-related death compared with placebo (2.62% vs. 6.41%, HR 0.40, 95% CI 0.20 to 0.80, $p=0.007$, number needed to treat of 26). However, there was no significant difference in the primary endpoint between the groups in the analysis of the full observation period (180 days). Rates of major bleeding were low (1.98% vs 0.99%, HR 1.96, 95% CI 0.59 to 6.49, $p=0.265$, number needed to harm of 101), as were rates of CRNMB (2.72 vs 1.98%, HR 1.34, 95% CI 0.54 to 3.32, $p=0.53$, number needed to harm of 135).

The results of these studies suggest that apixaban and rivaroxaban as primary thromboprophylaxis in high-risk patients may reduce the rate of VTE with largely favorable safety profiles, although these studies excluded patients with baseline organ dysfunction, which is common in real-life cancer patients.

DOACs for prevention or treatment of VTE in patients with acute leukemia

Direct thrombin inhibitors (gatrans) (e.g., dabigatran) or factor Xa inhibitors (xabans) (e.g., rivaroxaban, apixaban, edoxaban) do not rely on antithrombin (AT) for the inhibition of coagulation. This could be of great interest during the imbalance in hemostasis towards thrombosis and the particular decreases in AT levels occurring during asparaginase treatment in patients with acute lymphoid leukemia (ALL).²⁵

A phase III, multicenter, international, open-label, study has been launched, that randomizes pediatric patients (ages 1 to <18 years) with ALL or lymphoma treated with asparaginase to either the placebo group or the intervention group (Table 3). Patients who are 2 years old and above weighing less than 35 kg are given 0.07 mg/kg twice daily of apixaban as a 0.4 mg/ml solution and patients weighing ≥ 35 kg are given a 2.5-mg tablet twice daily. The primary endpoint of this study is a composite of non-fatal deep venous thrombosis, pulmonary embolism, cerebral venous sinus thrombosis, and VTE-related death up to 1 month after therapy. The researchers are planning to recruit a total of 700 subjects.

DOACs for prevention or treatment of VTE in patients with multiple myeloma

Multiple myeloma is associated with an increased risk of VTE. Risk factors can be patient-related, disease-related, and treatment-related.²⁶ The 1 to 2% baseline of incident VTE associated with conventional therapies as melphalan and prednisone is at least doubled by the use of doxorubicin or other chemotherapeutic agents. The VTE rate associated with immunomodulatory drugs (IMiDs) thalidomide or lenalidomide as monotherapy is similar, whereas combination with high-dose dexamethasone or multiple chemotherapeutic agents induces a multiplicative effect on the VTE rate up to 25%. LMWH, fixed low-dose warfarin, and aspirin are acceptable strategies for antithrombotic prophylaxis, reducing VTE to 5 to 8% in thalidomide-treated patients and 1 to 3% in lenalidomide-treated patients.²⁶ In a survey conducted in Ireland, most physicians involved in the treatment of patients with multiple myeloma employed LMWH or aspirin (82% and 71%, respectively). However, 3

of 28 (11%) had used dabigatran or rivaroxaban despite there was little evidence to support their use.²⁷

One population-based study extracted data from the French health insurance scheme database (SNIIRAM, Système National d'Information Inter-Régime de l'Assurance Maladie) for the Midi-Pyrénées area (South West France, 2.8 million inhabitants). In a cohort of 236 patients with multiple myeloma newly diagnosed from January 2012 to September 2013, 77 (32.6%) received antithrombotic prophylaxis for VTE: the most represented drugs were aspirin, LMWH, and VKA; only one patient (1.3%) received rivaroxaban.²⁸

In one retrospective survey 70 patients with multiple myeloma d who underwent first line therapy with IMiDs containing regimens received apixaban 2.5 mg twice daily for the first 4 months of therapy or until treatment was completed – whichever was shorter, and for a maximum of 6 months. Patients with severe renal failure (creatinine clearance <15 ml/min) or thrombocytopenia received alternative anticoagulants and were excluded from this study. No patients had venous thrombosis within 6 months of treatment initiation. Two patients (2.9%) had arterial thrombosis. One patient (1.4%) had major gastrointestinal bleeding [29]. In the MYELAXAT trial, myeloma patients requiring Melphalan-Prednisone-Thalidomide in first line, or Lenalidomide-Dexamethasone in relapse were enrolled between 2014 - 2016. All patients received apixaban, 2.5 mg x 2/day for 6 months as primary prophylaxis for VTE. Out of 104 patients, two VTE events were registered, i.e., an asymptomatic proximal deep vein thrombosis (DVT) and a symptomatic distal DVT; in the latter case, apixaban was stopped 14 days before. Only one major bleeding was reported.³⁰ Thus, apixaban used in a preventive scheme seems to be efficient and safe in preventing VTE in myeloma patients treated with IMiDs.^{29,30}

Other trials with apixaban in patients with multiple myeloma have been just completed or are ongoing (Table 3).

Table 3. DOACs (apixaban) trials in patients with hematological malignancies. DVT: deep venous thrombosis; VTE: venous thromboembolism.

Trial	Chalcrisks/ger identifier	Disease	Active arm	Comparator	Time frame	Primary outcomes
A phase III randomized, open label, multicenter study of the safety and efficacy of apixaban for thromboembolism prevention versus no systemic anticoagulant prophylaxis during induction chemotherapy in children with newly diagnosed acute lymphoblastic leukemia (ALL) or lymphoma (7 or 8 cell) treated with pegylated liposomation.	NCT02506553 (January 2015)	Lymphoma Acute Lymphoblastic Leukemia	Children assigned to the apixaban arm (<55 kg will get a dose of 0.07 mg/kg twice a day with the 0.4 mg/ml solution for 25-28 days)	Placebo No systemic anticoagulant prophylaxis	3 months	A composite of additional non-fatal DVT (either asymptomatic and symptomatic), pulmonary embolism, and cerebral venous thrombosis and VTE-related death Major bleeding
Evaluation of the use of an oral direct anti-Xa anticoagulant, apixaban, in prevention of venous thromboembolic disease in patients treated with IMiDs during myeloma: a pilot study	NCT02066454 (February 2014)	Multiple Myeloma	Apixaban 2.5mg x 2 per day for 6 months	Single arm	6 months	Total VTE and VTE-related death. Major and clinically relevant non-major bleeding
A pilot study investigating apixaban and desferrioxamine reduction in multiple myeloma	NCT02496177 (March 2016)	Multiple Myeloma	Apixaban 2.5mg x 2 per day for 3 months	Single arm	3 months	Anti Xa activity (measure of level of prophylaxis provided by apixaban)
Apixaban for primary prevention of venous thromboembolism in patients with multiple myeloma receiving immunomodulatory therapy	NCT02589661 (November 2016)	Multiple Myeloma	Apixaban 2.5 mg x 2 per day for 6 months	Placebo	6 months	Symptomatic VTE Major and clinically relevant non-major bleeding

DOACs for treatment of VTE in patients with Philadelphia-negative myeloproliferative neoplasms

In patients with myeloproliferative neoplasms, VKA treatment is highly effective in preventing recurrent VTE.³¹⁻³³ In a single-center registry of 760 MPN patients, 25 (3.3%) were treated with a DOAC. The reasons for prescribing DOACs were atrial fibrillation and thrombotic events for 13 and 12 patients, respectively.³⁴ In the German MPN registry of the Study Alliance Leukemia, 68 out of 454 patients (14.9%) had suffered from DVT or splanchnic vein thrombosis; only 8 patients (1.7% of the cohort) were treated with rivaroxaban. However, multivariate analysis revealed an odds ratio of major bleeding for patients on rivaroxaban of 1.61 (non-significant), which is lower than those for patients on VKA therapy (1.97), double platelet inhibition (3.05) and heparin (5.64, the only drug with a significant odds ratio for major bleeding).³⁵

In a monocenter retrospective survey, DOACs resulted of limited efficacy in MPN patients with thrombosis of cerebral or splanchnic veins,³⁶ further multicenter studies aimed to assess efficacy and safety

of DOACs in this setting on a statistically powered patient sample size are urgently needed.

Conclusions

The use of DOACs in the primary and secondary prevention of VTE in patients with cancer or hematological malignancies is promising. Cancer patients have increased risks of bleeding and VTE recurrence relative to other patients with VTE. While DOACs have emerged as an attractive alternative to LMWH, there may be an excessive bleeding risk in certain patient groups, particularly those with gastrointestinal malignancies. Recent studies have demonstrated that DOACs may be effective as primary thromboprophylaxis in high-risk cancer patients.

Some limitations in the field of malignant hematology can be anticipated. Patients on DOACs do not need routine coagulation monitoring, but in some challenging patients with hematologic malignancies drug titration could be necessary, such as patients with chemotherapy-induced or disease-related severe thrombocytopenia where anticoagulation is at high risk of bleeding.³⁷ In this setting LMWH dose-reduction according to the platelet count has been proposed by some experts,^{38,39} but the adoption of a similar strategy for DOACs is quite unexplored. The dosing regimen and dose adjustments for renal impairment are DOAC specific and could be critical in patients with multiple myeloma. Finally, the relative lack of drug interactions in DOACs is an advantage, but clinicians must be aware of the few critical drug interactions with some anti-fungal, anti-microbial and anti-viral medications involving the Cytochrome P450 3A4 and the P-glycoprotein metabolic pathways and largely employed by hematologists.⁴⁰ In conclusion, DOACs are effective and safe in the majority of patients with cancer, but controlled clinical trials targeted to patients with hematological neoplasms are urgently needed to explore advantages and pitfalls in this particular cancer setting.

References

- Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. *Arch Intern Med* 2000; 160: 809-15.
- Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *JAMA* 2005; 293: 715-22.
- Chew HK, Wun T, Harvey D, Zhou H, White RH. Incidence of venous thromboembolism and its effect on survival among patients with common cancers. *Arch Intern Med* 2006; 166: 458-64.
- Sorensen HT, Mellemkjaer L, Olsen JH, Baron JA. Prognosis of cancers associated with venous thromboembolism. *N Engl J Med* 2000; 343: 1846-50
- Heit JA, Spencer FA, White RH. The epidemiology of venous thromboembolism. *J Thromb Thromboysis* 2016;41:3-14.
- Elyamany G, Alzahran AM, Bukhary E. Cancer-associated thrombosis: an overview. *Clin Med Insights Oncol* 2014;8:129-37.
- Bick RL. Cancer-associated thrombosis. *N Engl J Med* 2003;349:109-11.
- Streff MB, Bockenstedt PL, Cataland SR, et al. Venous thromboembolic disease. *J Natl Compr Canc Netw* 2013;11:1402-29.
- Louzada ML, Carrier M, Lazo-Langner A, et al. Development of a clinical prediction rule for risk stratification of recurrent venous thromboembolism in patients with cancer-associated venous thromboembolism. *Circulation* 2012; 126: 448-54.
- Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008; 111: 4902-7.
- Prandoni P, Lensing AW, Piccioli A, et al. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. *Blood* 2002; 100: 3484-8.
- Lee AYY, Levine MN, Baker RI, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N Engl J Med* 2003;349:146-53.
- Lee AYY, Kamphuisen PW, Meyer G, et al. Tinzaparin vs warfarin for treatment of acute venous thromboembolism in patients with active cancer: a randomized clinical trial. *JAMA* 2015; 314: 677-86.
- Raskob GE, van Es N, Segers A, et al. Edoxaban for venous thromboembolism in patients with cancer: results from a non-inferiority subgroup analysis of the Hokusai-VTE randomised, double-blind, double-dummy trial. *Lancet Haematol* 2016; 3: e379-87.
- van der Hulle T, Den Exter PL, Kooiman J, van der Hoeven JJ, Huisman MV, Klok FA. Meta-analysis of the efficacy and safety of new oral anticoagulants in patients with cancer-associated acute venous thromboembolism. *J Thromb Haemost* 2014; 12: 1116-20.
- Schulman S, Goldhaber SZ, Kearon C, et al. Treatment with dabigatran or warfarin in patients with venous thromboembolism and cancer. *Thromb Haemost* 2015; 114: 150-7.
- Raskob GE, van Es N, Verhamme P, et al. Edoxaban for the treatment of cancer-associated venous thromboembolism. *N Engl J Med* 2018; 378: 615-24.
- Young AM, Marshall A, Thirlwall J, et al. Comparison of an oral factor Xa inhibitor with low molecular weight heparin in patients with cancer with venous throm-

- boembolism: results of a randomized trial (SELECT-D). *J Clin Oncol* 2018; 36: 2017-23.
19. McBane RD, Wysokinski W, Le-Rademacher J, et al. Apixaban, dalteparin, in active cancer associated venous thromboembolism, the ADAM VTE trial. *Blood* 2018; 132 (suppl. 1): 421 [abstract].
 20. Li A, Garcia DA, Lyman GH, Carrier M. Direct oral anticoagulant (DOAC) versus low-molecular-weight heparin (LMWH) for treatment of cancer associated thrombosis (CAT): a systematic review and meta-analysis. *Thromb Res* 2019; 173: 158-163.
 21. Rossel A, Robert-Ebadi H, Combescure C, et al. Anticoagulant therapy for acute venous thrombo-embolism in cancer patients: a systematic review and network meta-analysis. *PLoS One* 2019; 14: e0213940.
 22. Shimizu A, Sawada K, Shiomi M, et al. Direct oral anticoagulants are effective and safe for the treatment of venous thromboembolism associated with gynecological cancers. *Int J Gynaecol Obstet*. 2019 Aug 16. doi:10.1002/ijgo.12944. [Epub ahead of print]
 23. Carrier M, Abou-Nassar K, Mallick R, et al. Apixaban to prevent venous thromboembolism in patients with cancer. *N Engl J Med* 2019; 380: 711-719.
 24. Khorana AA, Soff GA, Kakkar AK, et al. Rivaroxaban for thromboprophylaxis in high-risk ambulatory patients with cancer. *N Engl J Med* 2019; 380: 720-728.
 25. De Stefano V, Za T, Cimminello A, Betti S, Rossi E. Haemostatic alterations induced by treatment with asparaginases and clinical consequences. *Thromb Haemost* 2015; 113: 247-61.
 26. De Stefano V, Za T, Rossi E. Venous thromboembolism in multiple myeloma. *Semin Thromb Hemost* 2014; 40: 338-47.
 27. Crowley MP, Kevane B, Eustace JA, O'Shea S, Gilligan O. Thromboprophylaxis in myeloma: what is happening outside of clinical trials? *Ir Med J* 2014; 107: 281-4.
 28. Palmaro A, Rougé-Bugat ME, Gauthier M, Despas F, Moulis G, Lapeyre-Mestre M. Real-life practices for preventing venous thromboembolism in multiple myeloma patients: a cohort study from the French health insurance database. *Pharmacoepidemiol Drug Saf* 2017; 26: 578-86.
 29. Storrar NPF, Mathur A, Johnson PRE, Roddie PH. Safety and efficacy of apixaban for routine thromboprophylaxis in myeloma patients treated with thalidomide- and lenalidomide-containing regimens. *Br J Haematol* 2019; 185: 142-144.
 30. Pegourie B, Karlin L, Benboukber L et al. Apixaban for the prevention of thromboembolism in immunomodulatory-treated myeloma patients: Myelaxat, a phase 2 pilot study. *Am J Hematol* 2019; 94: 635-640.
 31. De Stefano V, Za T, Rossi E, et al. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. *Haematologica* 2008; 93: 372-80.
 32. Hernández-Boluda JC, Arellano-Rodrigo E, Cervantes F, et al. Oral anticoagulation to prevent thrombosis recurrence in polycythemia vera and essential thrombocythemia. *Ann Hematol* 2015; 94: 911-18.
 33. De Stefano V, Ruggeri M, Cervantes F, et al. High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists. *Leukemia* 2016; 30: 2032-8.
 34. Ianotto JC, Couturier MA, Galinat H, et al. Administration of direct oral anticoagulants in patients with myeloproliferative neoplasms. *Int J Hematol* 2017 Jun 16. doi: 10.1007/s12185-017-2282-5. [Epub ahead of print]
 35. Kaifie A, Kirschner M, Wolf D, et al. Bleeding, thrombosis, and anticoagulation in myeloproliferative neoplasms (MPN): analysis from the German SAL-MPN-registry. *J Hematol Oncol* 2016; 9: 18.
 36. De Stefano V, Betti S, Za T, et al. Do direct oral anticoagulants provide a limited protection against recurrences in MPN patients with venous thromboembolism? 47th Congress of the Italian Society of Hematology, Rome 7-10 October 2019, abstract P151.
 37. Houghton DE, Key NS, Zakai NA, Laux JP, Shea TC, Moll S. Analysis of anticoagulation strategies for venous thromboembolism during severe thrombocytopenia in patients with hematologic malignancies: a retrospective cohort. *Leuk Lymphoma* 2017; 9: 1-9.
 38. Giordano P, Del Vecchio GC, Saracco P, Zecca M, Molinari AC, De Mattia D; Coagulation Defects AIEOP Working Group. A practical approach to diagnosis and treatment of symptomatic thromboembolic events in children with acute lymphoblastic leukemia: recommendations of the "Coagulation Defects" AIEOP Working Group. *Recent Pat Cardiovasc Drug Discov* 2007; 2: 53-62.
 39. Falanga A, Rickles FR. Management of Thrombohemorrhagic Syndromes (THS) in hematologic malignancies. *Hematology Am Soc Hematol Educ Program* 2007: 165-71.
 40. Di Minno A, Frigerio B, Spadarella G, et al. Old and new oral anticoagulants: Food, herbal medicines and drug interactions. *Blood Rev* 2017; 31: 193-203.

PATHOPHYSIOLOGY AND CLONAL EVOLUTION OF MYELOYDYSPLASTIC SYNDROMES

L. Malcovati

Department of Molecular Medicine, University of Pavia & Department of Hematology, IRCCS Fondazione Policlinico S. Matteo, Pavia.

Introduction

Myelodysplastic syndromes (MDS) are defined in the World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues as a group of clonal hematopoietic stem cell diseases characterized by cytopenia, dysplasia in one or more of the major myeloid cell lines, ineffective hematopoiesis, and increased risk of progression to acute myeloid leukemia (AML).

In the last few years, major advances have been made in the understanding of the genetic basis of MDS, and recurrent somatic mutations have been identified in several genes. This information has been contributing to unravel the pathophysiology of these disorders, and to open avenues of research leading to novel diagnostic and prognostic tools, and in perspective to novel therapeutic options.

Current concept of the pathophysiology of MDS: clonal expansion of myelodysplastic stem cell, ineffective hematopoiesis, and leukemic transformation

According to the current understanding of the biology of MDS, the occurrence of one or more somatic mutations in a hematopoietic stem cell provides survival and growth advantage, leading to expansion of a clone. Once the myelodysplastic clone has become fully dominant in the bone marrow, the disease may become clinically apparent as a consequence of abnormal differentiation/maturation of clonal hematopoietic progenitors and precursors. Then, the acquisition of subclonal driver mutations involves the expansion of subclones with increasingly impaired differentiation/maturation, eventually leading to progression into acute leukemia.¹ According to this working model, the somatic mutation responsible for gain-of-function at the stem cell level involves loss-of-function in hematopoietic precursors; alternatively, myelodysplasia may result from a combination of somatic mutation(s) that provide a proliferative advantage to the stem cell and mutation(s) responsible for the defective differentiation/maturation.

Spectrum of driver genetic lesions in MDS

Recurrent chromosomal abnormalities are detected in about half of patients with MDS, including del(5q), trisomy 8, del(20q), and monosomy 7 or del(7q).⁷ Most of these chromosomal abnormalities are likely secondary genetic events, deriving from genome instability. The only exception known so far is del(5q), which has been demonstrated to precede recurrent driver mutations in most cases, compatible with del(5q) being the initiating genomic lesion of the MDS associated with del(5q).

Recent studies performed comprehensive analyses of known or candidate genes relevant in myeloid neoplasms using massive parallel sequencing, and showed that up to 90% of MDS patients carry one or more oncogenic mutations (Table 1). Driver mutant genes include those of RNA splicing, DNA methylation, histone modification, transcription regulation, DNA repair, signal transduction, and cohesin complex. However, only 4 to 6 driver mutated genes, in addition to del(5q) cytogenetic abnormality, recur in a substantial fraction of MDS patients. These lesions typically occur earlier during clonal ontogeny, likely representing initiating events of the tumor process. Notably, the most frequently mutated genes are selective involved in two biological pathways, namely epigenetic regulation and RNA splicing.² The relative contribution of these distinct pathways to the pathogenesis of MDS, however, appear to be distinct. In fact, the available evidence is suggesting that mutations in epigenetic regulators involve the expansion of hematopoietic clones with the potential to progress in a broad range of myeloid neoplasms and the additional cooperative lesions drive the trajectory of evolution. Conversely del(5q) and spliceosome mutations appear to be selectively associated with myelodysplasia, and several lines of evidence support the concept that haploinsufficiency of a combination of key genes mapping to the commonly deleted region on the long arm of chromosome 5 or multiple splicing alterations consequent to splicing factor mutations may cooperatively contribute to the pathogenesis of MDS.

Driver mutations in epigenetic regulation genes, age-related clonal hematopoiesis and progression to MDS

Recent studies provided consistent evidence of age-related

hematopoietic clones in individuals who were unselected for cancer or hematologic phenotypes. These clones were found in up to 10% of persons 70 to 79 years of age, and 20% of persons 90 years of age or older. Persons with clonal hematopoiesis had a significantly higher risk of developing hematologic cancers compared to subjects without any detectable putative somatic mutations. This risk was further increased among persons with higher variant allele frequencies.³

Remarkably, detectable clonal expansions most frequently involve somatic mutations in three genes implicated in epigenetic regulation, *DNMT3A*, *TET2*, and *ASXL1*. The vast majority of subjects carrying detectable mutations had only one mutation in the set of examined genes, supporting the hypothesis that these persons had clones harboring only an initiating lesion.³

According to a multistep somatic genetic process, the occurrence of additional cooperating mutations is then resulting in the transformation of the hematopoietic cell and in the expansion of a malignant clone. A recent study on subject with unexplained anemia or cytopenia, including early manifestation of myelodysplasia, showed that mutations of *RUNX1*, *EZH2*, *CBL*, *TP53*, *NRAS*, *CUX1* or *IDH1/2* were the most frequent comutation associations, resulting in overt MDS (Figure 1).⁴

Table 1. Most frequently mutated genes in MDS and secondary AML.

Gene	Location	Ontology	Mutation frequency (%)	
			MDS	sAML
<i>SF3B1</i>	2q33	RNA splicing	28	11
<i>TET2</i>	4q24	DNA methylation	28	20
<i>ASXL1</i>	20q11	Histone modification	18	32
<i>SRSF2</i>	17q25	RNA splicing	15	20
<i>DNMT3A</i>	2p23	DNA Methylation	11	19
<i>RUNX1</i>	21q22	Transcription regulation	9	31
<i>U2AF1</i>	21q22	RNA splicing	8	16
<i>TP53</i>	17p13	DNA repair	8	15
<i>STAG2</i>	Xq25	Cohesin complex	6	14
<i>EZH2</i>	7q35-q36	Histone modification	5	9
<i>ZRSR2</i>	Xp22	RNA splicing	5	8
<i>CBL</i>	11q23	Signal transduction	4	5
<i>IDH2</i>	15q26	DNA methylation	4	11
<i>NRAS</i>	1p13	Signal transduction	4	23
<i>BCOR</i>	Xp11	Transcription regulation	4	8
<i>CUX1</i>	7q22	Transcription regulation	3	14
<i>JAK2</i>	9p24	Signal transduction	3	<1
<i>ETV6</i>	12p13	Transcription regulation	2	1
<i>IDH1</i>	2q33	DNA methylation	2	11
<i>KRAS</i>	12p12	Signal transduction	2	8
<i>PHF6</i>	Xq26	Transcription regulation	2	<1
<i>SETBP1</i>	18q21	Transcription regulation	2	5
<i>MPL</i>	1p34	Signal transduction	1	<1
<i>NF1</i>	17q11	Signal transduction	1	6
<i>PTPN11</i>	12q24	Signal transduction	1	5
<i>GATA2</i>	3q21	Transcription regulation	1	2
<i>NPM1</i>	5q35	Transcription regulation	1	5

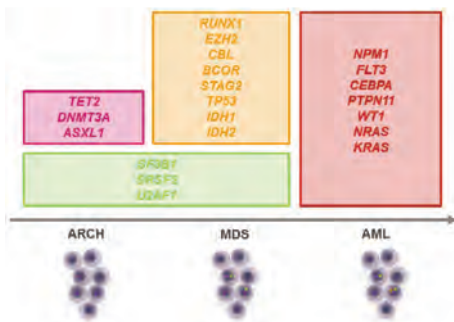


Figure 1. Gene mutations have stereotyped positions in the MDS clonal hierarchy. Several variables, either intrinsic to the clone, including founding somatic mutations or germline predisposition, or related to the context, including bone marrow microenvironment and immune abnormalities or exposure to cytotoxic therapies, contribute to shape the trajectories of clonal evolution of MDS, through constraints on the repertoire of subclonal genetic lesions or a Darwinian selection of subclones.

Haploinsufficiency of multiple deleted genes promotes MDS with *del(5q)*

The deletion of the long arm of chromosome 5 is the most common cytogenetic abnormality reported in MDS, and associated with a typical hematologic phenotype including macrocytic anemia, normal or elevated platelet count with hypolobated megakaryocytes, and a lower rate of progression to AML, currently recognized by the WHO classification as a distinct disease subtype.

The molecular basis of this MDS subtype was identified as the haploinsufficiency of selected genes that map in the common deleted region. These include the *RPS14* gene, which leads to activation of the p53 pathway and the macrocytic anemia characteristic of this disorder, miRNA-145 and miRNA-146a contributing to aberrant megakaryopoiesis, and *CSNK1A1* that induces hematopoietic stem cell expansion and a competitive repopulation advantage.⁵

This chromosomal deletion usually represent the founding genetic lesion of the MDS with isolated *del(5q)*. In addition, the available evidence is suggesting that it may represent the only genetic lesion required to propagate this MDS subtype.⁶ Accordingly, the WHO classification recognizes *del(5q)* as a disease-defining genetic lesion, providing presumptive evidence of MDS even in the absence of significant bone marrow dysplasia.

Spliceosome mutations: a direct link between abnormal splicing and myelodysplasia

More than 50% of patients with MDS carry somatic mutations in genes encoding splicing factors involved in the U2 small nuclear ribonucleoprotein complex, including *SF3B1*, *SRSF2* and *U2AF1*. Significant genotype-phenotype correlations have been established in patients carrying spliceosome mutations, *SF3B1* mutations being strongly associated with the occurrence of bone marrow ring sideroblasts, whereas *SRSF2* being significantly enriched in chronic myelomonocytic leukemia and multilineage dysplasia or excess blasts.⁷

Several lines of evidence consistently showed that spliceosome mutations may act as an initiating event. In particular, mutational analysis of the *SF3B1* gene in hematopoietic stem/progenitor cells demonstrated that the *SF3B1* mutation may occur alone or is the first event in most cases, whereas appears to be secondary to other oncogenic mutations in a minority of cases. In these latter, most frequently *SF3B1* mutations are occurring on the background of age-related hematopoietic clones carrying *TET2* or, less frequently, *DNMT3A* or *ASXL1* mutation.

RNA sequencing studies in hematopoietic cells carrying spliceosome mutations provided evidence that spliceosome mutations are associated with thousands of alternative splicing events. Most of the aberrant splicing events selectively observed in *SF3B1*-mutated samples are caused by misrecognition of 3' splice sites, resulting in a frameshift. These studies also suggested that approximately 50% of the aberrant mRNAs induced by *SF3B1* mutations undergo degradation by nonsense-mediated mRNA decay (NMD) pathway, resulting in down-regulation of canonical transcripts and protein expression. Notably, two genes involved in mitochondrial iron metabolism or heme biosynthesis, *PPOX* and *ABCB7*, uniformly showed reduced expression in *SF3B1*-mutated samples, consequent to abnormal splicing and NMD. Conversely, *SRSF2*- and *U2AF1*-mutated samples are characterized by alternative exon usage. Usage of an *EZH2* cryptic exon harboring a premature termination codon is increased in both *SRSF2*- and *U2AF1*-mutated samples.⁸

Trajectories of clonal evolution and effect of clonal and context-dependent variables

Genomic studies have shown that a multistep somatic genetic process typically drives clonal evolution of MDS, and emerging evidence is suggesting that recurrent mutations driving clonal dominance tend to have stereotyped positions in the clonal hierarchy. Several variables, either intrinsic to the clone or related to the environmental context, contribute to shaping the evolutionary trajectories of the myelodysplastic clones by skewing the spectrum of subclonal driver mutations.

Founding mutations constrain the spectrum of genetic events driving clonal progression

Founding mutations may shape the future trajectories of clonal evolution of a cancer through constraints on the repertoire of cooperating genetic lesions, a process termed as genetic predestination or canaliza-

tion. The magnitude of this process varies and appears to be specific of the founding mutation.

SF3B1-mutated clones are associated with a highly restricted spectrum of subclonal mutations driving clonal progression. *RUNX1* mutations may occur in up to 10% of *SF3B1*-mutated patients and have been reported to be significantly associated with increased risk of disease evolution.² In addition, a recent comprehensive transcriptomic analysis showed that a high proportion of *SF3B1* mutated cases clustering in the high risk category showed over-expression of *EVI1*, resulting from aberrant gene fusions or 3q26 abnormality. Accordingly, in a recent comprehensive study of leukemia supporting a fully genomic classification of AML, a clustering of *SF3B1*-mutated cases has been also reported in AML with inv(3) or t(3;3).

In addition, a significant co-occurrence has been reported between *SF3B1* mutations and JAK-STAT pathway activating mutations, including the classical *JAK2* (V617F) and less frequently *CALR* or *MPL* mutations. This mutation pattern is typically associated with the MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), currently recognized by the WHO classification as a distinct disease entity. The available evidence suggests that *SF3B1* mutations act as initiating lesions, responsible for myelodysplastic features, i.e. ineffective erythropoiesis and ring sideroblasts, whereas *JAK2*, *MPL* or *CALR* mutations drive the emergence of subclones conferring the myeloproliferative phenotype.

Germline predisposition to myeloid neoplasms

Although most cases of MDS are sporadic diseases, growing evidence is suggesting that a subgroup of cases is associated with germline mutations and occur on the background of a predisposing germ line mutation. The underlying germline mutations may have significant effect in selecting somatic genetic lesions driving the evolution into overt malignancy and later steps of clonal progression.

This mechanism is illustrated by monosomy 7 and interstitial deletions of chromosome 7, among the most frequent chromosomal aberrations found in essentially all types of myeloid tumors. The prevalence of such chromosomal abnormalities is significantly enriched in congenital diseases with a propensity to evolve into myeloid malignancies, such as those caused by germ line *GATA2* mutations, neurofibromatosis, severe congenital neutropenia and MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) syndrome associated with *SAMD9/SAMD9L* mutations.

Different mechanisms underlying the selective expansion of clones with somatically acquired monosomy 7 or del(7q) have been advocated. These include a relative survival advantage over the surrounding bone marrow cells sustained by enhanced responsiveness to cytokines, or an adaption by aneuploidy sustained by the loss of a germline mutated allele having an adverse effect on cell proliferation, recently described in children carrying a gain-of-function germline mutation of *SAMD9/SAMD9L*.

Environmental context-dependent factors

Exposure to cytotoxic therapy and therapy-related myeloid neoplasms

MDS that develops after chemotherapy or radiation for a non-myeloid disease are categorized as therapy-related myeloid neoplasm (t-MN). This definition is based solely on clinical history, and it is independent of the type or intensity of exposure and of the latency between exposure and t-MN diagnosis.

Recent genetic studies of t-MN have sought to better define a subgroup of patients who have true therapy-driven disease. Mutations in two genes involved in DNA damage response, *PPM1D* and *TP53*, were recently reported in 46% of the patients with t-MN, and they were the only gene mutations that were significantly associated with these malignancies. By contrast, the group of t-MN cases without *TP53* or *PPM1D* mutations appeared genetically similar to primary MDS and did not have adverse outcomes.⁹

Notably, these mutations can be identified in the peripheral blood of some patients who develop t-MN even before exposure to chemotherapy, suggesting that cytotoxic therapies can select for preexisting clones with acquired DNA damage response dysfunction. Experimental evidence in mice models support this clinical observation, showing that hematopoietic stem/progenitor cells harboring these mutated genes preferentially expand after exposure to chemotherapy. The strong as-

sociation between t-MN and *TP53* or *PPM1D* mutations as well as the shared biological role of these genes in the DNA damage response suggest that these mutations mark a subset of patients whose disease is directly driven or selected by therapeutic exposure.⁹

Abnormal bone marrow and immune environment

Several lines of evidence suggest that abnormal bone marrow and immune environment may give a critical contribution to MDS initiation and evolution. Disrupted inflammatory signaling from niche cells may facilitate the occurrence of somatic mutations, their selection, and subsequent clonal expansion through a Darwinian process.

Recent studies in aplastic anemia showed that approximately one third of patients had mutations in genes commonly affected in myeloid neoplasms. However, a substantial diversity was observed in mutation frequencies as compared to myeloid neoplasms, strongly supporting the concept of selection of restricted cell clones under the pressure of an abnormal bone marrow environment.¹⁰

Changes occurring during aging in the hematopoietic microenvironment are also likely selecting hematopoietic clones carrying specific mutations. Accordingly, recent evidence in persons without hematologic phenotype suggested that mutations affecting spliceosome genes are preferentially identified in individuals aged 70 years or older, implying that spliceosome gene mutations may drive clonal expansion under selection pressures particular to the aging hematopoietic system.

Conclusion

Recent genomic studies have proven that MDS is typically driven by a multistep somatic genetic process, resulting from a strict interaction between the hematopoietic clone(s) and the environmental context, which can actively influence the emergence, expansion and progression of clones resulting in MDS.

While massive genome sequencing studies have unveiled the landscape of driver genetic lesions, the study of extra-clonal components and their interaction with clones is emerging as a major challenge in the process of unveiling the complex pathophysiology of MDS.

These achievements have the potential to significantly influence the clinical management of patients with MDS, in all its key elements of diagnosis, classification, risk assessment and treatment, and have been opening avenues of research leading to novel therapeutic options and personalized treatment in the individual patient.

References

- Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. *Blood*. 2013;122:4021-34.
- Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-27.
- Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-98.
- Malcovati L, Galli A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. *Blood*. 2017;129(25):3371-8.
- Ebert BL, Pretz J, Bosco J, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. *Nature*. 2008;451(7176):335-9.
- Woll PS, Kjallquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer Cell*. 2014;25(6):794-808.
- Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011;478(7367):64-9.
- Shiozawa Y, Malcovati L, Galli A, et al. Aberrant splicing and defective mRNA production induced by somatic spliceosome mutations in myelodysplasia. *Nat Commun*. 2018;9(1):3649.
- Lindsley RC, Saber W, Mar BG, et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. *N Engl J Med*. 2017;376(6):536-47.
- Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. *N Engl J Med*. 2015;373(1):35-47.

IRON METABOLISM NAD IRON TOXICITY

D. Cilloni

Department of clinical and biological sciences University of Turin, Italy

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of the hematopoietic stem cells characterized by different degree of cytopenias and high propensity to transform into acute myeloid leukemia (AML). Although several treatments are available

nowadays to reduce the level of cytopenia and probably to increase the survival of MDS patients, the mainstay of therapy for most of them remains supportive care. Since one of the main clinical features of MDS is anemia, among the main supportive therapies the red blood cell (RBC) transfusion is the most applied.

Unfortunately, RBC transfusions cause iron overload that has been shown in many settings to impact on the survival of MDS patients. It was clearly shown that, not only the transfusion dependency but also the degree of units transfused every month has an impact on the development of comorbidities and finally on survival.

There is a correlation between multi-morbidity, in particular heart disease, and transfusion dependence in patients with MDS.¹ The mortality rate is greater in the transfusion-dependent MDS patients with iron overload. In the study by Castelli et al. has been identified a statistically significant correlation between heart failure, transfusion dependence and levels of ferritin.¹

In order to assess the extent of iron overload in patients with MDS is therefore important to evaluate both the number and the frequency of transfusions, and the saturation of transferrin. The value of saturated transferrin represents an indirect index of production of plasma free iron that can provide information on the exposure of the organs to this toxic form of iron: a saturation level higher than 60-70% was in fact associated with the presence of toxic forms of iron in plasma, labile plasma iron (LPI) and iron not bound to transferrin (NTBI).

In addition to transfusions, ineffective erythropoiesis is often associated with an increase in iron absorption due to an increased demand by erythroid precursors; this phenomenon contributes, together with saturation, to appear and accumulate in tissues the most reactive forms of iron (NTBI and LPI) which, by stimulating the production of reactive oxygen species (ROS), exert a biologically toxic action at the level of molecular, cellular and tissue.²

Coates and colleagues in an equation, summarized all the factors able to influence the toxicity of iron at the tissue level.² According to the concept of toxicity of the iron expressed in this formula, the toxicity of iron it can, therefore, worsen the comorbidities in place or generate new comorbidities. In this formula, the genetic of disease together with the environmental factors and the duration of exposure to toxic iron are responsible for the organ damage.²

To date, the clinical parameters used for the diagnosis of iron overload are: serum ferritin, saturation of transferrin, the number and frequency of transfusions and MRI. In the setting of transfusion-dependent thalassemias these parameters are well defined and validated with established threshold values that have been translated also to other pathologies characterized by iron overload.

Despite of this, it was understood that the experience accumulated with thalassemia cannot be adaptable to all the conditions characterized by iron overload, in particular to MDS. Furthermore, the interest of hematologists is progressively growing moving towards the dosage of "free iron" (LPI and NTBI) and markers capable of measuring stress cellular oxidative such as: reactive oxygen species (ROS), reduced glutathione (GSH), lipid peroxidase (MDA), 8-oxo-guanine (8-OHdG) and 8-oxo-guanine glycosylase, attributing to the concept of "iron toxicity" a primary role.

Based on these new concepts the iron chelation therapy is nowadays recommended for patients with transfusion dependent MDS.

The goal of iron chelation therapy is to reduce the levels of iron in the body, both by removing the deposit iron in excess (organ overload), and eliminating the highly reactive forms of free iron (LPI / NTBI). Iron chelation was associated with a prolonged survival in patients with MDS (in particular MDS a low risk).³

In a 5-year prospective analysis conducted on 599 patients with low-risk MDS and transfusional iron accumulation, a higher mortality rate was observed in the group of patients not subjected to chelation (73.3%) compared to that of patients treated with iron chelating agents (62.2%, $p=0.0039$) and treated with iron chelators for ≥ 6 months (59.6%, $p=0.001$). The survival of the chelated patients was (86.3 and 98.7 months, respectively) it was significantly longer than that of patients not chelated (47.8 months) (25). In this study the main reasons of death that affected the mortality rate observed in non-chelated patients were progression to acute myeloid leukemia (AML) and infections.⁴

Since the data on OS are mainly derived from retrospective studies, the reason why iron chelation is associated with prolonged survival was not clear. In order to demonstrate the benefit of iron chelation ther-

apy in a prospective way the TELESTO study was designed and launched in 2009.⁵ This was the first prospective study that randomized 225 patients with low/intermediate 1 MDS to receive placebo or iron chelation therapy with deferasirox; the primary endpoint was event free survival. The median EFS was 349 days longer with deferasirox (1440 days, 95% CI: 1167-1559) versus placebo (1091 days, 95% CI: 820-1348) with a reduction of the risk of 36.4% in EFS with deferasirox ($P=0.015$). Furthermore, the EFS estimated at 3 years was found 61.5% (95% CI: 52.2-69.6) with deferasirox e 47.3% (95% CI: 31.8-61.3) with placebo (26). The data of the TELESTO study confirmed in the setting of MDS the clinical benefit of removing iron.

Another result frequently observed with therapy iron chelator is the hematological response in different types of patients undergoing iron chelation: in patients subjected to hematopoietic stem cell transplantation (HSCT),⁶ in patients with myelofibrosis⁷ and in patients with MDS.⁸

An increase in progenitors has also been observed erythroid, Erythroid Burst-Forming Units (BFU-E), in patients affected by thalassemia major treated with deferasirox.⁹ The impact of iron chelation has been investigated also on the quality of life. In the patient population with MDS enrolled in the GIMEMA study MDS0306, the quality of life measured through the EORTC QLQ-C30 questionnaire remained constant throughout treatment with deferasirox.¹⁰ In general, in the patient affected by elderly MDS or with comorbidity, iron chelation should be interpreted as a tool to improve patient fitness.

In conclusion, the iron toxicity is well documented although the molecular mechanism of toxicity are still under investigation. It is now clear that the continuous exposure to iron induces organ damage and iron chelation can improve the survival and the quality of life of MDS patients.

References

- Castelli R, Schiavon R, Delilieri GL. The impact of anaemia, transfusion dependency, comorbidities and polypharmacy in elderly patients with low-risk myelodysplastic syndromes. *Med Oncol* 2018;35:33.
- Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radic Biol Med* 2014;72:23-40.
- Mainous AG 3rd, Tanner RJ, Hulihan MM, et al. The impact of chelation therapy on survival in transfusional iron overload: a meta-analysis of myelodysplastic syndrome. *Br J Haematol*. 2014;167:720-723.
- Lyons RM, Marek BJ, Paley C, et al. Relation between chelation and clinical outcomes in lower-risk patients with myelodysplastic syndromes: Registry analysis at 5 years. *Leuk Res* 2017;56:88-95.
- Angelucci E, Li J, Greenberg PL, et al. Safety and Efficacy, Including Event-Free Survival, of Deferasirox Versus Placebo in Iron-Overloaded Patients with Low- and Int-1-Risk Myelodysplastic Syndromes (MDS): Outcomes from the Randomized, Double-Blind Telesto Study. In: 60th American Society of Hematology Annual Meeting (Abstract 234), San Diego (CA, USA), 1- 4 dicembre 2018.
- Visani G, Guiducci B, Giardini C, et al. Deferasirox 27. Improves hematopoiesis after allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2014;49:585-587.
- Elli EM, Belotti A, Aroldi A, et al. Iron chelation therapy with deferasirox in the management of iron overload in primary myelofibrosis. *Mediterr J Hematol Infect Dis* 2014;6:e2014042.
- Gattermann N, Finelli C, Della Porta M, et al. Hematologic responses to deferasirox therapy in transfusion-dependent patients with myelodysplastic syndromes. *Haematologica* 2012;97:1364-1371.
- Forni GL, Podestà M, Musso M, et al. Differential effects of the type of iron chelator on the absolute number of hematopoietic peripheral progenitors in patients with β -thalassemia major. *Haematologica* 2013;98:555-559.
- Efficace F, Santini V, La Nasa G, et al. Health-related quality of life in transfusion-dependent patients with myelodysplastic syndromes: a prospective study to assess the impact of iron chelation therapy. *BMJ Support Palliat Care* 2016;6:80-88.

NEW THERAPEUTIC STRATEGIES IN MDS

M. Breccia, E. Scalzulli,

Hematology, Department of Translational and Precision Medicine, Sapienza University Az. Policlinico Umberto 1, Rome, Italy

Myelodysplastic syndromes (MDS) are a group of heterogeneous diseases mainly affecting elderly population and displaying a complex pathogenesis. For the advanced age, most of the patients cannot tolerate intensive approaches and the treatment is patient-centered and risk adapted¹. The current approach suggested for low risk patients is mainly focused on the improvement of cytopenias, in particular anemia-related symptoms and the consequences of transfusion burden. Although the erythropoiesis stimulating agents (ESAs) have been widely

used for years, only recently erythropoietin- α has been approved in Europe for the specific treatment of MDS. The results of two major phase 3 trials were published comparing darbepoetin and epoetin- α versus placebo: lower response rate was attested for darbepoetin (14.7%) as compared to epoetin- α (31.8%) that received the final approval¹². Some prognostic factors have finally been identified as predictive for response to ESAs, such as low endogenous EPO-level at baseline (inferior to 200 UI/L), transfusion burden less than 4 units within 8-weeks, IPSS-R and immunophenotype of myeloid cells². In patients with del(5q), ESAs were also the first option but the majority of patients do not respond: lenalidomide is the best strategy for this subset with about 70% of erythroid responses achieved. In the last years some biological findings allow to understand that patients with mutated TP53, but also TET2 and RUNX1 mutation, are less likely to reach a cytogenetic response with dismal prognosis and long-term increased risk of acute leukemia progression³. In patients without del(5q) after failure of ESAs, lenalidomide as possible approach was tested in a placebo-controlled phase 3 trial with 27% of patients reached red blood cell transfusion independence (RBC-TI) for more than 8 weeks and in another trial testing lenalidomide associated to ESA in resistant patients to ESAs alone improved erythroid responses (39.4%)³. Rarely used in Europe, but widely in US, hypomethylating agents (HMA) were also tested in ESA resistant patients with transfusion independence (RBC-TI) ranged between 16 to 30%. The oral formulation (CC-486) of azacitidine provides a possible more convenient way of administration allowing long-term administration: in a randomized trial with two different schedule of administration (14 vs 21 days) the overall response rate (ORR) was attained by 36% and 41% of patients, respectively, with similar rates of RBC-TI among the two group and a relatively good tolerance. However, a recent phase 3 trial placebo-controlled in intermediate-1 risk patients with RBC-transfusion dependence and thrombocytopenia has been prematurely closed for toxicity⁴. Indeed, new approaches to treat anemia in low risk MDS have been recently tested: luspatercept and sotatercept are activin receptor fusion proteins altering transforming growth factor- β signalling that, differently from ESAs that act on early progenitors, can regulate the late differentiation and maturation of erythroid progenitors. In particular, luspatercept is active for the treatment of anemia in lower risk patients (63% patients with erythroid response and 38% with RBC-TI), especially those with ring sideroblasts or with SF3B1 mutation. Recently, the results of a phase 3 randomized trial versus placebo (2:1), namely MEDALIST trial, were reported: a significant difference has been tested in RBC-TI rate with luspatercept (37.9%) as compared to placebo (13.2%). The median duration of RBC-TI response was 30.6 weeks and at week 48, 58.8% of patients achieved an erythroid improvement compared to 17% of patients randomized to placebo. No differences in AML progression between the two arms were observed⁵.

Roxadustat is a hypoxia-inducible factor prolyl hydroxylase inhibitor that promotes erythropoiesis by increasing endogenous EPO levels and, by modulation of hepcidin levels, improves iron regulation. It is currently being assessed in a phase 3 trial for the treatment of anemia in low-risk MDS and in patients with chronic kidney disease⁶.

Imetelstat is a telomerase inhibitor that acts on cells with hyperactive telomerase, a critical pathway to maintain normal hematopoiesis: a phase 2/3 trial is ongoing in ESAs relapsed/refractory low-risk patients and preliminary results showed that 37% of patients achieved a RBC-TI⁶. Pexmetinib (ARRY-614), a dual inhibitor of Tie2 and p38 MAPK has been tested in a phase 1 dose finding study in 45 low/int-1 risk patients: overall, 32% of patients, most of them pre-treated with HMA, achieved erythroid improvement (20%) or RBC-TI (12%)⁶.

In low-risk patients with thrombocytopenia, apart from platelet transfusions and HMA, TPO-receptor agonists have been tested. With Romiplostin about 36% of patients achieved a platelet response and survival benefit associated. Forty-seven % of patients achieved platelet response with eltrombopag and with both drugs was reported the same level of AML progression⁷.

Therapeutic approaches in higher-risk MDS are aimed at changing the natural course of the disease, improving overall survival and try to limit the disease progression. An appropriate evaluation of transplant eligibility should be performed whenever possible, because allogeneic procedure remains the only potentially curative option. Unfortunately, only 10% of patients can be candidate to this strategy due to presenting age, comorbidities and the higher mortality rate associated with this strategy. Two recent studies showed that early transplant improves life

expectancy in higher-risk but not in low risk MDS. No differences were indeed reported between different transplant sources, but a decreased relapse rate with myeloablative conditioning regimen compared to reduced-intensity regimen (reviewed in³). HMAs are preferred as cytoreduction before transplant and in non-transplant candidates: in Europe only azacitidine has been approved based on a randomized sponsored trial that showed survival advantage compared to conventional standard of care, even if responses are short-lived. The optimal schedule of azacitidine is 75 mg/mq for 7 days every 28, whereas for decitabine is 20 mg/mq for 5 days every 28 days. Responses are achieved slowly and a minimum of 4-6 cycles is required before to assess responses. Until yet is still critical the finding of prognostic factors at baseline predictive of response, such as markers of epigenetic regulation (TET2, DNMT3a) and have been indicated as possible clinical markers the poor performance status, high transfusion burden and poor cytogenetic risk⁸. After failure of HMAs, new possible strategies are available in clinical trial. Guadecitabine is a second generation HMA, resistant to degradation by citidine deaminase: for this reason has a longer half-life and can allow a prolonged exposure. It was tested in a phase I trial but only 19 MDS patients were enrolled and 6 of them achieved a clinical response, defining the possible maximum tolerated dose of 90 mg/mq for 5 days. Indeed, it was tested in relapsed/refractory and naïve AML patients in several phase 2 and 3 trial, ^{3,4,6}.

Azacitidine was associated as backbone to different partners such as lenalidomide, vorinostat, eltrombopag, vosaroxin, volasertib or HDAC inhibitors, but the results did not show increased rate of responses as compared to azacitidine single agent. Among HDAC agents, pracinostat associated to azacitidine showed contrasting inferior results in terms of efficacy in MDS patients as compared to AML patients. A novel inhibitor of NEDD8-activating enzyme, namely pevonedistat, shows in vitro synergistic activity when associated to azacitidine and a phase 3 trial is still ongoing evaluating the activity of the drug plus azacitidine versus azacitidine alone in high-risk MDS, chronic myelomonocytic leukemia (CMML) and oligoblastic AML ^{3,4,6}.

Venetoclax act as an orally selective inhibitor of BCL2 pro-survival protein that facilitates C-Myc induced transformation. This agent was approved for the treatment of naïve elderly AML patients after publications of several reports that demonstrating activity. Recently tested in 43 AML, MDS patients pre-treated with HMA showed an ORR of 21%, with 5% of patients reaching a CR and a median OS of 3 months. Several trials of venetoclax in combination with other drugs are currently ongoing in higher-risk MDS patients⁹.

IDH mutations are quite common in MDS (10-15% of patients) and initial responses in AML and MDS patients are extremely promising, allowing the possible use of these drugs in firstline. The results of phase 1 trial with ivosidenib in 12 relapsed/refractory MDS have been recently presented: 3 patients remained on treatment, whereas 6 patients discontinued due to progressive disease and 1 patient underwent transplant. The ORR was 92% at 21 months with 5 patients achieved CR (42%) and 5 patients achieved a mCR¹⁰. Enasidenib was also tested in a group of patients with IDH2 mutation: 14 MDS were enrolled and ORR was 50%, with 21% reached a CR. P53 mutation is associated with poor outcome even after allogeneic transplant: 10-days decitabine showed efficacy in mutated patients whereas less evidences were reported for 5-days regimen for azacitidine^{3,4,6}. APR-246 associated to azacitidine can modulate the activity of the transcriptional activity of P53 mutation: a phase 3 randomized trial is ongoing comparing the association of the drug with azacitidine vs azacitidine alone. A similar trial testing the association is ongoing by GFM group and another after allogeneic stem cell transplant. The drug received the orphan drug status and the designation for the possible association with HMA in US this year^{4,6}.

The multikinase inhibitor rigosertib binds the Ras domain of different kinases such as RAF, PI3K, therefore acting on PI3K pathway. It has been tested in the ONTIME trial, a phase 3 randomized study that investigated the efficacy of the drug versus best supportive care (BSC) in resistant/refractory patients to the previous HMA. The results failed to demonstrate the primary endpoint without differences in terms of OS between patients treated with rigosertib (8.2 months) and BSC (5.9 months). A subanalysis of the study showed efficacy in primary resistant patients to HMA and in very high risk IPSS-R patients: these latter categories represent the population requested for the subsequent ongoing trial called INSPIRE^{3,4,6}. Increased expression of programmed cell-death protein-1 (PD-1) and of its ligand PDL-1 has been described in

higher-risk MDS patients: the treatment with azacitidine leads to demethylation of PD-1 promoter and re-expression of PD-1. Checkpoint inhibitors have been tested in MDS alone or in combination with azacitidine in a phase 2 trial enrolling resistant patients to HMA. An update on 76 patients has been presented at ASH 2018 and showed higher response rates for the combinations with azacitidine of nivolumab (70%) or ipilimumab (62%) compared to checkpoint inhibitors alone³. Durvalumab was tested associated to oral azacitidine in resistant patients to azacitidine but the trial was prematurely closed due to the difficulties to find a correct dose for durvalumab. Actually is being tested together with subcutaneous azacitidine in untreated higher-risk MDS^{4,6}. Eighteen patients were treated with the combination of azacitidine and pembrolizumab, another humanized monoclonal antibody targeting PD1: of previously treated patients, 1 patient achieved CR and another a CRi, whereas of the untreated patients, 1 achieved CR and 2 patients showed haematological improvement. Atezolizumab was tested alone or in combination with azacitidine in higher-risk untreated MDS or after HMA failure: only 1 resistant patient achieved a clinical response to the combination of the drug plus azacitidine but 13 untreated patients obtained a response. The drug demonstrated increased toxicity with 21 deaths recorded among 42 patients enrolled^{6,8}.

Possible strategies in the future will include chimeric antigen receptor (CAR-t cells) and bispecific antibodies (DARTs): a phase 1 trial with Flotetuzumab, an antibody against CD3/CD123, enrolled MDS patients previously treated with HMA with more than 10% of bone marrow blasts. Of the 45 patients enrolled, 14 patients treated with 500-700 ng/kg/day have completed at least one cycle and 8 patients showed clinical responses for an ORR of 43%⁹.

In conclusions, due to the molecular heterogeneity of MDS a unique and common treatment is unlikely to be identified. With improved and continue understanding of molecular and immunological pathways of these diseases, in the next future emerging treatments will allow a personalized and tailored therapeutic strategies.

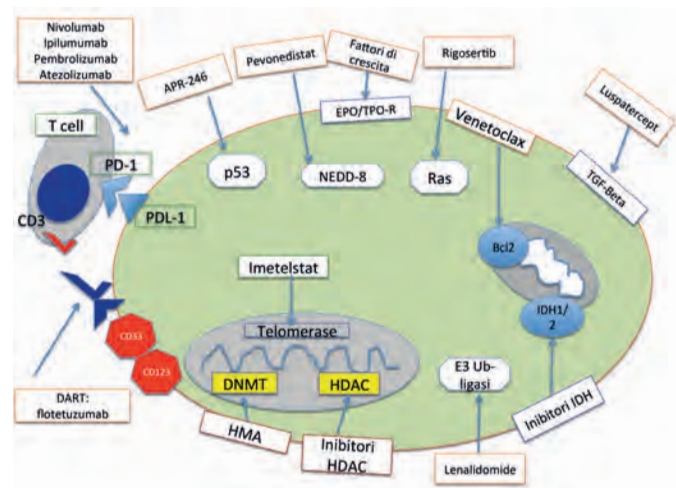


Figure 1. Possible new therapeutic approaches in MDS.

References

1. Montalban-Bravo G, Garcia-Manero G. Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management. *Am J Hematol* 2018; 93: 129-147
2. Park S, Greenberg P, Yucel A, Farmer C, O'Neill F, De Oliveira Brandao C, Fenaux P. Clinical effectiveness and safety of erythropoietin-stimulating agents for the treatment of low- and intermediate-1 risk myelodysplastic syndrome: a systematic literature review. *Br J Haematol* 2019; 184(2): 134-160
3. Platzbecker U. Treatment of MDS. *Blood* 2019; 133(10): 1096-1107
4. Germing U, Schroeder T, Kaiwers J, Kundgen A, Kobbe G, Gattermann N. Novel therapies in low and high-risk myelodysplastic syndrome. *Exp Rev Hematol* 2019; 31: 1-16
5. Fenaux P, Platzbecker U, Mufti GJ, Garcia-Manero G, Buckstein R, Santini V, et al. The Medalist Trial: results of a phase 3, randomized, double-blind, placebo-controlled study of luspatercept to treat anemia in patients with very low-, low-, or intermediate-risk myelodysplastic syndromes (MDS) with ring sideroblasts (RS)

who require red blood cell (RBC) transfusions. *Blood* 2018; 132: 1

6. Sockel K, Platzbecker U. Current and future treatment options for myelodysplastic syndromes: more than hypomethylating agents and lenalidomide? *Drugs* 2018; 78(18): 1873-1885
7. Stahl M, Zeidan AM. Management of lower-risk myelodysplastic syndromes without del5q: current approach and future trends. *Expert Rev Hematol* 2017; 10(4): 345-364
8. Montoro J, Yerlikaya A, Ali A, Raza A. Improving treatment for myelodysplastic syndromes patients. *Curr Treat Options in Oncol* 2018; 19: 66
9. DiNardo C, Rausch CR, Benton C, Kadia T, Jain N, Pemmaraju N, et al. Clinical experience with the BCL2-inhibitor venetoclax in combination therapy for relapsed and refractory acute myeloid leukemia and related myeloid malignancies. *Am J Hematol* 2018; 93: 401-407
10. Foran JM, DiNardo C, Watts JM, Stein EM, de Botton S, Fathi AT, et al. Ivosidenib (AG-120) induces durable remissions and transfusion independence in patients with IDH1-mutant relapsed/refractory myelodysplastic syndrome in a phase 1 dose escalation and expansion study. *Haematologica* 2019; abst 1337

NEW INSIGHTS IN WALDENSTROM MACROGLOBULINEMIA

A. Tedeschi, G. Zamproga, M. Deodato, P. Minga, A.M. Frustaci

ASST Grande Ospedale Metropolitano Niguarda, Niguarda Cancer Center, Milano, Italy

Waldenström Macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by the proliferation of lymphoplasmacytic elements in the bone marrow and the presence of monoclonal immunoglobulin M (IgM) gammopathy. WM is a rare disease representing 1–2% of all hematological malignancies and 6% of the lymphoproliferative disorders. The incidence is estimated at 3 new cases per 1 million individuals.

The recent discovery of two mutations, myeloid differentiation primary response 88 (*MYD88*) and C-X-C chemokine receptor type 4 (*CXCR4*) in WM patients has improved disease characterization allowing a deeper understanding of the biology of this disease and changing therapeutic scenario.

Disease characteristics

Clinical presentation of WM is extremely heterogeneous, despite a usually indolent disease course sometimes it may require prompt treatment to avoid irreparable organ damage or fatal complications. Signs and symptoms can be secondary to organ infiltration by clonal cells or due to specific immunological and physiochemical features of monoclonal IgM. Systemic symptoms common to all Non Hodgkin Lymphomas are also reported.

The most frequent clinical sign of bone marrow infiltration and most common indication for treatment initiation is anemia. Anemia may be related to absolute or functional iron deficiency, that can be distinguished by low iron saturation despite normal or high serum ferritin levels. This event has been related to hepcidin secretion by WM cells so that intravenous iron infusion, instead of oral supplementation can be useful in some selected cases. A hemolytic diagnostic workup is necessary in case of suspected hemolytic anemia. Thrombocytopenia and leucopenia are less common.

Compared to other lymphomas, the involvement of the lymph nodes, spleen or liver is less frequent (15–20% at initial presentation). Other organs have been described to be affected by the clonal lymphoid infiltration such as lungs, gastrointestinal tract (malabsorption, diarrhea, bleeding), kidneys, skin (cutaneous plaques), and eyes.

IgM paraprotein can be responsible for hyperviscosity syndrome, that variably manifests as spontaneous epistaxis, ocular and hearing disorders, such as blurred vision, headache, tinnitus and vertigo, and occurs in 10-15% of patients at the time of diagnosis. Usually, symptoms are observed when the serum IgM level is >30 g/l, although considering individual variability, a fundoscopic examination is warranted in these patients to reveal early signs of micro circular damage. In a recent retrospective study on 825 newly diagnosed WM patients, a serum IgM level >6000 mg/dl at diagnosis was associated with a median time to symptomatic hyperviscosity of 3 months, whereas the median time for patients with serum IgM level of 5000-6000 mg/dl was approximately three years. These findings support the use of serum IgM level >6000 mg/dl as a criterion for therapy initiation in an otherwise asymptomatic WM patient.

Amyloidosis, develops in 2% of WM patients with monoclonal IgM and is caused by the deposition of monoclonal light chain (mostly kappa) as fibrillar amyloid deposits (AL amyloidosis), mostly involving

kidneys, heart, liver and peripheral nerves.

Type I and II cryoglobulinemia can clinically emerge with skin alterations like purpura, ulcers and livedo, especially in the lower extremities. The presence of cryoglobulinemia can also worsen hyperviscosity manifestations.

Notably, peripheral neuropathy is present in a quarter of WM patients. The Last International Workshop on WM (IWWM) consensus panel, identified six distinct entities of paraprotein-associated neuropathies. The most frequent is characterized by the presence of anti-MAG antibodies, is typically distal and symmetrical, firstly affecting the sensory functions (paresthesias, dysesthesias, pains, ataxia), and in advanced stage motor functions (leg muscles atrophy). IgM antibodies directed to other neural antigens (such as GD1a, GD1b, GM2) can lead to demyelinating and slowly progressive predominantly distal neuropathy. High titer of anti-GM1 antibodies otherwise, can be associated with a multifocal motor neuropathy. High titer of antibodies against disialylated gangliosides (GQ1b, GT1a, GT1b, GD1b, GD2 and GD3) in the presence of neuropathy with ophthalmoplegia and ataxia may configure CANOMAD (Chronic ataxic neuropathy with ophthalmoplegia, M-protein, cold agglutinins and disialosyl ganglioside antibodies) syndrome.

Two different and distinctive syndromes, albeit rare, should always be considered. Bing-Neel syndrome identifies central nervous system involvement by WM, is a complication involving about 1% of patients. Heterogeneous neurological signs and symptoms may be investigated by cerebral and whole spine imaging and cerebrospinal fluid tests.

Schnitzler syndrome is a chronic autoimmune urticaria associated with IgM gammopathy and other rheumatic manifestations, such as recurrent fever, joint and bone pain.

Diagnosis of WM requires the histologic evidence of bone marrow infiltration of lymphoplasmacytoid elements and the serum presence of monoclonal IgM gammopathy. The need of at least 10% LPL infiltration as a cut-off to distinguish WM from IgM monoclonal gammopathy of undetermined significance (MGUS), was emphasized by the Mayo Clinic consensus. That is in contrast to the Second International Workshop Criteria that do not mandate a minimum BM involvement to confirm the diagnosis. Immunohistochemistry demonstrates lymphocytes and lymphoplasmacytic cells expressing IgM, with kappa or lambda restriction, *CD19*, *CD20*, weak *CD22* and *CD25*. *CD5*, *CD23* and *CD10* are usually negative.

Chromosomal abnormalities (detected by fluorescence in situ hybridization and/or conventional cytogenetics) are present in 45–50% of the cases although no specific aberration is associated with WM. Deletion of chromosome 6q appears to be the most frequent abnormality (30–60%), followed by 13q deletions (2–16%), del11q (8%) or trisomy 4 (8–20%), but without prognostic impact. Importantly, as in other lymphoproliferative disorders, deletion of chromosome 17p including TP53, found in about 8% of patients, is associated with a shorter progression-free survival (PFS) and disease-free survival, while TP53 mutation is rare.

Biological insights

In 2012 Treon et al. revealed the presence of *MYD88 L265P* mutation in more than 90% of WM patients by whole-genome sequencing, however it can't be considered as a unique marker of WM since detected in about 10% of MZL and other lymphoproliferative disorders. *MYD88* is absent in IgM multiple myeloma, hence can help with the differential diagnosis of these diseases. Interestingly, the presence of *MYD88* in the cerebral spinal fluid of patients with Bing Neel Syndrome may help as a diagnostic tool.

CXCR4 is a chemokine receptor that promotes WM cells survival, migration and adhesion to bone marrow stroma, mediated by its ligand CXCL12. Somatic *CXCR4* mutations are present in 30–40% of patients with WM and simultaneously harbor *MYD88* mutations in most cases. Two types of mutations have been observed in nearly equal proportions: the frame shift mutation (*CXCR4FS*) and nonsense mutation types (*CXCR4NS*).

WM patients with *CXCR4* mutation have specific clinico-biological features characterized by more aggressive disease features and adverse prognosis, in particular lower leukocyte, hemoglobin and platelet counts, higher serum IgM levels related to greater marrow involvement, higher International Prognostic Scoring System for WM (ISSWM) score with adverse features, and more symptomatic disease requiring therapy and hyperviscosity symptoms.

Based on the recurrence of *MYD88* and *CXCR4* mutations in WM, involving over 90% and around 30% of patients, three genotypes can be individuated: *MYD88* mutated (MT)/*CXCR4* wild type (WT), *MYD88 MT/CXCR4 MT*, and *MYD88 WT/CXCR4 WT* with important prognostic and therapeutic implications.

First line treatment

At diagnosis, 30–50% of WM patients do not require therapy, treatment should be considered only for symptomatic patients, except for patients with an IgM level > 60 g/L that should receive treatment to prevent hyperviscosity syndrome according to last ESMO guidelines. When symptomatic WM is related to hyperviscosity syndrome plasma exchange is the therapy of choice. The benefit from this procedure is time-limited so plasmapheresis should be always followed by a systemic treatment.

Given the rarity of WM, most of the current treatment regimens have been adopted from data derived from phase 2 studies and less often from prospective trials addressed to WM as well as to other indolent B-cell lymphomas.

There is no standard therapy, so treatment should be planned according to patient's age, comorbidities and disease characteristics. Furthermore, medication tolerance and avoidance of long-term toxic effects including the possible development of treatment related secondary malignancies should be considered.

ISSWM, which is the only score validated in Waldenström, stratify patients in three risk groups in respect of overall survival (OS) but should not be used to guide treatment choice.

Rituximab has been widely used as a single agent in WM. Two schedules have been evaluated, leading to an overall response rate (ORR) of 18–40% as standard regimen (375 mg/mq for a 4-week cycle) and 35–65% as extended course (375 mg/mq for additional 4 weeks administered 8 weeks apart). The median time to response with rituximab monotherapy is seven months, making this drug unsuitable for patients in need of a rapid disease control. Considering the risk of "IgM flare" after rituximab, defined as the transient increase of IgM serum level typically occurring after 1 to 4 months, this agent should be avoided in case of hyperviscosity syndrome and introduced when the serum IgM is < 4000 mg/dL. On the other hand, single-agent rituximab represents a valid option in the presence of immunologic disorders related to WM, such as symptomatic cryoglobulinemia, hemolytic anemia or isolated IgM related peripheral neuropathy. A recent publication demonstrated a significant clinical improvement in almost half of the patients with anti-MAG antibody neuropathy treated with rituximab monotherapy. Ofatumumab, approved for the treatment of Chronic Lymphocytic Leukemia, has been tested WM patients, including nine naive treatment cases, almost 60% of patients obtained a response with 35% achieving at least a PR.

In the past, oral chlorambucil was a commonly used agent, resulting in at least 50% IgM reduction in about 75% of patients. In one of the few randomized phase III trials, oral fludarabine was superior to chlorambucil in terms of progression free survival (PFS) and overall survival (OS). Importantly although a higher incidence of grade 3-4 neutropenia was observed among patients treated with fludarabine, second neoplasms, including hematologic malignancies, were significantly more frequent in the chlorambucil arm with a 6-year cumulative incidence rate of 20.6% versus 3.7% in the fludarabine arm.

Considering the slow response to chlorambucil and the possible risk of secondary malignancies and myelodysplastic syndromes, this therapy should be reserved for elderly frail patients, for whom combination therapy is considered inappropriate.

The combinations rituximab, dexamethasone and cyclophosphamide (DRC) or rituximab and bendamustine (BR) are currently considered standard first line options.

DRC appears to be highly effective, with an overall response rate (ORR) of 83%, 7% complete remissions (CRs), 35 months median PFS and a prolonged median time to next treatment of 51 months but most importantly with a favorable toxic profile. This regimen does not allow a rapid disease control due to the long median time to response of 4.1 months. In patients with high tumor burden the combination of BR may be preferred. BR was first compared to R-CHOP in a phase III trial also including patients with WM and although ORR was similar between the two regimens, BR was superior in terms of tolerability and PFS (69.5 vs 28.1 months). There is not a direct comparison between DRC and BR. A retrospective study at first line demonstrated a similar

ORR with a trend for a longer PFS with BR (2-year PFS 88% with BR and 61% with DRC, $p=0.07$). Importantly, the activity of BR and DRC appeared to be unaffected by *MYD88* mutational status. The full dosage of 90 mg/sqm of bendamustine of in the FILO study was associated with a high rate of neutropenia leading to dose reductions and treatment discontinuation although patients showed the same outcome when stratified according to the number of cycles administered (6 or less courses, 2 years PFS 87% versus 88%).

Purine analogue-based immuno-chemotherapy allowed to achieve good quality of responses with prolonged PFS and time to next treatment. Despite the high efficacy, purine analogues should be avoided as first-line due to the significant incidence of myelotoxicity, immunosuppression and possible impact on stem cell harvest. Furthermore there is general concern to avoid purine analogues because of the risk of secondary malignancies development.

Bortezomib rituximab combination with or without dexamethasone (BDR) have shown to be highly active. A long term follow-up of the European phase II study of BDR showed a median PFS of 3.5 years and an OS rate of 66% at 7 years. The major advantages of bortezomib combinations are low myelotoxicity and rapid reduction of IgM level so that they may be indicated when there is a need of immediate disease control. Furthermore, treatment with proteasome inhibitors overcome the negative impact of *CXCR4* mutations on survival. Treatment with bortezomib is associated with a high rate of peripheral neuropathy and neuropathic pain although subcutaneous administration at weekly intervals is better tolerated.

A retrospective analysis comparing primary treatment with CDR, BDR and BR showed a shorter median time to best response with BR or BDR when compared to CDR, furthermore BR and BDR were associated with better median progression-free survival than CDR (5.5, 5.8 and 4.8 years, respectively), and better 10-year OS rates (95%, 96% and 81%, respectively).

In a retrospective study maintenance therapy with rituximab lead to a PFS and OS improvement, but at present there is an insufficient evidence to support the use of rituximab maintenance because of an increased number of infections.

Table 1. Current Rituximab based regimens for WM.

Reference	N° of patients	ORR %	MR %	Follow up	
Combinations in treatment naïve patients***					
DRC	Dimopoulos et al., 2007	72	83	74	Median PFS 35 mo OS 47% at 8 yrs
	Kastritis et al., 2015				
Benda R	Rummel et al., 2013	22	93	40**	Median PFS 69.5 mo
Benda R	Laribi et al., 2019	69	97	96	OS 97% probability at 2 yrs PFS 87% probability at 2 yrs
Benda R vs DRC	Paludo et al., 2018	16 vs 50	93 vs 96	86 vs 87	PFS 88% vs 61% at 2 yrs
BDR	Treon et al., 2009	23	96	83	Estimated median TTP>30mo
Bortezomib R	Ghobrial et al., 2010	26	88	65	EFR 75% 1 yr
BDR	Dimopoulos et al., 2013	59	85	68	Median PFS 43 mo
	Gavriatopoulou et al., 2017				
Benda R vs BDR vs DRC	Castillo et al., 2018	182	98vs 90vs 89	94 vs 83 vs 84	median PFS 5.5 vs 5.8 vs 4.9 yrs
Combination in relapse/refractory patients***					
Benda R	Tedeschi et al., 2015	71	80	75	Median PFS NR at 19 mo
Benda R vs DRC	Paludo et al., 2018	44 vs 50	95 vs 87	81 vs 68	PFS 66% vs 53% at 2 yrs
Bortezomib R	Ghobrial et al., 2010	37	81	51	Median PFS 15.6 mo
FR	Treon et al., 2009	43	95	86	Median TTP 51.2 mo
FCR	Tedeschi et al., 2012	43	79	74	Median EFS 50.1 mo
FCR	Souchet et al., 2016	82	81	74	Median PFS 79%at 3yrs

*ORR= overall response rate; MR= partial response (PR)+ very good partial response (VGPR)+ complete response (CR). ** 40% of CR. *** DRC= dexamethasone, rituximab, cyclophosphamide; R= rituximab; Benda R= bendamustine, rituximab; BDR= bortezomib, dexamethasone, rituximab; FR= fludarabine,rituximab; FCR= fludarabine, cyclophosphamide, rituximab.

Table 2. Trials with ibrutinib in WM.

Regimen	Reference	Patient's characteristics*	N° of patients	ORR %	MR%**	Follow up
Ibrutinib monotherapy	Treon et al., 2015	R/R	63	90.4	77.7	5yr PFS 73 and 42 *** 5yr OS 87 %
	Treon et al., EHA 2018					
Ibrutinib monotherapy	Dimopoulos et al., 2017	R/R	31	90	71	PFS 86% estimated at 18mo OS 97% estimated at 18 mo
Ibrutinib Rituximab vs Rituximab monotherapy	Dimopoulos et al., 2018	TN and R/R	150	92 vs 47	72 vs 32	PFS 82% vs 28% at 30 mo OS 94% vs 92% at 30 mo
Ibrutinib monotherapy	Treon et al., 2018	TN	30	100	83	PFS 92% at 18 mo OS 100% at 14.6 mo

*TN= treatment naïve, R/R= relapsed/refractory disease. **MR= partial response (PR)+ very good partial response (VGPR)+ complete response (CR). *** MYD88 Mut/CXCR4 WT and MYD88Mut/CXCR4 Mut.

Treatment for relapse/refractory

Type of therapy used at the time of relapse is determined by the response to initial therapy and again, as for initial therapy, treatment should be planned according to patient's age, comorbidities and disease characteristics. There is a general consensus, as for other lymphoproliferative disorders, to repeat the original treatment according to response duration. In symptomatic patients relapsing more than 3 years after rituximab combination therapy, the same treatment may be repeated. DRC, BR, and bortezomib based immunotherapy have been successfully used in the salvage setting.

The BTK inhibitor ibrutinib is the first drug to receive approval for WM even in treatment-naïve patients, unsuitable for immunochemotherapy, with different reimbursement issues according to the country.

The approval was based on a study addressed to a relapsed refractory population in which ibrutinib showed to be effective leading to an ORR of 90 %, with a major response rate (MRR) of 73%, and a median time to response of 4 weeks. In the longer follow up at 48 months the 5-year PFS for all patients was 60% (95% CI 46-71%). Notably, response rate and quality significantly resulted to be associated with the *MYD88 MT/CXCR4 WT* genotype, MRR were 92% versus 62% of the *MYD88 MT/CXCR4 MT*, similarly PFS was not reached at 48 month for patients carrying *MYD88* mutation, was 42 months for those having *MYD88 MT/CXCR4 MT* genotype, and was 5 months for *MYD88 WT* patients.

Overall, 71% of patients with *MYD88 WT* status achieved a response, that was a minor response in all the cases.

The 48 months follow up also showed that dose reduction or holding for more than 8 days are associated with shorter PFS.

Clinically meaningful and rapid responses were seen with ibrutinib monotherapy in 30 treatment-naïve *MYD88* mutated patients with an 18 months PFS of 92%. *CXCR4* mutation in this series of patients exerted a negative impact resulting in a significantly slower response rate.

The 31 rituximab-refractory heavily pretreated patients enrolled in the multicentric phase 3 iNNOVATE trial receiving ibrutinib monotherapy achieved an ORR and MRR of 90% and 71%, respectively, and an estimated 18-months PFS and OS of 86% and 97% respectively confirming the BTKi treatment favorable outcome.

In the same trial treatment naïve and pretreated WM cases patients were randomly assigned to receive ibrutinib plus rituximab or placebo plus rituximab. At 30 months, the progression-free survival rate was 82% with ibrutinib-rituximab versus 28% with placebo-rituximab. Importantly the benefit in the ibrutinib-rituximab group was independent of the *MYD88* or *CXCR4* genotype. The rate of major response was higher with ibrutinib-rituximab than with placebo-rituximab (72% vs.32%, $P<0.001$).

As in the other lymphoproliferative disorders acquired BTK mutations have been detected in WM patients that have shown clinical progression on ibrutinib therapy. The most frequent is BTKCys481 mutation and is associated with mutated *CXCR4*. Some other muta-

tions involved *PLCG2* and *CARD11*.

Ibrutinib showed to be well tolerated even in pretreated patients, the most commonly reported grade 3-4 adverse events were neutropenia, thrombocytopenia, anemia, atrial fibrillation and infection.

New perspectives

Studies with second generation BTK inhibitors acalabrutinib, zanubrutinib, are ongoing with preliminary data showing high efficacy and a more favorable toxic profile.

The second generation proteasome inhibitor, carfilzomib, in combination with rituximab, has shown promising results leading to deep responses (35% of VGPR or better) translating in better PFS. Similarly the combination of ixazomib, dexamethasone and rituximab demonstrated to be highly effective (ORR 96%) and well tolerated. Both proteasome inhibitor-based regimens lead to limited peripheral neuropathy. Responses to proteasome inhibitors, including both first and second generation inhibitors, are achieved irrespective of the *MYD88* and *CXCR4* status.

Phase II trials using Multiple Myeloma approved drugs thalidomide and lenalidomide in monotherapy or in combination with rituximab, resulted in good responses in spite of excessive neurotoxicity often leading to treatment discontinuation.

Venetoclax, a *BCL2* inhibitor, has been tested in a set of pretreated WM patients leading to 80% ORR regardless relapsed or refractory disease state, prior BTKi exposure or *CXCR4* mutational status, with a median time to response of 9 weeks.

Autologous and allogeneic stem cell transplantation are feasible for fit patients with a clinically aggressive disease course, following at least 2 therapeutic lines, and for those patients not tolerating or not responding to ibrutinib.

Data from EBMT showed that allogeneic transplantation either by myeloablative or reduced intensity conditioning is feasible and effective, with 5 years PFS and OS of 56% and 62% respectively in myeloablative, and of 49% and 64% for reduced intensity conditioning. Transplant related morbidity and mortality should always be taken into account, especially considering that WM is a disease of elderly that may per se exclude the application of such intensive treatments.

At present individual treatment decisions are mostly based on comparisons of phase II data suggesting different approaches according to patients' age and disease characteristics. In the future it would be desirable to base therapeutic choices on results of larger trials that may help to direct the use of molecular markers to develop personalized medicine in this field.

References

- Kastritis E, Leblond V, Dimopoulos MA, et al. Waldenstrom's macroglobulinaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29 (Suppl 4):iv41–iv50.
- Leblond V, Kastritis E, Advani R, et al. Treatment recommendations for the Eighth International Workshop on Waldenstrom's Macroglobulinemia. *Blood.* 2016;128:1321–1328.
- Hunter ZR, Yang G, Xu L, et al. Genomics, signaling, and treatment of waldenström macroglobulinemia. *J Clin Oncol.* 2017; 35:994–1001.
- Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. *N Engl J Med.* 2012;367: 826–833.
- Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenström Macroglobulinaemia. *Br J Haematol.* 2016;172:735–744.
- Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenstrom's macroglobulinemia. *N Engl J Med.* 2015;372:1430–1440.
- Treon SP, Meid K, Gustine J, et al. Ibrutinib shows prolonged progression-free survival in symptomatic, previously treated patients with MYD88 mutated Waldenstrom's Macroglobulinemia: long term follow-up of the pivotal trial (NCT01614821). *Proc Eur Soc Hematol.* 2018;Abstract PS1185.
- Castillo JJ, Gustine JN, Meid K, et al. Response and survival for therapy combination regimens and maintenance rituximab in Waldenstrom macroglobulinemia. *Br J Haematol.* 2018; 181:77–85.
- Dimopoulos MA, Tedeschi A, Trotman J, et al. Phase 3 trial of ibrutinib plus rituximab in Waldenstrom's Macroglobulinemia. *N Engl J Med.* 2018;378:2399–2410.
- Kastritis E, Dimopoulos MA. Proteasome inhibitors in Waldenstrom Macroglobulinemia. *Hematol Oncol Clin North Am.* 2018;32:829–840.

CLINICAL MANAGEMENT OF ACUTE AND CHRONIC PAIN IN PATIENTS WITH SICKLE CELL DISEASE

G. Finco, S. Sardo

Department of Medical Sciences and Public Health, University of Cagliari, Cagliari; Department of Surgery, Azienda Ospedaliera Universitaria di Cagliari, Cagliari, Italy

Abstract

Sickle cell disease (SCD) is a rare hereditary red cell disorder. One of the main clinical manifestations of SCD are the acute painful vaso-occlusive crisis, which are responsible for frequent accesses of SCD patients to the emergency departments (EDs). Multiple pathophysiological mechanisms cause the onset and the maintenance of pain. In SCD patients, three different pains might be recognized: the acute pain related to VOCs, the inter-VOC pain and the chronic pain. A significant number of patients develop chronic pain with characteristic of centralized or neuropathic pain. Pharmacologic treatment is a valid option in SCD pain, even if, in chronic pain it is often unsatisfactory, particularly in patients where pain is sustained by a neuropathic mechanism. Opioids don't represent the only therapeutic choice, and their use don't seem to satisfy all the patients or physicians. A multimodal analgesia is the correct approach to SCD pain, and the choice of drug must always take into consideration the site and mechanism of action of it. The non-pharmacological treatments may be an alternative and they might represent very efficacious approaches in several patients, particularly considering cognitive-behavior therapy. In this narrative review will be analyzed the mechanisms of pain involved in the SCD and the current management of acute and chronic pain.

Introduction

Sickle cell disease (SCD) is a world-wide distributed hereditary red cell disorder, emerging as global problem of public health. Patients with SCD complain pain during their all life. Indeed, Smith WR et al observe that almost 54% of SCD patients report pain on more than half of days (51%) in the studied period of time¹. SCD is characterized by both acute and chronic pains. In acute vaso-occlusive crisis (VOCs), the entrapment of dense red cells, sickled red cells and neutrophils associated with the local amplified inflammatory response result in tissue ischemic damage, promoting intense acute pain. The recurrence of VOCs in the same districts promotes the development of chronic pain. Studies in different settings indicate that the acute VOC is the most frequent cause of hospitalization of adult patients with SCD². In addition, the over half of SCD patients generally presents 1-2 episodes annually, and one percent presents more than 10 episodes annually³. Previous studies have identified SCD related acute pain of somatic and visceral origin, whereas chronic SCD related pain of neuropathic origin. VOCs are characterized by sudden onset of strong pain that involves extremities, chest, low back or joints. During VOCs can be identified four phases: prodromal, initial, established and resolution⁴. In most of SCD patients, pain is present between acute VOCs, becoming more neuropathic pain than nociceptive pain⁵.

Clinical management of pain in SCD is still a challenge and data from clinic trials are limited with several limitation, showing a partial control of both acute and chronic SCD related pains. To identify the molecules and the therapeutic strategies to be used to control pain in SCD, it is important to know the mechanisms involved in SCD pain and to define the therapeutic targets and the timing of intervention. The main scope of the present review is to discuss the main pharmacologic strategies to control acute or chronic pain in SCD based on its pathophysiological mechanisms.

Pathophysiology of SCD related pain and analgesic treatment

Sickle Cell pain (SCP) can present as acute recurrent painful crises (VOC), chronic pain syndromes and isolated neuropathic pain. These three different conditions are sustained by different mechanisms and they might all be experienced by SCD patients during their life⁶. The ischemic-reperfusion damage that characterizes acute VOCs is associated with local release of (i) pro-inflammatory mediators such as bradykinin, H⁺, serotonin, histamine, potassium ions, norepinephrine, which are able to stimulate the peripheral nociceptors (PNs); and (ii) pro-inflammatory mediators, such as leukotrienes, prostaglandins, Calcitonin Gene Related Peptide and substance P, which are able to sensitize the PNs to others inflammatory mediators. The peripheral

nociceptor activation produces an action potential that conveys the painful stimulus to the spinal cord⁵. In SCD, a proper analgesic treatment might start from the PNs and from the control and elimination of the chemical mediators that stimulate the PNs. So, it would be possible to abolish the acute pain sensation and to prevent the peripheral sensitization that establishes when the peripheral nociceptors is activated by repeated strong stimuli or by chronic stimuli. The peripheral sensitization is believed a significant cause of chronic pain⁷. Some non-opioid drugs such as Non-Steroidal Anti-Inflammatory-Drugs (NSAIDs) are able to inhibit the chemical mediators that activate PNs and, in this way, they concur to abolish pain and to prevent it becomes chronic. NSAIDs represent the first choice in the treatment of VOC, because they act on prostaglandins and there are distinct molecules and various formulations available to the physicians. This is very useful in clinical practice, because it's possible to choose the appropriate drug for each patient and to select the right administration route in the different context. Corticosteroids are mentioned in VOC treatment for their strong inhibitory activity on leukotriene, other than prostaglandin, production. However, the use of corticosteroid should be limited and used with caution since they can promote acute VOCs or severe complications of acute VOCs. A second line drug is represented by anti-histamines for their inhibitory activity on histamine. The presence of tissue peripheral opioid receptors in presence of tissue inflammation has suggested a peripheral activity of opioids⁸, which might account for the efficacy of a single dose of fentanyl buccal tablet administered during VOC episode reported in a study by De Franceschi L *et al.*⁹ Local anesthetic inhibits the transmission of action potential to spinal cord and, then, they can represent a valid choice in VOC pain with radicular distribution or limited to hands or feet or single joint. During VOC, pain is transmitted through the delta e C fibers to the dorsal horn of the spinal cord where the painful stimulus is processed and modulated based on its frequency and severity before to be transmitted to thalamus and sensory cortex and limbic system. Weak stimuli trigger the alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), while stronger and frequent pain stimuli trigger the N-methyl-D-aspartic acid receptors (NMDA). NMDA receptor activation progressively facilitates the transmission of painful stimuli to brain and it causes a neuronal overactivity. Glutamate, released by peripheral neurons, binds to NMDA receptor and causes the expelling magnesium from the receptor. The activated NMDA receptors is highly permeable to the influx of calcium. Intracellular calcium triggers a series of cellular signaling cascades that increase the neuron sensibility to pain stimuli⁶. In addition, spinal cord activity is deeply modified, since its neuronal transmission to the brain is increased, promoting the central sensitization. This results from excessive peripheral nerves stimuli, inducing neuronal hyperactivation and glia activation. Glial activation is a phenomenon depending by excessive production of glutamate by neurons, which initially activates microglia and then astrocytes. This promotes spinal inflammatory mediator production (TNF-alfa, IL-6, IL-1beta) and spinal inflammation. In turn, spinal inflammation is able to change the activity of neurons of the spino-thalamic tract and the activity of cerebral descending tract. The spinal cord loses the ability to modulate the cortical transmission of pain. The presence of central sensitization results in continuous amplification of pain sensation. Thus, patients present a reduced pain threshold with altered perception of non-painful peripheral stimuli. These patients express like hyperalgesia and allodynia. These two findings characterized the neuropathic pain, too. Today, there is an important discussion on new third mechanistic descriptor of pain in literature: the nociplastic pain¹⁰. In SCD, it might be intriguing to consider the presence of nociplastic pain, because the diagnosis of chronic neuropathic pain in SCD does not fully meet the criteria of neuropathic pain according to international guidelines.

Treatment of SCD related acute pain tries to avoid the development of chronic pain. This might be possible with the control of modulation different mechanisms at spinal cord and brain. NMDA receptors play a very important role in the central sensitization, and so they represent a major target for pain therapy. The use of ketamine, gabapentin and pregabalin could be considered because they act on NMDA receptors. Ketamine is noncompetitive antagonist of NMDA receptors, and it's considered a safe adjuvant to opioid analgesia. There is some evidence that ketamine is useful in VOC pain, but it is not clear its role in chronic SCD pain⁶. Gabapentin and pregabalin bind to the alfa-2-delta subunits of the voltage-dependent calcium ion channels, and this is the reason they block the development of hyperalgesia and central sensitization.

However, no strong evidence of a real efficacy of gabapentin and pregabalin on pain in VOC and on SCD pain are available. Thus, their use might be reserved to SCD patients with signs of hyperalgesia and in combination with other drugs working on other mechanisms of pain. Another antagonist of NMDA receptors is magnesium sulfate. Magnesium has been proven to be a successful drug in some studies for the treatment of SCD pain in children⁵.

Opioids represent the second line of therapy for acute VOC pain, but there are few evidences of efficacy on prevention of chronic SCD pain. Opioids inhibit the glutamate release by the spinal neurons and increase the activation potential of neurons, but, on the other hand, the prolonged use of opioids is one of the causes of microglia activation. Microglia activation is cause of chronic pain, and for this reason the chronic used of opioids might be considered with attention, and reserved to patients non-responding to other treatment. A different consideration deserves tramadol and tapentadol. These are opioids, but both present a two-way mechanism of action. They act on mu opioid receptors and potentiates the mono-aminergic system acting on the reuptake of mono-aminergic mediators (norepinephrine for tapentadol and norepinephrine and serotonin for tramadol) at the level of the inhibitory pain pathways. The latter is considered a very efficacious therapeutic strategy in chronic pain control, either nociceptive than neuropathic pain. There are evidence that tramadol is effective in VOC pain where it can be used in iv infusion and orally in chronic SCD pain¹¹. Tapentadol might be used per os in chronic SCD pain and for longer period of time without risk of addition¹².

Finally, NSAIDs might be extremely useful against pain of neuroinflammatory origin. In fact, spinal neuroinflammatory pain is characterized by the local release of prostaglandins and COX2. The following profile of action is required: to be able to cross the BBB and to display a major action on COX2. The large part of NSAIDs such as diclofenac, indometacin, ketorolac, ibuprofen, celecoxib, etorocoxib has these properties, thus physician might choose between one of them based on patient's profile.

Therapeutic strategies for clinical management of pain in SCD patients

Thus, in SCD patients are present three different types of pains:

1. acute pain during VOC crisis
2. inter episode VOC crisis pain
3. chronic pain

These three types of pains need a different analgesic strategy since pain with distinctive phenotypical characteristics respond to specific pharmacological treatments (Table 1).

1. Acute pain during VOC crisis. During VOC crisis, pain is predominantly nociceptive (somatic and visceral), acute and severe. However, the ischemic-reperfusion damage locally related to vaso-occlusive event, increases the complexity of clinical management of pain in SCD patients. The development and viability of protocols/recommendations or guidelines to treat acute pain in SCD patients is crucial to avoid more severe complications such as the acute chest syndrome. NSAIDs are key molecules to deal with acute pain in SCD patients. The infusion route should be chosen to gain a more rapid analgesic effect. Among NSAIDs, ketorolac seems to be effective in controlling acute pain in SCD. However, the combination with an opioid (i.e. tramadol or morphine or fentanyl) as multimodal analgesia represent an interesting strategy to control acute pain of different origins in SCD. Intravenous infusion of opioid(s) is the route generally used during acute VOCs. Whereas oral route is generally used in home-therapy (i.e codeine or oxycodone). Noteworthy, opioids are generally prescribed at sub-therapeutic or low-therapeutic range, which might be responsible for partial efficacy of opioid in pain control⁶. The revision of the literature points out that there is not clear advantage between the different opioid-sparing modalities (i.e.: intermittent, continuous or patient controlled analgesia). We recently show that transbuccal fentanyl used as pain breaking drug in combination with multimodal analgesia with ketorolac and tramadol showed a better management of pain and a greater satisfaction of patients compared with ketorolac and tramadol alone. Similar results were also reported with intranasal fentanyl in SCD children presenting in the emergency department for VOC. In Italy, the scientific-Italian-Society for the study of Thalassemias and Hemoglobinopathies (SITE) algorithm for treatment of VOC pain rec-

commend as first line the use of intravenous tramadol. Tramadol is a centrally acting analgesic drug with a dual mechanism of action as a weak opioid agonist and an inhibitor of serotonin and noradrenaline re-uptake. The latter action might reduce the central sensitization to pain, and it might justify the powerful analgesic activity of tramadol as reported in literature by several studies^{9, 11}. Other drugs may be also used during VOC, as ketamine or gabapentin or pregabalin, to control the pain and to avoid excessive opioid doses. Particularly, ketamine, a non-competitive antagonist of NMDA receptors, is considered a useful adjuvant to opioid analgesia in acute and chronic pain. In literature there are few studies reporting the efficacy of intravenous ketamine in acute VOC pain, and the results, even if it was reported a good pain control, were insufficient to recommend its use. The same considerations must be done for gabapentin and pregabalin, two antiepileptic drugs employed in acute and chronic pain that have synergism with opioids. There are no data on their use in SCD patients, but they may represent a new treatment strategy because they inhibit central sensitization acting on calcium ion channels that modulates NMDA receptor activity. An interesting non-pharmacological intervention is represented by transcutaneous electrical nerve stimulation. This procedure seems to ensure a beneficial analgesia during VOC and it reduces the opioid doses.

2. Inter episode VOC crisis pain. The large part of patients with SCD presents continuous pain between acute VOC. The pain is usually moderate in intensity but it is "stiffing"⁹. The first choice in treatment of this pain is the use of weak opioid such as codeine or tramadol. These drugs present different pharmacological characteristic: codeine has only an opioid activity, tramadol has a dual activity as above mentioned. Their efficacy is ameliorated by the addition of acetaminophen or NSAID. Their use is for oral route, which might be continuous at the minimum effective dose or intermittent at patient demand. A possible non-pharmacological intervention might be also considered cognitive behavioral therapy, biofeedback and hypnosis are efficacious in these patients and they might always be tried in the therapeutic approach.

3. Chronic pain. The pain in SCD patients often becomes chronic. The characteristics of pain change during the time and this is due to peripheral and central sensitization. So, the pain progressively presents features of neuropathic type. Today, there is a great discussion on the definition of this kind of pain, because the pain presented by some patients not satisfies completely the features reported in the definition of neuropathic pain. Several patients do not present evidence "for disease or lesion of the somatosensory system causing the pain" (International Association for the Study of Pain – IASP- Taxonomy 2017). Neither they present a pain that arises from altered nociception due to clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors. In these patients we see the presence of pain with these features: "Pain that (1) arises from altered nociception despite no (2) clear evidence of actual or threatened tissue damage causing activation of peripheral nociceptors or (3) evidence for disease or lesion of the somatosensory system causing the pain". This is the definition of a third mechanistic descriptor of pain introduced by IASP Taxonomy: NOCIPLASTIC PAIN.

This definition of pain is very interesting for SCD, because it satisfies the necessity to classify the chronic pain presented by several pain and to choose the best pharmacological treatment. In presence of nociplastic pain, the first line of therapy might be represented by drugs that modulate the central sensitization and peripheral sensitization. The first line of treatment is antiepileptic drugs, as gabapentin and pregabalin. They block the NMDA receptor activation binding the calcium ion channels. Other antiepileptic drugs binding the sodium ion channels on primary afferent sensory nociceptive neurons could be useful to reduce and modulate the afferences of painful stimuli to spinal cord. The inhibitors of monoamine neurotransmitter re-uptake are the first and the second line treatment. These are efficacious because they strengthen the modulatory activity of brain on pain. Data from the literature suggest that duloxetine, amitriptyline, imipramine are more efficacious than other anxiolytic or tricyclic drugs in the treatment of chronic pain. Opioids are a third line of treatment, because their efficacy is not sustained by univocal data in literature and they present a lot of side effects that restrict their long period use. However, tapentadol, an opioid agonist, is a valid therapeutic choice, because it potentiates the mono-aminergic system acting on the re-uptake of norepinephrine mediator and its employment is characterized by a low incidence of side effects¹².

Finally, a non-pharmacological intervention such as cognitive behav-

ioral therapy, biofeedback and hypnosis might represent an interesting and important tool in management of chronic pain in patients with SCD. These strategies might be complementary to classic pharmacologic approaches. Thus, the therapy of chronic sickle cell related pain might be again a multimodal analgesia, because each drug acts on different mechanisms of pain and it's important to block as much as possible its pathogenesis, reducing drug side effects.

Conclusion

The treatment of sickle cell related pain represents a challenge in several patients. The pharmacological therapy for acute pain in VOC is effective by using multimodal analgesia. Whereas, the treatment of chronic SCD pain is often unsatisfactory, particularly in patients where pain is sustained by a neuropathic mechanism. A multimodal analgesia is the correct approach to acute and chronic SCD pain, and the choice of drug must always take into consideration the pain site and the mechanism of action. The non-pharmacological treatments may be an alternative and very efficacious approaches in several patients, and, their use and knowledge might be disseminated within physician and the SCD patient community.

Table 1. Treatment of pain in SCD patients.

Type of pain	Analgesic regimen	Route and mode of administration	
Acute pain during VOC crisis	- NSAIDS	- Intermittent i.v.	
	- NSAID plus tramadol	- Continuous i.v.	
	- NSAID plus tramadol plus fentanyl	- Continuous i.v. and sublingual	
	- NSAID plus morphine	- Continuous i.v.	
	- Opioids (morphine, oxycodone, hydrocodone)	- Oral in home setting Intermittent or continuous i.v. in Emergency Department	
Inter episode VOC crisis pain	- Weak opioids (codeine, tramadol)	- Oral, around-the-clock	
	- Weak opioids plus acetaminophen or NSAID	- Oral, around-the-clock	
	- Non-pharmacological intervention: cognitive behavioural therapy biofeedback hypnosis		
Chronic pain	1. Nociceptive pain	- Weak opioids (codeine, tramadol)	- Oral, around-the-clock
		- Weak opioids plus acetaminophen or NSAID	- Oral, around-the-clock
		- Opioids (morphine, oxycodone, hydrocodone, fentanyl, buprenorphine)	- Oral, transdermal, around-the-clock
		- Anxiolytics, antidepressants (duloxetine, amitriptyline)	- Oral, around-the-clock
		- Non-pharmacological intervention: cognitive behavioural therapy biofeedback hypnosis	
	2. Neuropathic or Nociplastic pain	- Antiepileptics (gabapentin, pregabalin)	- Oral, around-the-clock
		- Anxiolytics, antidepressants (duloxetine, amitriptyline)	- Oral, around-the-clock
		- Opioids (morphine, oxycodone, hydrocodone, fentanyl, buprenorphine)	- Oral, transdermal, around-the-clock
		- Non-pharmacological intervention: cognitive behavioural therapy biofeedback hypnosis	

References

- Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman D, Aisiku IC, Levenson JL and Rosef SD. Daily Assessment of Pain in Adults with Sickle Cell Disease. *Annals of Internal Medicine* 2008; 148: 94-101.
- Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology, and prognostic significance. *Am J Hematol.* 2005; 148 (2): 17-25.
- Platt OS, Thoringhton BD, Brambilla DJ, Milner PF, Rosse WF, Vichinsky E and Kinney TR. Pain in sickle cell disease. Rates and risk factors. *N Engl J Med.* 1991; 325: 11-16].
- Ballas SK, Gupta K, Adams-Graves P. Sickle cell pain: a critical reappraisal. *Blood.*

- 2012; 120(18): 3647-56.
5. Wang ZJ, Wilkie DJ and Molokie R. Neurobiological mechanisms of pain in sickle Cell Disease. *Hematology Am Soc Hematol Educ Program*. 2010; 2010: 403-08.
 6. Puri L, Nottage KA, Hankins JS, Angheliescu DL. State of art management of acute vaso-occlusive pain in sickle cell disease. *Pediatr Drugs* 2018; 20: 29-42.
 7. Ji RR, Xu ZZ, Gao YJ: Emerging targets in neuroinflammation-driven chronic pain. *Nat Rev Drug Discov* 2014; 13:533-48.
 8. Stein C and Lang LJ. Peripheral mechanisms of opioid analgesia. *Curr Opin Pharmacol* 2009; 9(1): 3-8.
 9. De Franceschi L, Mura P, Schweiger V, Vencato E, Quaglia FM, Delmonte L, Evangelista M, Polati E, Olivieri O, Finco G. Fentanyl buccal tablet: a new breakthrough-pain medication in early management of severe vaso-occlusive crisis in sickle cell disease. *Pain Pract* 2016;16(6):680-7.
 10. Kosek E, Cohen M, Baron R, Gebhart GF, Mico JA, Rice ASC, Rief W, Sluka AK. Do we need a third mechanistic descriptor for chronic pain states? *PAIN* 2016;157:1382-86.
 11. de Franceschi L, Finco G, Vassanelli A, Zaia B, Ischia S, Corrocher R. A pilot study on the efficacy of ketorolac plus tramadol infusion combined with erythrocytapheresis in the management of acute severe vaso-occlusive crises and sickle cell pain. *Haematologica* 2004; 89(11): 1389-91 [de Franceschi 29015.
 12. Finco G, Mura P, Musu M, Deidda C, Saba M, Demelas I, Evangelista M, Sardo M. Long-term, prolonged-release oral tapentadol for the treatment of refractory chronic low back pain: a single-center, observational study. *Min Med*. 2018; 109(4): 259-65.

CLINICAL MANAGEMENT OF SICKLE CELL DISEASE IN PREGNANCY

V. Pinto, G.L. Forni

Centro della Microcitemia e delle Anemie Congenite, E.O. Ospedali Galliera, Genova, Italy

Background

Sickle cell disease (SCD) is an inherited red cell disorder caused by a single amino acid substitution at the sixth residue of the beta-globin subunit (Glu6Val), which results in the production of the characteristic defective form of hemoglobin S (HbS).

SCD is one of the most important hemoglobinopathies worldwide in terms of frequency and social impact. Indeed, SCD has recently been recognized as a global public health problem by the World Health Organization (WHO) and the United Nations (UN). In the last decade, the burden of SCD has continued to grow due to: (i) the improvement in infant and child survival in high prevalence, low and medium income countries; and (ii) the migration of carriers and sickle subjects from endemic areas to higher income areas such as Europe and North America.¹ It has been estimated that approximately 300,000 newborns are affected each year, and that 75% of them are born in Sub-Saharan Africa where SCD is endemic and the carrier frequency ranges from 10 to 40%. In Western countries, 100,000 SCD patients have been reported in the US alone. In Europe, the number of subjects affected is estimated to be between 20,000 and 25,000, but numbers are steadily rising due to migration patterns.²

The main clinical manifestations of SCD are chronic hemolytic anemia and recurrent acute vaso-occlusive crisis (VOC), which are characterized by pain and ischemic/reperfusion organ damage. These are related to the entrapment of the sickled, dense red cells in the microcirculation associated with increased inflammatory response, vascular endothelial cell damage, and activation of the coagulation system. The combination of these factors promotes a prothrombotic state that characterizes SCD. Previous studies have shown: (i) enhanced thrombin generation; (ii) accelerated factor VII turnover; and (iii) activation of intrinsic coagulation pathway with decreased levels of factor XII and IX in steady state conditions. The prothrombotic profile of SCD is also enhanced by low levels of protein C and S, and an increase in plasma levels of plasminogen activator inhibitor (PAI-1).

Pregnacy and Sickle Cell Disease

In Western countries, improvements in the clinical management of SCD patients have meant that the percentage of affected infants who survive to adulthood in industrialized countries has risen to over 95%. Therefore, the number of SCD women reaching reproductive age is increasing.

Previous studies have shown that SCD-related mortality rates during pregnancy are higher in lower income countries, even if clinical management, obstetric care and intensive post-natal support have had a beneficial effect on pregnancy in SCD. More admissions to neonatal intensive care units (NICU) and an increase in perinatal mortality rates have also been reported in large SCD population studies.³⁻⁴ Therefore,

pregnancy in SCD women is considered high risk for patients and newborns. All women with SCD should be made aware of the potential morbidity and mortality associated with pregnancy, ideally prior to conception.

In SCD women, a limited number of studies are available on the pathophysiology of pregnancy. Pregnancy is characterized by increased metabolic demand, increased tendency toward vascular stasis and a pro-coagulant state. In addition, decreased functional residual capacity and residual volume, together with increased cardiopulmonary demand may promote underlying cardiopulmonary dysfunction in SCD women which compromises the cardiopulmonary system. In addition, ischemic-reperfusion damage due to placenta vaso-occlusive events (villous sclerosis, intravillous fibrin deposit, and sickling) leads to uteroplacental insufficiency, intrauterine growth retardation (IUGR), and low birthweight. Thus, pregnancy in SCD requires a multidisciplinary management program including the early detection and treatment of complications during pregnancy and post-partum, follow up by obstetric and sickle cell teams, and appropriate pain management protocols.

Based on the revision of the literature, three main groups of complications have been identified:

a) the severe clinical manifestations related to SCD such as recurrent VOCs or acute chest syndrome (ACS);

b) the medical complications of pregnancy, such as infections (i.e., urinary tract infection, pneumonia, sepsis) or thromboembolic events (i.e. deep vein thrombosis, pulmonary embolism, cerebral vein thrombosis);

c) the obstetric complications, such as gestational hypertension (PIH), pre-eclampsia, eclampsia, abruption, IUGR, small-for-gestational-age newborns, miscarriage, stillbirth, and preterm labor.

The course of pregnancy in SCD patients is very difficult to predict given the extreme variability of the clinical phenotypes involved. Factors such as frequent hospitalizations for VOC and/or episodes of ACS, renal insufficiency, prior thromboembolism and pulmonary hypertension may correlate with poorer overall health and increased risks during pregnancy. Thus, ideally, an assessment for chronic disease complications should be made prior to conception, e.g. screening for pulmonary hypertension with echocardiography, for iron overload with MRI-T2*, for red cell antibodies, for hypertension and/or proteinuria, for nephropathy and/or hepatic dysfunction, for proliferative retinopathy. These will help differentiate between women with SCD with mild phenotype from those with a more severe phenotype (i.e. with organ damage) who require closer monitoring and a more aggressive therapeutic approach.

Perinatal mortality and stillbirth rates are high in SCD patients, ranging from 0 to 20 events per 100 births. The literature reports large variations in maternal mortality, ranging from 0.07% in the US to over 9% in less developed countries where incidence of stillbirth or perinatal death also appear to be higher.

Cinical care of SCD women during pregnancy

Sickle cell disease women should be encouraged to attend regular consultation sessions with hematology and obstetric-sickle clinics bi-weekly up to 28 weeks of gestation, and weekly thereafter. Regular monitoring should include urinalysis and blood pressure monitoring. Urinalysis and urine culture should be performed once monthly to screen for asymptomatic bacteriuria, which should be treated immediately should the woman test positive. Monthly monitoring should be continued thereafter and suppressive treatment prescribed if necessary. Since SCD women are at high risk of pre-eclampsia and eclampsia, their blood pressure should be checked daily and a monthly 24-hour urine protein test should be performed. Although there is no specific evidence for the role of aspirin in SCD, it has been shown to decrease the risk of pre-eclampsia in high-risk pregnancies and a daily 75-mg dose of oral aspirin may be appropriate after week 12 of gestation unless there are contra-indications to its use; however, there is still no consensus on this.

The risk of venous thromboembolism (VTE) increases 5-fold during pregnancy with SCD women showing a higher prevalence of deep vein thrombosis. Placental pathology indicates the presence of ischemic-reperfusion damage and local thrombosis. However, there is no consensus on indication and timing of anticoagulant medications to prevent thrombotic complications of SCD, and studies have included a small sample size with no control group. Women with a history of VTE

or hospitalized with acute medical problems should receive anticoagulation prophylaxis with low molecular weight heparin (LMWH) during pregnancy and the post-partum period. During pregnancy, severe infection might result in life-threatening SCD-related VOC. Therefore, prompt antibiotic treatment of urinary tract infection is required, i.e. amoxicillin-sulbactam renal adjustment. Daily administration of 5 mg folic acid is appropriate throughout pregnancy. Iron supplementation is indicated only to those with proven iron-deficiency anemia. During the pregnancy, any ongoing iron-chelating therapy should be discontinued.

Careful monitoring of fetal growth by serial ultrasonography combined with determination of arterial impedance (umbilical arterial Doppler) is recommended for the risk of fetal growth restriction.

Transfusion regimens during pregnancy

The prophylactic role of transfusion regimens (single transfusion, partial exchange or automatic erythrocytapheresis, ECP) to reduce sickle cell-related clinical complications during pregnancy and improve fetal and maternal outcome is based on small and non-prospective controlled trials. This makes statistical evaluation of the data difficult and no consensus has yet emerged. A literature review reveals that studies were retrospective, except for one prospective randomized controlled study, and involved groups of between 14 and 128 SCD women. A recent meta-analysis demonstrated an association with prophylactic transfusion and reduction in maternal mortality and sickle-specific and neonatal complications, including acute pain crises, pulmonary complications, pyelonephritis, perinatal mortality, neonatal death and pre-term birth.⁵ Starting time of transfusion regimens differed widely (22-28 weeks of gestation). More recently, a double-center retrospective cross-sectional study on 46 single pregnancies in SCD women with history of severe sickle cell-related clinical manifestations treated with early (10.7±5.2 weeks of gestation) and anticoagulation prophylaxis with enoxaparin showed that an early ECP program was associated with fewer medical and obstetric complications compared with previous studies on pregnant SCD women treated with transfusion regimens starting between 22 and 28 weeks of gestation.⁶

On current evidence, prophylactic transfusion is not recommended as routine practice in pregnant women with SCD with mild hematologic phenotype. Prophylactic transfusion should be reserved for women with severe sickle cell-related clinical manifestations or pre-eclampsia and eclampsia.

In the acute setting, transfusion may be required for the management of anemia or for the treatment of acute complications such as ACS.

Testing women with SCD who have been transfused and are anticipating pregnancy for red blood cell (RBC) alloantibodies is recommended to promote the search for an extended RBC phenotype match, especially in a European context where blood donors are mainly Caucasian for recipients of African descent.

Management of vaso-occlusive crisis and vascular complications during pregnancy

During pregnancy, approximately 27-50% of SCD patients experience painful episodes. These are more frequent in the third trimester and are associated with poor perinatal outcomes. A pregnant woman with SCD who presents with pain should be evaluated for other complications. She should be hospitalized, and receive prompt and aggressive pain relief medication. Bed rest and adequate fluid intake are required; paracetamol and non-steroidal anti-inflammatory drugs may be given, if necessary, between weeks 12 and 18 of gestation. Narcotic analgesics may be used in case of persistent pain.

Acute chest syndrome is the most common severe complication in SCD women during pregnancy occurring in 7-20% of SCD patients who are not on a chronic transfusion regimen. Prompt antibiotic therapy, hydration, oxygen support and transfusion (simple transfusion or exchange transfusion according to Hb level) are all required.

In case of stroke, exchange transfusion should be performed as soon as possible. Manual RBC exchange transfusion is preferable in case of brain hemorrhage to avoid bleeding risk due to the anticoagulants used in an automatic separator. The Italian Society of Thalassemia and Hemoglobinopathies (SITE) has published comprehensive and detailed guidelines for the management of VOC and SCD complications.^{7,8}

Management of hematologic complications during pregnancy in Sickle Cell Disease women

Anemia and reduced reticulocyte count should drive suspicion of parvovirus infection with related fetal involvement. Thrombotic thrombocytopenic purpura (TTP) should be differentiated from HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome and treated *via* plasma exchange and emergency delivery.

Delivery

Decisions as to timing and mode of delivery should be made in consideration of obstetric indications. If there are no contraindications or severe SCD-related organ damage, SCD women might go into labor spontaneously with normal vaginal delivery. However, continuous fetal heart monitoring is recommended due to an increased rate of still births, placental abruption and insufficiency. Oxygen support should be provided for the mother during labour to maintain oxygen saturation ≥94% and avoid fetal distress. The woman should be kept warm, and adequate hydration should be maintained. Use of steroids should be avoided unless these are required for fetal health. There appears to be an increase in painful episodes and ACS in the intrapartum period in SCD which increases if labor is prolonged. In 2008, Villers *et al.* reported a mortality rate for women with SCD of 72.4 deaths per 100,000 deliveries, compared with 12.7 deaths per 100,000 deliveries for women without SCD; an increased risk of intrauterine fetal death was not found in the study. In addition, the adverse social context of pregnant SCD women, the large part of whom are immigrants from Sub-Saharan African countries, contributes to the increased risk of maternal death.⁹ Transfusion is indicated to control bleeding according to obstetric indications. The rate of cesarean delivery is higher in women with SCD than in women without. In case of cesarean delivery, preoperative transfusion therapy to increase hemoglobin levels to 10 g/dL and HbS <40% is strongly recommended in patients with SCD. Hydration and oxygen support should be maintained in patients with chronic pulmonary disease and in those who have experienced cerebrovascular events or multi-organ failure. Women with SCD have a risk of thrombosis comparable to that of patients with high-risk thrombophilia. Thromboprophylaxis is recommended; LMWH should be given and compression bandages applied after cesarean section.

Post-partum care

Risk of VOC, VTE and infection continues into the postnatal period. Therefore, close monitoring of these adverse events, their early detection and prompt therapy is recommended. Women with SCD should receive ten days post-partum thromboprophylaxis with LMWH, while women with SCD who received antenatal LMWH should receive six weeks of post-partum thromboprophylaxis. Pulmonary rehabilitation programs should be encouraged to prevent atelectasis after delivery. There is no contraindication to breastfeeding. However, women who were taking hydroxyurea before conception should not recommence this until they stop breastfeeding.

Conclusions

Due to improvements in the clinical management of SCD patients, a growing number of SCD women wish to become pregnant. They should be supported in this by promoting screening of their partner and reviewing their own disease status, ensuring that chronic complications of SCD are under control. Couples considering pregnancy should be counseled about the potential risk of having a child affected with SCD if the partner has SCD, HbS and/or beta-thalassemia trait or is carrier of another abnormal hemoglobin such as HbC. They should be informed of the possibility of prenatal diagnostic testing for SCD by chorionic villus sampling in the first trimester or by amniocentesis in the second trimester. Another risk that should be discussed with the couple is the potential fetal and neonatal effects of maternal medications, i.e. chronic opioids and angiotensin-converting enzyme inhibitors, which are used respectively to treat pain and microalbuminuria and should be discontinued during pregnancy. Hydroxyurea is teratogenic in animals but has not been associated with an increased risk of birth defects in infants under *in utero* exposure; nonetheless, it should be discontinued during pregnancy, and ideally

prior to conception. During pregnancy, SCD poses problems to both mother and fetus. Despite the improvement in maternal and fetal outcomes in SCD seen in recent years, it must be remembered that women with SCD remain at high risk of adverse events that can arise from chronic underlying organ dysfunction such as renal disease or pulmonary hypertension, from acute complications of SCD such as VOC and ACS, and/or from pregnancy-related complications. Risks of adverse fetal outcomes are reduced but not eliminated through fetal surveillance. The key point is that pregnancy in SCD requires a multidisciplinary management program for early identification and treatment of acute and chronic clinical manifestations and an experienced team of specialists in family medicine, general internal medicine, pediatric and adult hematology, psychiatry and mental health, transfusion medicine, obstetrics and gynecology, and dedicated nursing staff.

Table 1. Management of SCD Patients During Pregnancy.

INITIAL EVALUATION OF PREGNANCY	
<ul style="list-style-type: none"> Collect patient's history and data, including complications related to SCD (episodes of ACS, stroke, renal dysfunction, pulmonary hypertension) and prior VOC with hospitalizations, history of transfusions and alloimmunizations, VTE, spleen status and vaccinations. Evaluate smoking habits, alcohol use, opioid dependence. Evaluate ongoing medication, and discontinuation of hydroxyurea therapy, chelating agents, and ACE inhibitors. Assess iron stores and prescription of iron supplementation only in case of iron-deficiency anemia, and administration of 5 mg folic acid. Genetic testing and counseling of the couple. 	
CARE DURING PREGNANCY	
Timing of consultations and tests	<ul style="list-style-type: none"> Regular consultation with hematology and obstetric-sickle clinics, bi-weekly up to 28 weeks of gestation, then weekly. Monthly urinalysis and urine culture in asymptomatic patients. Daily monitoring of blood pressure and monthly 24-hour urine protein test. Ultrasonography combined with determination of arterial impedance to monitor fetal growth.
Prophylaxis and treatment	<ul style="list-style-type: none"> Prompt antibiotic treatment of infection (particularly urinary tract infection) and fever.¹⁰ Prophylaxis with daily 75-mg dose of oral aspirin after week 12 of gestation unless there are contraindications to its use. Anticoagulation prophylaxis with LMWH in case of history of VTE or hospitalization with acute medical problems.
Transfusion regimens	<ul style="list-style-type: none"> Prophylactic transfusion regimens (single transfusion or ECP) in case of SCD-related pre-eclampsia/eclampsia or organ damage, before delivery by cesarean section. Note: target Hb 9-11 g/dL, Target HbS < 40%. Transfusion regimens (single transfusion or ECP) in acute setting: ACS, anemia, splenic sequestration. <p>Note: an extended RBC phenotype match is recommended (risk of alloimmunization and hyperhemolytic syndrome)</p>
Management of VOC and vascular complications	<ul style="list-style-type: none"> VOC:^{7,8} evaluate pain by visual analog scale (VAS), bed rest and adequate hydration 2l/24 hours; paracetamol and non-steroidal anti-inflammatory drugs between 12 and 18 weeks of gestation, narcotic analgesics may be used in case of persistent pain; LMWH (prophylaxis dosage). The patient should be monitored every 30 min for respiratory rate, sedation status, and pain. Hospitalization is recommended. ACS:⁹ antibiotics (intravenous broad spectrum), oxygen support to maintain oxygen saturation >95%, hydration 2l/24 hours (avoid overhydration), LMWH (prophylaxis dosage), single transfusion if Hb is < 7 g/dL or ECP with Hb target 30% if Hb > 8 g/dL, pain management.⁷ Note: consider transfer to ICU in case of increasing respiratory distress, signs of multisystem organ failure, decline in Hb concentration (despite simple transfusion), onset of confused state, sepsis. Stroke:⁸ hydration i.v. 1500 cc/24hours (avoid overhydration), consult neurological specialist, ECP. Note: in case of hemorrhagic stroke, manual erythroexchange is preferable to avoid bleeding risk due to anticoagulants used in an automatic separator.
DELIVERY	
Vaginal delivery	<ul style="list-style-type: none"> During labor check hemodynamic parameters, oxygen saturation. Adequate i.v. Hydration. Keep warm. Avoid use of steroids (increased risk of VOC) unless fetal health requires. Oxygen support should be provided during labor (to maintain oxygen saturation ≥ 94%). Transfusion is indicated to control bleeding according to obstetric indication.
Cesarian section	<ul style="list-style-type: none"> Preoperative transfusion therapy to increase Hb levels to 10 g/dL and HbS <40% is strongly recommended. Follow the intra- and post-operative management guidelines for SCD to minimize hypoxia, hyperthermia, acidosis, and intravascular volume depletion.⁷
POSTPARTUM CARE	
<ul style="list-style-type: none"> Maintain i.v. hydration (20 mL/Kg/die) and oxygen support. Thromboprophylaxis with LMWH for 10 days post partum (6 weeks post partum) in women who received antenatal thromboprophylaxis). Application of elastic compression bandages for varicose veins. Prompt antibiotic treatment of fever.¹⁰ Early mobilization. Pulmonary rehabilitation programs. 	

SCD: sickle cell disease; ACS: acute chest syndrome; VOC: vaso-occlusive crisis; VTE: venous thromboembolism; ACE: angiotensin-converting enzyme; LMWH: low-molecular-weight heparin; ECP: erythrocytapheresis; VAS: visual analog scale; RBC: red blood cell; Hb: hemoglobin; ICU: intensive care unit; HbS: hemoglobin S; i.v.: intravenous; min: minutes.

References

- Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global Burden of Sickle Cell

- Anaemia in Children under Five, 2010–2050: Modelling Based on Demographics, Excess Mortality, and Interventions. *PLoS Med.* 2013;10(7):e1001484.
- De Franceschi L, Lux C, Piel FB, et al. Access to emergency department for acute events and identification of sickle cell disease in refugees. *Blood.* 2019;133(19):2100-2103.
- Villers MS, Jamison MG, De Castro LM, James AH. Morbidity associated with sickle cell disease in pregnancy. *Am J Obstet Gynecol.* 2008;199(2):125.e1-5.
- Oteng-Ntim E, Meeks D, Seed PT, et al. Adverse maternal and perinatal outcomes in pregnant women with sickle cell disease: systematic review and meta-analysis. *Blood.* 2015;125(21):3316-3325.
- Malinowski AK, Shehata N, D'Souza R, et al. Prophylactic transfusion for pregnant women with sickle cell disease: a systematic review and meta-analysis. *Blood.* 2015;126(21):2424-2435.
- Vianello A, Vencato E, Cantini M, et al. Improvement of maternal and fetal outcomes in women with sickle cell disease treated with early prophylactic erythrocytapheresis. *Transfusion.* 2018;58(9):2192-2201.
- De Franceschi L, Graziadei G, Rigano P, Cianciulli P, Forni GL. [Raccomandazioni per la gestione del paziente adulto affetto da anemia falciforme della Società Italiana Talassemie ed Emoglobinopatie - SITE.] *Collana Scientifica S.I.T.E.* Available from: http://site-italia.org/file/collana_scientifica/libretto_2_2014/download.php?file=Collana_scientifica_SITE_n.2_2014 Last accessed 25 Feb 2019.
- Forni G, Balocco M, Casale M, et al. [Algoritmo per la gestione in pronto soccorso degli eventi acuti nei pazienti affetti da Anemia Falciforme.] *Collana Scientifica S.I.T.E.* Available from: http://www.site-italia.org/file/Triage_SCD Last accessed 25 Feb 2019.
- Lesage N, Deneux Tharax C, Saucedo M, et al. Maternal mortality among women with sickle-cell disease in France, 1996-2009. *Eur J Obstet Gynecol Reprod Biol.* 2015;194:183-188.
- Casale M, De Franceschi L, Balocco M, et al. [Algoritmo interattivo Gestione del rischio infettivo nel paziente splenectomizzato o con asplenia funzionale.] *Collana Scientifica S.I.T.E.* Available from: http://site-italia.org/download.php?file=Management_Asplenici Last accessed 25 Feb 2019.

UP TO DATE: ALLOIMMUNIZATION

D. Venturelli

Department of Transfusion Medicine, University Hospital Modena, Modena, Italy

Abstract

Alloimmunization is a major complication of transfusion therapy in sickle cell disease (SCD). The antigen disparity between donors, who are primarily of Caucasian origin and patients, who are primarily of African ancestry, is the major cause of alloimmunization in SCD. Red blood cell (RBC) alloantibodies are produced in up to 60% of adult patients with SCD despite prophylactic phenotype matching (e.g., C/c, E/e, K antigens). Evidence suggests that SCD individuals may be high- or low- responders depending on their propensity to produce antibodies. Thus, besides RBC transfusion burden/antigen exposure other variables such as chronic inflammation influence alloimmunization in SCD. In addition, alloimmunized SCD subjects are prone to developing severe delayed hemolytic transfusion reactions (DHTRs), which are often unrecognized or lately diagnosed because their clinical presentation can mimic a classic sickle cell related vasoocclusive crisis. Currently, there are no standardized approaches for clinical management of alloimmunization in SCD. The development of strategies aimed at improving the frequency of blood donations among individuals of African origin is essential to enhance the donor pool of antigen-matched blood and to prevent the development of alloimmunization in SCD patients. This review will discuss the main progresses in the knowledge of alloimmunization in SCD, the clinical management of DHTRs and the risk/contingency plans to reduce alloimmunization in SCD patients requiring transfusion regimen.

Background

Red blood cell (RBC) transfusion, either simple or by exchange, is an established treatment for both acute and chronic complications of sickle cell disease (SCD). Its potential benefits include correction of anemia, reduction of hemolysis, suppression of HbS synthesis, and decrease in the number of circulating sickle cells.¹ Thus, the majority of SCD patients receive RBC transfusions during their lifetime.

In SCD, RBC transfusions represent a significant challenge in clinical transfusion medicine because alloimmunization is a serious and frequent complication of transfusion therapy. Previous studies have shown that the effectiveness of the antibody response in SCD patients is determined by a complex interplay of genetic and circumstantial factors at the time of transfusion such as the existence of chronic inflammatory

conditions with significant immune activation.²

In SCD, the prevalence of alloimmunization ranges from 7% to 59% compared to 2-3% of sporadically transfused non-SCD patients and it is significantly higher than any other heavily transfused population, such as transfusion-dependent thalassemia patients. The major cause of alloimmunization in patients with SCD is the diverse distribution of red cell antigens between donors, who are primarily of European/Caucasian ancestry and SCD patients who are primarily of African ancestry.³ Indeed, studies in Uganda and Jamaica, where donors and SCD patients are ethnically more homogeneous, show an alloimmunization rate respectively of 6.1% and 2.6%. This are comparable to alloimmunization frequencies reported for the general population of these 2 countries (1%-6%).⁴

Different factors have been recognized to be responsible for the higher alloimmunization rate of SCD patients and are reviewed and discussed below.

Different levels of RBC polymorphism(s) can be detected in SCD patients related to common antigens such as C, E, Fya, Jkb and S

Compared to donors, who are mostly of Caucasian descent. The corresponding antibodies are readily detectable in pre-transfusion screening tests. Noteworthy, over two-thirds of alloantibodies produced by SCD patients have RH blood group specificities. Genetic analysis of SCD patients who develop antibodies despite RH- matched RBCs has revealed that these patients carry altered Rh alleles. Individual of African descent have a high degree of genetic variation in the RH locus and have RH variants encoding the so-called partial Rh antigens that lack certain immunogenic epitopes of the normal antigen. These "partial D patients" may therefore develop anti-Rh antibodies against those missing epitopes when transfused with RBCs carrying the entire RH antigen. In patients with SCD, many partial D, C and E antigens have been described and high-resolution RH genotyping revealed variant alleles in 87% of individuals which contribute to Rh immunization.⁵

Specific rare blood groups are also present in these SCD patients

Since they could lack an antigen that is expressed in almost all donor RBCs (defined as high-incidence antigen). The main rare blood groups in individuals with SCD are in the RH (absence of Hrs, Hrb or HR46), KEL (absence of JsB) and MNS (absence of U) systems. Individuals with such rare blood groups are at increasing risk of alloimmunization because of the high prevalence of the missing antigen in the donor population and these antibodies may be associated with possible development of severe post-transfusion complication such as the delayed hemolytic transfusion reaction (DHTR).⁴

Clinical evidence indicates that the risk for the first alloimmunization increases with transfusion exposure. Half of the alloimmunized SCD patients produce their first alloantibody before the 8th transfusion. Receiving the first lifetime transfusion after the age of 5 is an independent risk factor for alloimmunization. In addition, in patients with SCD episodic transfusions as compared to chronic transfusions were associated with an increased risk of alloimmunization.⁶ In SCD, the peculiar presence of RBC autoantibodies might be the strongest risk factor for the development of RBC alloantibodies compared to general population. Table 1 summarizes the main factors known to be involved in increasing the alloimmunization rate in SCD.

State of the art

In SCD, management of alloimmunization has not been standardized yet and remains a widely debated topic/issue within the scientific community. Up to now, extended antigen matching for Rhesus (Rh) phenotype and Kell (K) has significantly reduced the rate of alloimmunization, and additional antigen matching for Fy, Jk, and MNS appears to be even more effective. Receiving the first transfusion even after extended matching for at least RhCE and K appeared to be protective for alloimmunization in SCD. A study by Chou et al demonstrated that 58% of chronically transfused SCD patients were alloimmunized despite phenotypic matching for Rh phenotype and K, because of the high genetic variation in Rh genes in both the SCD population and African American donors.⁵

A promising strategy to reduce alloimmunization in SCD is prophylactic matching including the use of genotype-matched RBCs. Extended red cell antigen profiling of both patients and blood donors by DNA-array platforms allow identification of altered Rh antigens not detected

serologically.⁷ In order to reduce costs and make this logistically feasible, the strategy should be reserved to selected-high risk SCD patients. Up to now, it is not yet possible to define the cutoff between potential high and low responders for developing alloantibodies. The only factor known with any certainty is the prior immune-hematological status of the patient. A patient who has already developed one or more antibodies following transfusion can certainly be considered as a high-responder.

Delayed Hemolytic Transfusion Reaction (DHTR)

Delayed hemolytic transfusion reactions (DHTRs) are a major concern in SCD. This results from re-stimulation of clinically significant antibodies against transfused (RBCs), occurring up to three weeks after RBC transfusion. The primary exposure, which may have even occurred many years earlier, results in the formation of an alloantibody to a RBC antigen that the patient is lacking, and usually there is no clinical evidence for alloimmunization other than the detection of a new antibody on a blood bank evaluation (if done). If the patient receives no further transfusions with RBCs containing that antigen, the antibody may disappear (or "evanesce") from the patient's serum. More than 35% of RBC alloantibodies can be transient in patients with SCD because they evanesce over time or fall below the level of detection by traditional blood bank methodologies.⁸ Therefore, the antibody will not be present on future antibody screens or crossmatches. However, if a future transfusion contains RBCs with the putative antigen, an anamnestic response is likely to occur with memory lymphocytes activation and subsequent production of large amounts of antibody, resulting in rapid destruction of the transfused cells. The phenomenon of RBC alloantibodies evanescence makes RBC alloimmunization extremely problematic in patients with SCD.

Three factors have been shown to increase the risk of DHTR: i) a history of alloimmunization (high-responder status); ii) history of previous DHTRs; and iii) transfusion in the setting of an acute sickle-related complication. 4.4-7.7% of transfused adult SCD patients experience at least one life-time DHTR. The clinical presentation of a DHTR in patients with SCD may be quite similar to that of a vaso-occlusive crisis (VOC) with or without aplastic crisis and for this reason are frequently missed in this population. DHTRs are particularly consequential in patients with SCD due to hyperhemolysis (often termed bystander hemolysis), a life-threatening complication in which the transfused RBCs and the patient's own erythrocytes are destroyed, potentially leading to severe and fatal hemolysis in up to 6% of cases. It is extremely important to early recognize DHTR, as additional transfusions may exacerbate the hemolysis and the clinical symptoms.

The most common presenting symptoms include: hemoglobinuria (98%), pain which most often is indistinguishable from a typical VOC (89%), fever (64%), symptoms of anemia (44%), which typically occur on average 9-10 days (\pm 5 days) after the inciting transfusion.⁸ Reticulocytopenia (defined as $<$ 150,000/uL) was reported to be present in approximately 40% of cases. However, low-normal absolute reticulocyte counts (defined as 150,000 to 250,000/uL) were detected in an additional 40% of patients. Additional laboratory findings included elevated LDH (median value: 13351 U/mL), and a significant decrease in total Hb levels ($>$ 30%) and/or in HbA ($>$ 50%) relative the post-transfusion values within 3-5 days after transfusion. Progression to acute chest syndrome, hepatic impairment, and renal failure (reported at 50%, 35% and 10% respectively) is not uncommon, and mortality is 6% in all adult SCD patients presenting with DHTRs. While alloantibodies are often associated with inciting a DHTR, sometimes a new alloantibody is not identified, leading to the diagnosis of alloantibody-negative DHTR (30% of cases). The mechanism underlying the reaction in such cases remain unknown.

A careful monitoring of transfusion outcomes in SCD is needed to ensure a correct diagnosis of DHTR and a two-step diagnostic approach has been proposed. This is divided into:

- Recognition of recent transfusions at the appearance of clinical manifestation of VOC. In case of RBC transfusion within three weeks evaluations for hemoglobinuria, hyper-hemolysis (via LDH, Hb, and HbA/HbS), and reticulocytopenia. Antibody screening monitoring at time of presentation and for the following three months.
- Determination of the extent of HbA concentration drop relative to the immediate post-transfusion values. SCD patients should have total Hb and HbA/HbS obtained within 48 hours post-transfusion. Diagnosis of DHTR can be made when a significant decrease in HbA

(> 50%) and/or in total Hb levels (> 30%) relative the post-transfusion values are observed associated with symptoms concerning for a DHT.⁹⁻¹⁰

Table 1. Factors influencing RBC alloimmunization in SCD patients.

FACTOR	MECHANISM	OUTCOME ALLOIMMUNIZATION	REFERENCE
Recipient inflammatory state at time of transfusion	RBC transfusion during a proinflammatory complication (particularly ACS and VOC)	Enhanced	Fasano RM, Booth GS, Miles M, et al. Red blood cell alloimmunization is influenced by recipient inflammatory state at time of transfusion in patients with sickle cell disease. <i>Br J Haematol</i> 2015;168(2):1-300.
Genetic factors related to B lymphocyte signal modulation	Association CD81 polymorphisms	Enhanced	Tatar Calderone Z, Tamouza R, Le Boudier GP, et al. The association of CD81 polymorphisms with alloimmunization in sickle cell disease. <i>Clin Dev Immunol</i> 2013;2013:937846. Bao W, Zhong H, Manwani D, et al. Regulatory B-cell compartment in transfused alloimmunized and non-alloimmunized patients with SCD sickle cell disease. <i>Am J Hematol</i> 2013; 88:736-740.
Chromosome 5	Variant of regulatory locus of African ancestry, cis-acting enhancer predicted to regulate transcription of ADRA1B and the lncRNA LINC01847	Enhanced	Lesedi M, Williams, Zhuina Qi, Ken Batai, Stanley Hooker, Nancy J. Hall, Roberto F. Machado, Alice Chen, Sally Campbell-Lee, Yipglan Guan, Rick Killen, Neil A. Hancock. A locus on chromosome 5 shows African ancestry-limited association with alloimmunization in sickle cell disease. <i>Blood Adv</i> . 2018 Dec 26; 2(24): 3637-3647
Inpartite Motif protein (TRIM)	TRIM21 Polymorphisms	Enhanced	Tatar-Calderone Z, Minniti GP, Kralovic T, Spasivovic M, Volmer A, Bakajovic I, Zhang E, Hoang A, Luban N, Vukobratovic S. rs660 polymorphism in Ro52 (SSA1; TRIM21) is a marker for age-dependent tolerance induction and efficiency of alloimmunization in sickle cell disease. <i>Mol Immunol</i> . 2009 Nov;47(1):64-70
HLA alleles	HLA DRB1 Polymorphisms HLA-B*55 HLA-DQB1	Enhanced Protective	Alant L, Castro O, Ofosu M, Dunston G, & Scott, R.B. (1986) HLA-B*55 is associated with red cell alloimmunization in sickle cell disease. <i>Clinical Immunology and Immunopathology</i> , 38, 178-85. Chiappoli J, Dentoni I, Ferrara V, Logrand D, Toscani M, Mercier P, de Micco P, & Reut-rod D. (2006) HLA-DRB1 polymorphism is associated with Kell sensitization. <i>British Journal of Haematology</i> , 132, 374-376. Rawcox D, Dentoni I, Ferrara V, Logrand D, Toscani M, Mercier P, de Micco P, & Reut-rod D. (2008) HLA-DRB1 alleles and (k) immunization. <i>Transfusion</i> , 48, 858-859. Tatar-Calderone Z, Gordish-Dressman H, Fasano R, Riggs M, Forster C, Cambell A.D., Charon D., Gordeuk V.R., Luban, N.L., Vukobratovic S. & Tamouza R. (2016) Protective effect of HLA-DQB1 alleles against alloimmunization in patients with sickle cell disease. <i>Human Immunology</i> , 77, 36-40. Sippert E.A., Vasantiner J.E., Alves H.V., Rodrigues C., Gil, S.C., Adas-Carvalho M., Saad S.T., Costa F.F. & Castilho L. (2017) Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms. <i>Transfusion</i> , 57, 379-389.
Proinflammatory cytokine genes	IL1B-TNFA polymorphisms	Enhanced	Sippert E.A., Vasantiner J.E., Alves H.V., Rodrigues C., Gil, S.C., Adas-Carvalho M., Saad S.T., Costa F.F. & Castilho L. (2017) Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms. <i>Transfusion</i> , 57, 379-389.

genes of the Toll-like receptor (TLR) pathway and genes that were previously associated with antibody-mediated diseases	TRL Single Nucleotide Polymorphisms (SNP) TANK (SNP) STAM (signal transduction adaptor molecule) (SNP)	Enhanced Enhanced Protective	Meinders SM, Gerritsma JJ, Sins JWR, de Boer M, van Leeuwen B, Bascagli BJ, Rijneveld AW, Kerkhoffs JH, Hebbi A, van Boven R, Kuipers TW, van der Schoot E, Elenga F, Eijvoordat K, Tanck MW, van den Berg TK. Identification of genetic biomarkers for alloimmunization in sickle cell disease. <i>Br J Haematol</i> . 2019 Jun;5-
Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)	Gene Polymorphisms	Enhanced	Oliveira V.B., Dezan M.R., Gomes F.C.A., Mendes Quilodora S.F., Krieger J.E., Pereira A.C., Marziska J.D., Livi J.E., Rocha V., Men-drone-Junior A, Sabino E.C. & D’Orazio C.L. (2017) 318C/T polymorphism of the CTLA-4 gene is an independent risk factor for RBC alloimmunization among sickle cell disease patients. <i>International Journal of Immunogenetics</i> , 84, 219-224.
Fcγ receptors (FcγRs) for immunoglobulin G (IgG)	Single Nucleotide Polymorphisms (SNP) and copy number variation of FCGR2/3 gene cluster	Protective	Meinders, S.M., Sins, J.W.R., Eijvoordat, K., Napolitano, S.Q., Geisler, J., Tanck, M.W., Rougemont, C., Blomend, B.J., Rijneveld, A.W., Kerkhoffs, J.H., Eijvoordat, S., Hebbi, A., van Boven, R., Kuipers, T.W., Elenga, F. & van den Berg, T.K. (2017) Nonclassical FCGR2C haplotype is associated with protection from red blood cell alloimmunization in sickle cell disease. <i>Blood</i> , 130, 2121-2130.
T cells	Phenotypic differences of CD4(+) T cells	Enhanced	Bao W, Zhong H, Li X, et al. Immune regulation in chronically transfused allo-antibody responder and nonresponder patients with sickle cell disease and beta-thalassemia major. <i>Am J Hematol</i> 2011;88:1001-8. Vingard B, Tamargo M, Habibi A, et al. Phenotypic differences of CD4(+) T cells in response to red blood cell immunization in transfused sickle cell disease patients. <i>Eur J Immunol</i> 2015;45:1868-79. Vingard B, Tamargo M, Desmarais M, et al. Partial dysfunction of Tr1g activation in sickle cell disease [published correction appears in <i>Am J Hematol</i> . 2015;90(1):84]. <i>Am J Hematol</i> . 2014;89(3):261-266.
Dendritic cells	Altered mediated-name modulation	Enhanced	Zhong H, Bao W, Friedman D, et al. Hematopoietic T cell polarization in sickle cell alloimmunization. <i>J Immunol</i> 2014;193:102-10. Coadroy E, Liu Y, Shi P, Mitchell WB, Cohen D, Chau ST, Manwani D, Yazdankhah K. Altered hematopoietic modulation of dendritic cell function in sickle cell alloimmunization. <i>Haematologica</i> . 2018 Sep;101(9):1028-38

In addition to supportive treatment and temporarily withholding additional transfusion to avoid further hemolysis, therapeutic strategies for the clinical management of DHTR are currently available.^{9,11} These are summarized below:

- EPO to promote/enhance erythropoiesis
- Immunosuppressive treatment. (i) Immunoglobulins for prevention of antibody-mediated immune-destruction; (ii) High-dose corticosteroids for immune system modulation; (iii) Rituximab for circulating CD20+ B-cell depletion and to prevent formation of additional antibodies; (iv) Eculizumab to block complement activation and to prevent irreversible multiorgan failure (MOF) previous anti-meningococcal vaccination
- Plasma-exchange might be considered to rapidly remove toxic free heme or free Hb, which might further amplify sickle cell related inflammatory vasculopathy.

Since DHTR is a life-threatening complication and has an impact on the use of future transfusions to treat clinical manifestation of SCD, risk reduction strategies for transfusion support should be considered (Table 2).

- The main risk/contingency plans are:
- Minimizing the risk of alloimmunization with appropriate use of RBC transfusions (careful evaluation of the indication for transfusion) and providing prophylactic antigen matched RBCs when transfusions are

necessary;

- Matching of RBCs for RH (D, C, E, c, e) and KEL (K) status should be included in standard care of SCD patients; (iii) extended matching to other blood groups (FY, JK, MNS) and matching partial RH phenotypes in alloimmunized patients. In addition, given the life-threatening nature of DHTR, taking into account the possibility of partial D expression in SCD patients is recommended. The possible collection of matched RBC units, provided by donors from the same ethnic background as the patients should be considered as additional tool.
- Minimizing the likelihood of missing the detection of “transient” alloantibodies and re-exposure to “evanesced” alloantibodies through reliable intra- and inter-institutional communication and discouraging multi-site transfusion;
- Identifying high risk patients for DHTR. If transfusion cannot be avoided prophylaxis with rituximab is recommended^{9,11} (Table 2).

Table 2. Transfusion strategy for preventing DHTR in low-high risk patients with SCD (modified from Pirenne F) (9)

Immunization status of the patient before transfusion	Patient Low Risk of DHTR	Patient Intermediate/High Risk of DHTR
	<ul style="list-style-type: none"> Chronic transfusion program, With No history of DHTR Episodic transfusion With only one of the following factor <ol style="list-style-type: none"> history of DHTR cumulative number of transfused Unit <12 antibody history (one significant antibody, only Rh/k and/or non significant antibody) 	<ul style="list-style-type: none"> Patient receiving episodic transfusion with at least 2 of following factors <ol style="list-style-type: none"> history of DHTR cumulative number of transfused Unit <12 antibody history (one significant antibody, only Rh/k and/or non significant antibody)
<ul style="list-style-type: none"> No antibodies Only antibodies RH/K Non mayor antibodies 	Rh/K matched RCBs	Rh/k and extended matched (Fy, Jk, MNS) Intraracial matched donor/patient
Significant antibodies (Fy, JK, MNS, DO, high frequency, other)	Rh/K and matched to antibody specificity + if possible extended matched (Fy, Jk, MNS)	Rh/k and Extended matched (Fy, Jk, MNS) Intraracial matched donor/patient + Rituximab (case by case decision)

The national experience

The Italian Society of Thalassemia and Hemoglobinopathies (SITE), in collaboration with the Italian Society of Transfusion Medicine and Immunohematology (SIMTI) and the Italian Association of Hematology and Pediatric Oncology (AIEOP) conducted in 2018 a national survey to collect information on transfusion approaches in clinical management of SCD.

Data were collected from 15 Italian Center and 1,579 patients were analyzed.

Allimmunization was documented in 8.5% patients; 45% patients were already alloimmunized at their arrival at the comprehensive SCD center. Red blood cell antibodies were identified in 124 patients; 69 had a single antibody, 44 had multiple antibodies (>2), and in 11 patients the antibodies were not correctly identified. The data showed an alloimmunization index of 8.5% and no association was found with gender or with HbSS or HbS/B⁰ or HbS/B⁺ genotype. The majority of patients (61%) had a single antibody; in the Caucasian cohort, the single antibody was prevalently found in males (60%) while there were no significant differences in gender in the African cohort. The antibodies identified were prevalently related to RhCDE, (44%), Kell 15%), and MNS systems (11.9%), Lower frequencies were detected in other blood group systems: Duffy (6.2%), Lewis (4.7%), Kidd (4.1%), Lutheran (2.6%); antibodies not identified (8.8%); antibodies versus minor anti-

gens (0.8%) Red blood cell alloimmunization rate was low in respect to literature and most patients had antibodies anti-Rh and Kell systems with different distribution related to ethnicity, gender and age.¹²

A single center experience using molecular genotyping of RH system in SCD population from Ghana and Nigeria, living in Italy, shows some discrepancy between the molecular characterization of *RHD-CE* and serological typing for frequent variant alleles (RHD 26% and RHCE 86%). RBC cross match strategies were modified accordingly. Alloantibodies were detected in three patients only and low immunization rate (6%) was reported (Venturelli D, Chou ST, Westhoff C. personal communication).

Conclusion and Remarks

RBC transfusions play an important role in managing and preventing clinical complications in SCD patients. A careful evaluation of whether RBC transfusions are necessary is crucial and should be taken into account the risk of DHTR and the availability of RBC units for these patients. Transfusion protocols in SCD patients should also include strategies to minimize alloimmunization. Prevention has centered on using phenotypically-matched RBCs but the effectiveness of this approach is limited by the incidence of RHDCE or other blood group variants, as well as the supply of RBC units with appropriate phenotype. Promoting RBC donation in African populations and performing an extended phenotype in all patients with SCD prior first transfusion could be an effective approach. *RHD* and *RHCE* genotyping strategy should be implemented in selected African patients. Monitoring and analyzing cases of DHTR is a new and substantial challenge for hemovigilance. The record of transfusion history, alloimmunization and DHTR of each patient should be kept up to-date and available to medical staff. Implementation of National registries of SCD patients including their immunohematological profile, i.e RBC typing (molecular and serological), should be strengthened. RBC alloimmunization is likely to become an even more important issue for individuals with SCD as they are living longer and indication for transfusion are expanding.

References

- Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 2010;376:2018-2031.
- Yazdanbakhsh K, Shaz B.H, and Hillyer D.C Immune Regulation of sickle Cell Alloimmunization. *ISBT Sci Ser.* 2017 February ; 12(1): 248-253.
- Vichinsky EP, Earles A, Johnson RA, Hoag MS, Williams A, Lubin B. Allo-immunization in sickle cell anemia and transfusion of racially unmatched blood. *New Eng J Med* 1990; 322:1617-21.
- Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood* 2012;120:528-37.
- Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DE, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. *Blood.* 2013;122(6):1062-1071.
- Sins JW, Biemond BJ, van den Bersselaar SM, Heijboer H, Rijnveld AW, Cnossen MH, Kerkhoffs JL, van Meurs AH, von Ronnen FB, Zalpuri S, de Rijke YB, Ellen van der Schoot C, de Haas M, van der Bom JG, Fijnvandraat K. Early occurrence of red blood cell alloimmunization in patients with sickle cell disease. *Am. J. Hematol.* 91:763-769, 2016.
- Chou ST, Westhoff CM. Application of genomics for transfusion therapy in sickle cell anemia. *Blood Cells Mol Dis.* 2017 Sep;67:148-154
- Fasano RM, Miller MJ, Chonat S, Stowell SR. Clinical presentation of delayed hemolytic transfusion reactions and hyperhemolysis in sickle cell disease. *Transfus Clin Biol.* 2019 May;26(2):94-98
- Pirenne F. Prevention of delayed hemolytic transfusion reaction. *Transfus Clin Biol.* 2019 May;26(2):99-101
- Dean CL, Maier CL, Chonat S, Chang A, Carden MA, El Rassi F, McLemore ML, Stowell SR, Fasano RM. Challenges in the treatment and prevention of delayed hemolytic transfusion reactions with hyperhemolysis in sickle cell disease patients. *Transfusion.* 2019 May;59(5):1698-1705.
- Pirenne F and Yazdanbakhsh K. How do I safely transfuse patients with sickle cell disease and manage delayed hemolytic transfusion reactions. *Blood* 2018;131(25) 2273-2781.
- Graziadei G, Sainati L, Bonomo P, Venturelli D, et al Transfusion therapy in a multi-ethnic sickle cell population real-world practice. A preliminary data analysis of multicentre survey. *Blood* 2018 132:2389;

HYPEREOSINOPHILIC SYNDROMES AND MASTOCYTOSIS BETWEEN MORPHOLOGY AND MOLECULAR BIOLOGY: THE 2016 WHO CLASSIFICATION

C. Elena

Department of Hematology Oncology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy

Eosinophilia, hypereosinophilic syndrome and tissue eosinophilia

Eosinophilia is observed in a wide range of reactive and clonal diseases and may be associated with organ damage consequent to both tissue infiltration by eosinophil cells and release of cytokines and granular contents.

In 2012, an international consensus group published modified criteria for the definition of peripheral blood and tissue hypereosinophilia (HE),¹ and in 2016 the World Health Organization (WHO) classification has been updated based on the more recent acquisitions in terms of molecular pathogenesis and better definition of reactive conditions.²

Mild eosinophilia is defined by an absolute peripheral eosinophil count between 0.5 and 1.0x10⁹/L, is a common finding in the general population, and is more frequently associated with reactive conditions.

Conversely, severe eosinophilia or hypereosinophilia (HE), defined as a persistent (at least 2 examinations with interval > 1 month) absolute peripheral eosinophil count higher than 1.5 x 10⁹/L, is relatively rare and could be associated, independently from the generating process, either reactive or clonal, with life-threatening organ damage. When HE is associated with end organ manifestations, a diagnosis of hypereosinophilic syndrome (HES) is formulated. In the presence of severe eosinophilia, a prompt comprehensive diagnostic and clinical evaluation is warranted, in order to establish a correct diagnosis and, in case of organ damage, to choose and start the best therapeutic approach as soon as possible. Tissue eosinophilia (TE) is defined as 1) eosinophils > 20% of all nucleated cells in a bone marrow aspirate, or 2) eosinophilic tissue infiltration that in the opinion of an experienced pathologist is markedly increased, or 3) extensive extracellular deposition of eosinophil-derived proteins in tissue as demonstrated by immunostaining.

Reactive eosinophilia

In the majority of cases eosinophilia is reactive, secondary to the production of cytokines such as IL-3, IL-5, or GM-CSF. In reactive conditions eosinophilia could range from mild to severe. The most common causes of reactive eosinophilia include infections (helminths, ectoparasites, protozoan, fungal, HIV), atopic diseases, drug hypersensitivity, autoimmune disorders, and hematological (especially Hodgkin's and non Hodgkin's lymphomas) or solid malignancies (mainly cervical or lung cancer) (see Table 1).

Table 1. Reactive and clonal eosinophilias.

SECONDARY (REACTIVE) HE	PRIMARY (CLONAL) HE
<ul style="list-style-type: none"> Allergy/atopy (asthma, atopic dermatitis) 	<ul style="list-style-type: none"> Chronic eosinophilic leukemia (CEL), NOS (MPN)
<ul style="list-style-type: none"> Infections (helminths, ectoparasites, protozoan, fungal, HIV) 	<ul style="list-style-type: none"> Eosinophilia associated WHO-defined myeloid neoplasm (SM, CML, AML, MDS, MDS/MPN)
<ul style="list-style-type: none"> Drug hypersensitivity (eosinophilia-myalgia, DRESS) 	<ul style="list-style-type: none"> Myeloid/lymphoid neoplasms with eosinophilia and rearrangements of <i>PDGFRA</i>/<i>PDGFRB</i>/<i>FGFR</i>/<i>PCM1-JAK2</i>
<ul style="list-style-type: none"> Collagen-vascular disease 	
<ul style="list-style-type: none"> Immunodeficiency disorders (Hyper-IgE syndrome, Omenn syndrome) 	
<ul style="list-style-type: none"> Lymphocyte variant (L-HES) 	
<ul style="list-style-type: none"> Solid malignancies (adenocarcinomas) 	
<ul style="list-style-type: none"> Lung eosinophilic diseases 	
<ul style="list-style-type: none"> Lymphoproliferative disorders (NHL, HL, ALL) 	

Lymphocytic variant hypereosinophilia (L-HE/L-HES) can be included among the reactive forms of eosinophilia, as it is sustained by the secretion of different eosinophilopoietic cytokines by a clonal or oligoclonal immunophenotypically aberrant (CD3⁻ CD4⁺ or CD3⁺ CD4⁻ CD8⁻) population of T-lymphocytes, characterized by the presence of a clonal TCR rearrangement.³

Clonal eosinophilia

Classification principles

Primary (clonal) eosinophilia is usually severe at presentation and includes a broad range of disorders, currently classified by the revised 2016 WHO classification of hematologic neoplasms across different categories. Clonal eosinophilias encompass myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA*/*PDGFRB*/*FGFR1* or with *PCM1-JAK2*, the myeloproliferative neoplasm (MPN) designated as chronic eosinophilic leukemia not otherwise specified (CEL-NOS), and WHO -defined myeloid neoplasms associated with eosinophilia (see Table 1).

Myeloid/lymphoid neoplasm with eosinophilia and rearrangements of *PDGFRA*/*PDGFRB*/*FGFR1* or *PCM1-JAK2*

Following the identification of recurrent, genetically defined cases of HE associated with rearrangements and constitutive activation of several tyrosine kinase (TK) genes, a distinct disease category was introduced in the 2008 WHO classification under the designation of "Myeloid/lymphoid neoplasm with eosinophilia and abnormalities of platelet-derived growth factor receptor alpha (*PDGFRA*), platelet-derived growth factor receptor beta (*PDGFRB*), or fibroblast growth factor receptor 1 (*FGFR1*)", with the inclusion of *PCM1-JAK2*-positive neoplasm as provisional entity in the revised version of 2016.²

The cytogenetically occult *FIP1L1-PDGFRA* rearrangement, generated from a submicroscopic 800-kb deletion on chromosome 4, del(4)(q12q12),⁴ must be identified with a specific FISH for the *CHIC2* deletion and/or reverse transcription polymerase chain reaction (RT-PCR). Conversely, the presence of alternative *PDGFRA*, *PDGFRB*, *FGFR1* or *JAK2* fusion genes can be suspected when specific breakpoints at chromosome 4 (4q12, *PDGFRA*), chromosome 5 (5q31-33, *PDGFRB*), chromosome 8 (8p11-12, *FGFR1*), and chromosome 9 (9p24, *JAK2*) are identified with conventional cytogenetic analysis.

So far, more than 70 different fusion genes associated with eosinophilia have been identified, also including alternative rearrangements of *FLT3* and *ABL* genes. However, despite the significant improvement in the understanding of the molecular basis of these disorders, the final diagnosis still relies on a combination of histomorphologic, clinical and laboratory criteria. In fact, none of the above-mentioned molecular markers is associated with a specific phenotype, which may encompass a wide spectrum of myeloid and lymphoid neoplasms ranging from chronic and indolent forms to very aggressive disorders. Notably, peripheral eosinophilia may also be absent.

Chronic eosinophilic leukemia

CEL-NOS is a MPN characterized by the independent proliferation of an eosinophil clone that results in persistently increased numbers of eosinophils in peripheral blood (PB), bone marrow (BM) and peripheral tissues, with eosinophilia being the dominant hematological abnormality. According to the WHO classification, together with the exclusion of *BCR-ABL*, *PDGFRA*, *PDGFRB*, *FGFR1*, *PCM1-JAK2*, *ETV6-JAK2* and *BCR-JAK2* rearrangements and in absence of WHO criteria for other MPN or MPN/MDS, the diagnosis of CEL-NOS requires an increase in PB (≥2%) or BM (≥5%) blasts, and/or the presence of a non-specific clonal cytogenetic or molecular abnormality.²

Therefore, so far the category of CEL-NOS still includes rearrangements of *FLT3*, *ABL* and those of *JAK2* other than *PCM1-JAK2*, *ETV6-JAK2* and *BCR-JAK2*.

WHO -defined myeloid neoplasm associated with eosinophilia

Clonal eosinophilia is most frequently associated with chronic myeloid neoplasms, including chronic myeloid leukemia (CML), myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and more rarely with aggressive forms (i.e. acute myeloid leukemia, AML). The available evidence indicates that in these disorders, eosinophils are part of the primary malignant clone. Systemic mastocytosis (SM) may present with peripheral HE; however, in approximately 20% to 30% of cases eosinophilia could accompany systemic mastocytosis (SM) as an associated hematological neoplasm (SM-CEL): in these cases the *KIT*^{DB16V} mutation has been demonstrated both in mast cells and eosinophils.

Hypereosinophilia of undetermined significance

If none of the aforementioned conditions is identified and HE is persistent for more than 6 months, a provisional diagnosis of HE/HES of undetermined significance (HE/HES_{UG}) is rendered until a cause of eosinophilia is identified.²

Recently, several groups identified with NGS techniques recurrent molecular alterations in a fraction of patients undergoing diagnostic work-up for HE.⁵ However, taking into account that some clonal molecular genetic abnormalities (e.g. *DNMT3A*, *ASXL1*, *TET2* mutations) can be identified in healthy elderly people without hematological phenotype in the context of clonal hematopoiesis of indetermined significance, it remains essential to exclude all possible causes of reactive eosinophilia before making the diagnosis of clonal eosinophilia in presence of a non-specific genetic aberration.

Mastocytosis

Mastocytosis is a rare disease characterized by the presence, proliferation and accumulation of atypical neoplastic mast cells (MC) in various organ systems, including skin, bone marrow, spleen and gastrointestinal tract. It is characterized by an abnormal MC infiltrate, which often contains multifocal compact clusters or cohesive aggregates. The clinical presentation is very heterogeneous, ranging from a childhood-onset skin-limited disease, that in most cases spontaneously regress before puberty, to highly aggressive neoplasms associated with systemic involvement, multiorgan failure and poor survival.

Due to its peculiar features, the 2016 WHO classification has distinguished mastocytosis from the other MPNs, and created a distinct disease category that encompasses a cutaneous (CM) and a systemic (SM) form of mastocytosis (see Table 2).

Table 2. WHO classification of mastocytosis variants (2016).

Cutaneous Mastocytosis
• Urticaria pigmentosa/maculopapular cutaneous mastocytosis
• Diffuse cutaneous mastocytosis
• Mastocytoma of the skin
Systemic mastocytosis
• Indolent systemic mastocytosis (including bone marrow mastocytosis)
• Smouldering systemic mastocytosis
• SM with an associated haematological neoplasm
• Aggressive systemic mastocytosis
• Mast cell leukemia
Mast cell sarcoma

Cutaneous mastocytosis

In cutaneous mastocytosis, clonal MC infiltrates are limited to the skin. Depending on the clinical presentation, the WHO classification recognizes three different forms of CM: 1) urticaria pigmentosa/maculopapular CM, 2) diffuse CM, and 3) mastocytoma of the skin (see Table 2).² Diagnosis is based on the presence of characteristic skin lesions, associated with typical histological infiltrates of MC in a multifocal or diffuse pattern in an adequate skin biopsy, in absence of diagnostic criteria for SM.⁷

Systemic mastocytosis

According to WHO classification, the diagnosis of SM relies on histological, morphological, cytofluorimetric, molecular and biochemical criteria (see Table 3). A diagnosis of SM can be made when one major criterion AND at least one minor criterion are present, OR at least three minor criteria. The histological pattern is characterized by the presence of multifocal dense infiltrates of mast cells (≥ 15 mast cells in aggregates). Neoplastic MC in biopsy section or BM smear can show an atypical morphology (e.g. spindle-shaped, degranulated MC), and express aberrant cytofluorimetric markers, such as CD25 with or without CD2.

When highly sensitive methods are applied (e.g. RT-PCR), a somatic point mutation of *C-KIT* is found in the majority of cases: the most frequent mutation is D816V (> 95% of cases), but more rarely both other mutations in exon 17 and in the transmembrane and extracellular domain could be identified. If *KIT*(D816V) mutation is not found, but clinical suspicion of SM remains very high, the *KIT* gene should be sequenced. Five variants of SM are recognized and their subclassification is based on the determination of B and C criteria, on the quantifi-

cation of MC in the BM aspirate, and on the presence of diagnostic criteria for an associated hematological neoplasm (AHN) (see Table 3).

Table 3. WHO diagnostic criteria for systemic mastocytosis; B and C findings definition.

WHO DIAGNOSTIC CRITERIA FOR SYSTEMIC MASTOCYTOSIS	
MAJOR CRITERION:	
• Multifocal dense infiltrates of MCs (≥ 15 MCs in aggregates) in BM sections and/or other extracutaneous organ(s)	
MINOR CRITERIA:	
• > 25% MCs in the infiltrate (biopsy sections of BM or other extracutaneous organ) are spindle-shaped or have atypical morphology or > 25% of all MCs in BM aspirate smears are immature or atypical	
• Detection of an activating point mutation at codon 816 of <i>KIT</i> in the BM, blood or extracutaneous organ	
• MC in BM, blood or other extracutaneous organ express CD25, with or without CD2, by flow or immunohistochemistry, in addition to normal MC markers	
• Serum total tryptase is persistently > 20 ng/ml, unless there is an associated myeloid neoplasm (in this case this parameter is not valid)	
B AND C FINDINGS IN SYSTEMIC MASTOCYTOSIS	
B findings (Burden of disease)	
• High MC burden: MCs infiltrates in BM > 30% AND sTryptase > 200 ng/mL	
• Signs of dysplasia or myeloproliferation in non mastcell lineage(s), without definitive criteria for AHN	
• Hepatomegaly without impairment of liver function, palpable splenomegaly without hypersplenism and/or lymphadenopathy on palpation or imaging	
C findings (Consider Cytoreduction)	
• BM dysfunction caused by MC infiltration, manifested by ≥ 1 cytopenia (ANC $< 1.0 \times 10^9/L$, Hgb < 10 g/dL, and/or PLT $< 100 \times 10^9/L$).	
• Palpable hepatomegaly with ascites, portal hypertension and/or impaired liver function	
• Palpable splenomegaly with hypersplenism	
• Malabsorption with weight loss due to gastrointestinal MC infiltrates	
• Skeletal involvement, with large-sized osteolytic lesions with or without pathologic fractures (pathologic fractures caused by osteoporosis do not qualify as a C finding)	
• Life-threatening organ damage due to MCs infiltrates	

Indolent systemic mastocytosis

Indolent systemic mastocytosis (ISM) is characterized by a low MC burden (no more than one B finding and no C findings), and skin lesions are found in most patient. Overall survival (OS) is as good as that of general population.⁸

In bone marrow mastocytosis (BMM), which has been included in the 2016 WHO classification as a provisional entity, all the criteria of ISM are satisfied, but there is no skin involvement.

Smouldering systemic mastocytosis

In smouldering systemic mastocytosis (SSM) the MC burden is high (2 or more B findings should be present), but no C findings are documented.

In this category the OS is very heterogeneous: the clinical course is often stable for many years, but progression to advanced forms of mastocytosis may occur.⁸ *KIT* mutations are found in the majority of cases, and show a multilineage involvement.

Systemic mastocytosis with an associated hematological neoplasm

SM with an associated hematological neoplasm (SM-AHN) fulfils both the diagnostic criteria for SM and those for an AHN. In most cases a myeloid neoplasm is diagnosed (the most frequent being chronic myelomonocytic leukemia, followed by MPN, MDS and AML), but more rarely the AHN presents a lymphoid phenotype. *KIT* mutations are identified in the majority of patients, not only in the MC but also in the AHN compartment. Moreover, recurrent additional somatic mutations typical of myeloid neoplasms could be detected in a high percentage of cases.^{9,10}

Aggressive systemic mastocytosis

For the diagnosis of aggressive systemic mastocytosis (ASM) at least one C finding must be documented. The MC burden is usually high, with development of end-organ damage; MC in BM smear may be increased but no more than 20% of all the nucleated cells. In most patients there is no skin involvement. As in SM-AHN, *KIT* mutation can be found in association with other somatic mutations that may show a significant prognostic role.

Mast cell leukemia

Mast cell leukemia (MCL) represents the leukemic variant of SM, MC in BM smear accounting for more than 20% of all nucleated cells. These cells can be both more mature atypical MC, and highly immature MC (metachromatic blasts). If circulating MC in the PB are less than 10%, the aleukemic form of MCL is diagnosed. In general, the clinical course of MCL is very aggressive and, before the recent introduction of targeted therapies in the clinical setting, its prognosis was very poor, with OS inferior than one year.

Mast cell sarcoma

Mast cell sarcoma (MCS) is a very rare disease characterized by de-streant growth of highly atypical MC. The MCS is usually initially localized, but spread to other organ and tissues is frequent during the natural history of the disease, resembling MCL after a short interval of time.

References

1. Valent P, Klion AD, Horny HP et al. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. *J Allergy Clin Immunol.* 2012 Sep;130(3):607-612.
2. Arber DA, Orazi A, Hasserjian R et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016 May 19;127(20):2391-405
3. Cogan E, Schandene L, Crusiaux A et al. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. *N Engl J Med.* 1994 Feb 24;330(8):535-8
4. Cools J, DeAngelo DJ, Gotlib J et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hyper-eosinophilic syndrome. *N Engl J Med.* 2003 Mar 27;348(13):1201-14.
5. Pardanani A, Lasho T, Wassie E et al. Predictors of survival in WHO-defined hypereosinophilic syndrome and idiopathic hypereosinophilia and the role of next-generation sequencing. *Leukemia.* 2016 Sep;30(9):1924-6
6. Wang SA, Tam W, Tsai AG et al. Targeted next-generation sequencing identifies a subset of idiopathic hypereosinophilic syndrome with features similar to chronic eosinophilic leukemia, not otherwise specified. *Mod Pathol.* 2016 Aug;29(8):854-64.
7. Hartmann K, Escribano L, Grattan C et al. Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergy and Clinical Immunology. *J Allergy Clin Immunol.* 2016 Jan;137(1):35-45
8. Lim KH, Tefferi A, Lasho TL et al. Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors. *Blood.* 2009 Jun 4;113(23):5727-36
9. Garcia-Montero AC1, Jara-Acevedo M, Teodosio C, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood.* 2006 Oct 1;108(7):2366-72.
10. Schwaab J, Schnitger S, Sotlar K et al. Comprehensive mutational profiling in advanced systemic mastocytosis. *Blood.* 2013 Oct 3;122(14):2460-6

HYPEREOSINOPHILIC SYNDROMES AND MASTOCYTOSIS: MULTIDISCIPLINARY CLINICAL MANAGEMENT

R. Zanotti^{1,2}, P. Bonadonna^{1,3}, M. Bonifacio^{1,2}, M. Rossini^{1,4}, L. Scaffidi^{1,2}, D. Schena^{1,5}, M. Tebaldi^{1,6}

¹Gruppo Multidisciplinare per lo studio della Mastocitosi (GISM), Azienda Ospedaliera Universitaria Integrata di Verona, Italy; ²Hematology Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Italy; ³Allergy Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Italy; ⁴Rheumatology Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Italy; ⁵Dermatology Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Italy; ⁶Gastroenterology Unit, Azienda Ospedaliera Universitaria Integrata di Verona, Italy

HYPEREOSINOPHILIC SYNDROMES (HES)

Hypereosinophilia (HE) is defined by a marked increase in absolute eosinophils count (AEC) in peripheral blood ($\geq 1.5 \times 10^9/L$). If HE is persistent (≥ 6 months) and there is associated tissue damage, the disorder is classified as hypereosinophilic syndrome (HES). A history of 6 months may not be necessarily enforced if the diagnostic work-up is adequate and treatment is needed to minimize organ damage by the eosinophilic infiltrate.¹

According to an International Consensus Group the classification of HE include primary clonal/neoplastic HEs (HE_N), secondary eosinophilia (HE_R), Hereditary (familial) HE (HE_{FA}) and HE of undetermined significance (HE_{US}).¹

HE_N is distinguished from HE_{US} by the presence of histologic, cytogenetic, or molecular evidence of an underlying myeloid malignancy. The WHO classification recognized 2 distinct subcategories of clonal eosinophilia: myeloid/lymphoid neoplasms with abnormalities of *PDGFRalpha*, *PDGFRbeta*, *FGFR1*, or *PCM1-JAK2*, and chronic eosinophilic leukemia not otherwise specified (CEL NOS).² Clonal eosinophilia may also accompany other WHO-defined myeloid malignancies, including chronic myelogenous leukemia, myelodysplastic syndromes, chronic myelomonocytic leukemia, and systemic mastocytosis (SM).² Eosinophilia accompanies SM in approximately 20% to 30% of cases, above all in advanced variants: the clonal relationship has been convincingly proven both in most advanced and indolent disease by the presence of the *KIT D816V* mutation also in eosinophils.³

HE_R has numerous causes that may require diagnostic evaluation by a team of different sub-specialty consultants. In developing countries, eosinophilia most commonly derives from infections, particularly parasites. Allergy/atopy, drug reaction, collagen-vascular disease (e.g., Churg-Strauss Syndrome, granulomatosis with polyangiitis, systemic lupus erythematosus), pulmonary eosinophilic diseases (e.g., idiopathic acute or chronic eosinophilia pneumonia, tropical pulmonary eosinophilia, allergic bronchopulmonary aspergillosis, etc), allergic gastroenteritis and adrenal insufficiency are diagnostic considerations in the appropriate clinical context. HE_R may be associated with non-myeloid malignancies as a result from the production of cytokines (e.g, T-cell lymphomas, Hodgkin lymphoma, and acute lymphoblastic leukemias).²

Lymphocytic variant HES (L-HES) is characterized by polyclonal eosinophil expansion in response to marked overproduction of IL-5 by deregulated T cells. Other rare conditions associated with eosinophilia include familial eosinophilia (HE_{FA}), an autosomal dominant disorder.²

HEs of undetermined significance (HE_{US}) include the HE after the exclusion of previous variants.¹

Eosinophils have been implicated in the pathogenesis of tissue fibrosis, thrombosis, vasculitis, and allergic inflammation both through direct cytotoxic effects and by recruitment and activation of other inflammatory cells. These effects depend on a number of factors, including the AEC, their location, and degree of activation. Although these factors may be influenced by the underlying etiology of the eosinophilia, the consequences of eosinophilic inflammation can be identical despite markedly different clinical diagnoses. For example, clinically indistinguishable eosinophilic endomyocardial fibrosis has been described in patients with *PDGFRalpha*-positive myeloproliferative neoplasm, idiopathic HES, and helminthic infection.⁴

Patients with HES may present with various combinations of symptoms and signs of organ damage mediated by eosinophils. In many patients the onset of symptoms is insidious and eosinophilia is detected incidentally. Sometimes, on the other hand, initial manifestations can also be serious and life-threatening due to the rapid evolution of cardiac and/or neurological complications. Clinical manifestations may be either non-specific (cough, night sweats, fatigue, loss of appetite, weight loss) or directly related to the affected organ and/or organs.⁴

Essentially all organ systems may be susceptible to the effects of sustained eosinophilia.² In a study including 188 patients the most common presenting manifestations of HES were dermatologic (37%), followed by pulmonary (25%), and gastrointestinal (14%). During follow-up the most common clinical manifestations were: dermatologic, pulmonary and gastrointestinal (69%, 44%, 38% respectively), while cardiac diseases and neurologic complications were identified in about 20% of patients (only 5-6% at the time of initial presentation).²

In advanced SM with eosinophilia, end-organ damage, e.g. hepatopathy or osteolysis, is distinctively different from organopathies recorded in other clonal eosinophilia with end-organ damage. The eosinophilia in SM is often not pathologic and does not necessitate treatment.⁵

The clinical work-up of HE can be challenging due to the broad and complex underlying causes of HE. The clinician confronted with a patient presenting with HE must first and foremost address 2 questions. First, is hypereosinophilia secondary to a common and treatable underlying condition, such as parasitic infections or adverse drug reactions? Second, is hypereosinophilia in itself causing rapidly progressive damage? Table 1 reports the suggested diagnostic investigations in patients with eosinophilia and systemic symptoms or those with persistent eosinophilia (at least $1.5 \times 10^9/l$) aimed to diagnosing secondary and primitive HE and organ damage.^{4,6}

In the absence of symptoms, the best suggested approach is to postpone treatment until the diagnostic work-up is completed and the specific diagnosis is made, regardless of eosinophil count, with the exception of clonal eosinophilia associated with imatinib-sensitive molecular markers. In absence of treatment it is important to monitor serum troponin levels, and perform periodically echocardiography and lung function tests. Because validated surrogate marker for disease activity in HES are lacking, the AEC remains a key laboratory test that is used by experts to monitor disease activity.⁴

Large retrospective studies have shown that about 5% of all patients with HE (regardless of etiology) will eventually develop a hematologic malignancy (at a median time after the diagnosis of 30 months); therefore all patients with HE should be monitored for potential symptoms and laboratory evidence of malignancy with regular clinical exams, complete blood count with differential, and other tests based on the evolution of signs or symptoms.⁴

In the treated patients the frequency of further serial evaluations of organ function is determined by the severity and extent of organ compromise and/or by worsening of the eosinophilia.⁴

Cardiac damage

Eosinophilic myocarditis is an important cause of morbidity and mortality in HES patients, especially in those with the *PDGFRalpha* gene mutation. Cardiac involvement in patients with HES is unpredictable and not clearly correlated with the degree of AEC. An increased level of serum cardiac troponin has been shown to correlate with the presence of cardiomyopathy in patients with HES. Electrocardiogram may show non-specific and diffuse T-wave inversion. Typical echocardiographic findings in such patients include ventricular apical thrombus, posterior mitral leaflet or tricuspid valve abnormality, endocardial thickening, dilated left ventricle, and pericardial effusion.

Table 1. Clinical, laboratory and other diagnostic evaluations in patients with eosinophilia and systemic symptoms or those with persistent eosinophilia (at least $1.5 \times 10^9/l$), with or without suspected organ damage.^{3,6}

Complete history	History travel. Exposure to animals, insects, raw food, or untreated water Recent use of drugs, although a delayed onset (2-6 weeks) may be seen in some cases
Complete physical examination	Lung abnormalities Lymph abnormalities Skin lesions, edema, or erythema Nails splinter hemorrhages, nail fold infarcts Gastrointestinal symptoms Lymphadenopathy and/or splenomegaly
Tests as indicated by history, signs and symptoms	Parasitic serologies, (e.g. strongyloidiasis, schistosomiasis, filariasis, toxocariasis) and stool examination Serum immunoglobulin levels, including IgE IgG4 level Antinuclear activity (ANA), antibodies to cyclic citrullinated peptide (CCP), ANCA HIV serology Serum troponin, NT-proBNP
Complete blood count and differential	To assess the presence of increased neutrophils, monocytes, anemia, thrombocytopenia
Peripheral blood smear	To look for dysplastic eosinophils, circulating myeloid precursors or blasts
Routine chemistries	Renal and liver function tests, muscle enzyme
Inflammation parameters	Sedimentation rate, ferritin, and C-reactive protein.
Electrocardiogram, echocardiogram	If abnormal, cardiac MRI should be considered as this may show characteristic features
Pulmonary function tests	Chest X-ray, CT, bronchoscopy with bronchoalveolar lavage/endobronchial ultrasonography
Chest/abdomen/pelvis CT	To assess for splenomegaly, lymphadenopathy and occult neoplasms
Neurologic/complementary tests	If indicated by symptoms
Serum B12 levels, Serum tryptase	Increased levels suggest HES.
FIP1L1/PDGFRalpha analysis by FISH or RT-PCR (PB)	To be performed unless clear indications for secondary forms and/or indications for HES.
Bone marrow biopsy, including cytogenetics	Recommended in all patients with AEC $> 5.0 \times 10^9/L$, features of HES, or L-HES; should be considered in other patients
Biopsies of affected tissues (if possible)	e.g. skin, gastrointestinal tract
T and B cell receptor rearrangement studies	If suspected L-HES or to exclude B cell lymphoproliferative disorders
Lymphocyte phenotyping by flow cytometry	To assess for aberrant CD3-CD4+, CD3-CD4-CD8+ and CD3-CD4-CD8- populations and B cell lymphoproliferative disorders

Neurological manifestations

HES can be complicated by recurrent stroke, encephalopathy and peripheral sensory neuropathies. Peripheral neuropathy is the more common neurological manifestation of HES patients. It can involve the motor nerves and/or sensory nerves, and can manifest as mono-neuritis multiple or as radiculopathy and muscular atrophy

Skin manifestations

The most common cutaneous manifestations in HES are eczema (which often involves the hands or areas of flexion), erythroderma, lichenification, dermatographism, recurrent urticaria and angioedema. Oral, nasal, pharyngeal, esophageal or genital mucosal ulcers less frequently develop in HES.

Lung manifestations

Pulmonary involvement is common in HES and may result from an eosinophilic infiltration of pulmonary tissue resulting in fibrosis and pulmonary embolism. The more common symptoms at presentation were dyspnea (45%), cough (39%) and wheezing (24%). In 43% of patients radiological changes were observed.

Gastrointestinal manifestations

Eosinophilic gastritis, enteritis and/or colitis can occur during HES and can cause weight loss, abdominal pain, vomiting and/or severe diarrhea. Liver involvement can take the form of chronic active hepatitis, of hepatic focal lesions, of cholangitis eosinophilic, or Budd-Chiari syndrome.

Conclusions

The evaluation of patients with eosinophilia may be complex, costly, needs time and a multidisciplinary approach. The involvement of allergist, hematologist, pathologist and the infectious diseases specialist may be recommended in diagnosis and management of eosinophilic disorders. The main steps include application of diagnostic algorithm, exclusion of the most frequent secondary causes, and evaluation of organ damages and their severity. Moreover, the hematological evaluation of HE may be difficult, due to many possible abnormalities, with uncertain aspects and a need for highly specialized complementary tests. There is a clear need for multidisciplinary specialized centers in eosinophilic disorders.

SYSTEMIC MASTOCYTOSIS

Systemic Mastocytosis (SM) is a clonal disease characterized by an accumulation of mast cells (MC) in various organs (skin, bone marrow (BM), gastrointestinal tract, lymph nodes and spleen) other than skin and it is correlated in the majority of cases to a point mutation of the gene that codes for the KIT receptor.^{5,7} According to the WHO classification, SM can be divided into: indolent SM (ISM); smoldering SM (SSM); SM with an associated hematologic neoplasm (SM-AHN); aggressive SM (ASM); and MC leukemia (MCL).⁷ From the prognostic point of view, SM is a heterogeneous disorder, which varies from non-advanced forms, with an excellent prognosis, to advanced variants (ASM, SM-AHN, MCL) with very poor prognosis.

The term "mastocytosis in the skin" (MIS) is an operational term pending completion of the staging workup to establish whether the patient with typical lesions has systemic or cutaneous mastocytosis. MIS is present in the majority of ISM patients, and less frequently in advanced variants. However the indolent variants without MIS are underestimated and characterized by peculiar onset symptoms such as mainly hymenoptera, drug, food or idiopathic anaphylaxis, unexplained osteoporosis or gastrointestinal symptoms (see Table 2).⁸ The majority of these cases are classifiable as BM mastocytosis (BMM), a provisional sub-variant of SM where MC infiltration is largely restricted to the BM, the serum tryptase (sT) level is low or normal, and no skin lesions can be detected.⁷

Clinical manifestations

Symptoms of SM could be related to the acute or chronic mediators release or to organ damage. MC-mediator related symptoms are mainly cutaneous, gastrointestinal (GI), allergic, musculoskeletal and neuropsychological. Other less frequent symptoms are cardiovascular (hypertension, palpitations, pre-syncope episodes, syncope) or constitutional (asthenia, weight loss, fever, profuse sweating). Symptoms due to MC degranulation are often unpredictable and can be triggered by physical factors, such as heat/cold variation and physical exertion, the consumption of certain food and/or alcohol, the use of drugs, such as non-steroidal anti-inflammatory drugs, emotional stress and hymenoptera venoms. The mediator release symptoms in some patients are very modest and sometimes absent, while in others they are severe or even life-threatening and require continuous therapy and, in some cases, cytoreductive treatment.^{7,8}

Signs and symptoms due to from tissue infiltration are hepatosplenomegaly, ascites, malabsorption, pain due to bone lysis and/or pathological fractures, lymphadenomegaly, cytopenias.⁷ Both constitutional symptoms and clinical manifestations of tissue infiltration are present almost exclusively in advanced variants.

Evaluation of patient with Systemic Mastocytosis

Recommended clinical, laboratory and radiologic parameters to investigate in patients with SM are detailed in Table 3.^{8,9}

Table 2. Clinical and laboratory features that argue for a bone marrow examination in adult patients with suspected mast cell disease without mastocytosis in the skin (MIS).^{8,9}

<p>Absolute indications: Elevated basal serum tryptase level (>20 ng/mL or >30 ng/mL as isolated finding) that is not based on a known familial hypertryptasemia or other myeloid neoplasm Expression of <i>KIT</i> D816V in circulating blood leukocytes Mast cell activation syndrome patients in whom the basal serum tryptase level is clearly elevated (>20 ng/mL) with or without known familial hypertryptasemia Unexplained splenomegaly and/or lymphadenopathy Unexplained blood count abnormalities, e.g., eosinophilia and/or cytopenia Anaphylactic shock after bee or wasp stings (-/- known allergy) and REMA score >=2</p> <p>Relative indications: Unexplained (idiopathic) mast cell activation syndrome Unexplained osteoporosis—especially in men Non-specific colitis and/or unexplained hepatopathy with ascites Histamine—symptomatology, e.g., otherwise unexplained headache + diarrhea Unexplained weight loss Unexplained gastrointestinal symptoms responding to H2R blockers Unexplained elevated serum alkaline phosphatase activity</p>

Table 3. Clinical, laboratory and other diagnostic evaluations in patients with Systemic Mastocytosis.^{8,9}

<p>Accurate history of:</p> <ul style="list-style-type: none"> allergic reactions mediator symptoms and related triggers previous and ongoing drug treatment, previous fractures weight loss
<p>Physical examination</p> <ul style="list-style-type: none"> presence and distribution of typical skin lesions hepato-splenomegaly lymphadenomegaly
<p>Laboratory tests:</p> <ul style="list-style-type: none"> Basal serum tryptase dosage Blood count with differential Bilirubin, transaminase, creatinine, uric acid <p><i>Suggested:</i></p> <ul style="list-style-type: none"> prothrombin time, partial thromboplastin time alkaline phosphatase, lactate dehydrogenase, beta2microglobulin albumin, cholesterol, serum B12, folate and iron, ferritin 25-OH-vitamin D, serum C-terminal telopeptide of type I collagen, parathormon, calcemia, phosphoremia, 24-hour calciuria and phosphaturia
<p>Radiological investigations:</p> <ul style="list-style-type: none"> Evaluation of the abdomen by ultrasound or NMR Lumbar spine and femur densitometry Study of the skeleton in whole or limited to Rx column in toto and pelvis Bone scintigraphy, or PET-TAC or TC (only in selected cases)
<p>Endoscopy of the stomach and colon (only in selected cases)</p>

Skin manifestations

Maculopapular cutaneous mastocytosis (MPCM) is the most common form of MIS in adults. It presents as symmetrically distributed red-brown macules or papules with the highest density of lesions on the trunk, whereas the palms, soles, face, and head are often spared. Mechanical irritation may cause reddening and urticarial swelling of the lesions, the so-called Darier's sign, which is pathognomonic for MIS. Typical skin symptoms in SM are pruritus, flushing, blistering, urticaria; their severity appear unrelated to sT level and extent of lesions. Recurrent flushing could be the also the onset symptoms in SM without MIS. The clinical severity of MIS can be assessed using the SCORMA (SCORing Mastocytosis) system according the extent of MIS, severity of representative cutaneous lesions and symptom severity. Recent series demonstrated that only a minority of adult patients have skin-limited disease.⁹

Allergic manifestations

In adults patients with mastocytosis the prevalence of anaphylaxis has been reported to be 22% to 49%, which represents almost a 100 fold increased risk compared to the general population.¹⁰ The most common elicitors of anaphylaxis appear to be hymenoptera sting, mainly in BMM (10), followed by idiopathic anaphylaxis. Although less frequently, drugs and food can also be elicitors in patients with SM. The clinical course of anaphylactic reactions is often severe and includes hypotensive syncope. At least SM patients with previous allergic reactions (or all SM according to recent published guidelines) must be advised to carry two epinephrine self-injector-pens.^{8,9,10}

In patients with BMM, anaphylaxis represents the most frequent symptom leading to the diagnosis of mastocytosis and is characterized in the majority of cases by the absence of angioedema and erythema and the predominance of cardiovascular symptoms, such as hypotension leading to loss of consciousness. These patients typically show prevalence of the male sex, lower MC burden and slightly elevated or normal sT level. The recently established REMA score (based on sex, sT level, presence or absence of urticaria/angioedema and hypotension during allergic episode) is a helpful tool that can suggest the presence of SM with sufficient precision and sensitivity, to justify a BM investigation.¹⁰

Because MCs in BMM associated with HVA often represent a very small fraction of all nucleated BM cells and *KIT* mutation is usually restricted to the neoplastic MC population, diagnosis relies on BM evaluation through multiparametric flow-cytometry and highly sensitive methods such as ASO-qPCR molecular analysis for detecting D816V *KIT* mutations.¹⁰

Diagnosis of SM in HVA patients has significant consequences because specific immunotherapy should be continued long-life, due to the reports of severe anaphylaxis, even lethal, after discontinuation.¹⁰

It is very important to refer all patients with adverse reactions to drugs to an allergy specialist for appropriate counseling and, if necessary, testing (e.g., skin testing, provocation test) under close supervision. In patients who have never experienced adverse reactions to drugs, previously and continuously tolerated medications are allowed. The anesthetic procedures must be managed as high-risk procedures in mastocytosis patients, since it is difficult to predict the presentation of MC mediator related symptoms. In this line, it is recommended to give prophylactic anti-mediator therapy. Multidisciplinary collaboration is needed for all anesthesia/surgery, endoscopy or other invasive radiologic procedures.

Bone manifestations

Bone involvement is one of most common expressions of SM in adults. The range of clinical pictures is wide. The more frequent manifestation is osteoporosis with or without fragility fractures reported from 8% to 41%. Diffuse osteosclerosis is reported from 2.5% to 8% of SM patients and generally associated with markedly high sT levels. Osteolysis are not frequent complications of SM, reported in variable

percentage (5-11%), generally associated with patchy osteosclerotic lesions and osteoporosis. Large osteolytic lesions and pathologic fractures due to diffuse infiltration of MCs, often immature, represent one of the C finding defining the ASM according to WHO classification. On the contrary small asymptomatic lytic lesions (<0,5 cm) are reported in about 2% of ISM. As recommended by the guidelines it is always desirable in the case of large bone lesions to perform a biopsy in order to exclude other hematologic or non-haematologic neoplasia.¹¹

Dual-energy X-ray absorptiometry (DXA) technique represents the gold standard for assessing bone mineral density (BMD) in SM patients, with evaluation of T score and Z scor.¹¹

Because half of the patients have a fragility fracture without a pathologic BMD value, a total spine radiography is strongly recommended for early detection of vertebral asymptomatic fractures. A whole skeleton radiograph might be suggested to exclude other focal lytic or sclerotic lesions.¹¹

Serum bone turnover markers (BTM) such bone alkaline phosphatase, osteocalcin and N-terminal of procollagen type I, and bone resorption markers such as serum C-terminal telopeptide of collagen I (CTX) are only partially reliable for assessing bone health in SM patients. In our experience serum CTX at baseline could predict BMD change, being negatively correlated to BMD change at lumbar spine.¹¹

Repeated DXA is recommended in all SM patients, even in asymptomatic cases: our suggestion is to perform a DXA scan once a year until stability is achieved, and thereafter between 12 and 24 months in osteoporotic patients. In SM patients without osteoporosis and with normal serum CTX a DXA screening every 24-36 months could be sufficient.¹¹

It is important to state that computed tomography, magnetic resonance imaging, scintigraphy, or positron emission tomography (PET) studies may sometimes add information to the staging in SM, but are not standard in the evaluation of osteopathy.⁸ Our suggestion is to perform a scintigraphy in patients with elevated BTM to detect focal asymptomatic bone lesions and target radiographs examination.

Indeed, a vitamin D deficiency is regarded as an important co-morbidity in the context of SM-mediated osteopathy, and its monitoring and supplementation if needed is mandatory.¹¹

Osteoporosis in SM has been attributed directly to the infiltration of bone marrow by MCs and local release of their known multiple mediators (eg histamine, heparin, sT).

Therefore osteoporosis may be the only manifestation of a latent SM. Any form of osteoporosis of unknown etiology, especially in the male, should be evaluated for the suspicion of SM, using sT level as a screening method.^{8,11}

Neuropsychological manifestations

Headache, anxiety, depression, disorders of attention and memory are frequent. These patients may need additional expert advice and support in order to control their symptoms. Whether these symptoms are primarily triggered by MC-derived mediators and/or are mainly caused by comorbidities and/or psychological impact of disease-related signs/symptoms remains at present unknown.

Gastrointestinal manifestations

Many patients with SM have significant GI symptoms, such as diarrhea, abdominal pain, gastrointestinal ulcerative disease, which are thought in most cases to be due to the release of mast cell mediators. In patients with involvement of the GI tract, symptoms may also be due to direct infiltration of the mucosa by MCs, with resultant malabsorption and/or enhanced local effects of mediator release. The frequency of GI symptoms in patients with systemic mastocytosis (SM) is estimated around 60-70%.

Because involvement is often multifocal and is frequently not associated with endoscopic abnormalities, taking multiple biopsies at different sites throughout the colon (or other sites in the GI tract) is the optimal approach at the time of endoscopy. GI involvement does not necessarily correlate with aggressive disease, as many patients with GI

involvement follow an indolent clinical course. MC density in colonic mucosa is highly variable with high overlap with patients with irritable bowel disease and other inflammatory diseases. Instead, the presence of aggregates or sheets of mast cells is the key diagnostic finding, which should prompt confirmation of mastocytosis by documenting the aberrant expression of CD25, which is, together with KIT mutation, an invaluable marker for the diagnosis.

Conclusions

The rarity of the mastocytosis, the multi-organ involvement and the variable clinical course require a multidisciplinary diagnostic approach and a multidisciplinary treatment strategy. It is strongly recommended to send patients to specialized centers with experience in mastocytosis.

References

1. Valent P, Klion AD, Horny HP et al. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. *J Allergy Clin Immunol.* 2012;130:607-612.e9
2. Gotlib J. World Health Organization-defined eosinophilic disorders: 2017 update on diagnosis, risk stratification, and management. *Am J Hematol.* 2017;92:1243-1259
3. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood.* 2006;108(7):2366-2372
4. Klion AD. Eosinophilia: a pragmatic approach to diagnosis and treatment. *Hematology Am Soc Hematol Educ Program.* 2015;2015:92-7
5. Kovalszki A, Weller PF. Eosinophilia in mast cell disease. *Immunol Allergy Clin North Am.* 2014 ;34:357-6
6. Butt NM, Lambert J, Ali S et al. Guideline for the investigation and management of eosinophilia. *Br J Haematol.* 2017;176:553-572
7. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood.* 2017;129:1420-1427.
8. Valent P, Akin C, Gleixner KV et al. Multidisciplinary Challenges in Mastocytosis and How to Address with Personalized Medicine Approaches. *Int J Mol Sci.* 2019;20 (12)
9. Gotlib J, Gerds AT, Bose P et al. Systemic Mastocytosis, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2018;16:1500-1537
10. Bonadonna P, Bonifacio M, Lombardo C, Zanotti R. Hymenoptera Allergy and Mast Cell Activation Syndromes. *Curr Allergy Asthma Rep.* 2016;16:5
11. Orsolini G, Viapiana O, Rossini M et al. Bone Disease in Mastocytosis. *Immunol Allergy Clin North Am.* 2018;38:443-454

THE TREATMENT OF SYSTEMIC MASTOCYTOSIS AND EOSINOPHILIC DISORDERS: FROM SUPPORTIVE TO TARGETED

F. Mannelli

CRIMM, Centro di Ricerca e Innovazione per le Malattie Mieloproliferative, Azienda Ospedaliera Universitaria Careggi, Firenze, Italy

Systemic mastocytosis (SM) and eosinophilic disorders are rare diseases sharing the increasing need for integrated approaches involving onco-hematologic competences for appropriate clinical management and treatment. The wide variability of clinical manifestations and disease course claims for an appropriate risk-stratification, embedding more objective parameters such as comprehensive molecular profiles. The development of novel targeted drugs is extending progressively the range of available therapeutic alternatives.

Systemic Mastocytosis

General considerations

Current paradigm of treatment of SM envisions a conservative approach for indolent forms, where life expectancy is not impaired significantly, while cytoreductive treatment is indicated for advanced variants, where the need to control manifestations of myeloproliferation and prevent damage to target organs overtake the potential side effects of therapies. Although the clinical manifestations of advanced SM often mandate early initiation of treatment, in other cases the distinction between mediator-related signs/symptoms and "true" organ damage may be complicated.

In this context, the risk is on one side to overtreat with cytoreductive drugs, on the other to overlook subtle disease-related issues that might deserve a timely therapeutic intervention to avoid further damage. Several biomarkers, including clinical, hematologic and molecular variables,

have been explored, with the aim to provide robust support to clinical management. The inclusion of biological parameters into prognostic models follows this direction; currently, main therapeutic decisions still rely on the clinical appraisal of the benefit/risk ratio of treatment modalities within indolent and advanced variants according to WHO Classification (Figure 1).

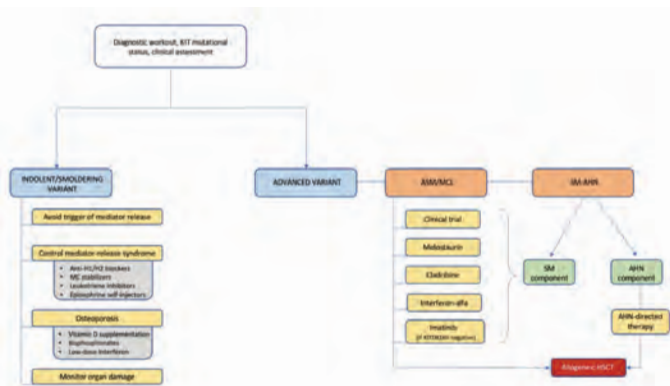


Figure 1. Treatment algorithm in systemic mastocytosis.

Indolent/Smoldering variants

According to WHO, indolent systemic mastocytosis (ISM) is defined by the absence of organ damage, on turn highlighted by the so-called C-findings, and includes the large majority of patients. Smoldering (SSM) form has been separated from ISM based on features of high disease burden, and a watchful monitoring in order to catch early signs of progression is recommended. The general indication for ISM/SSM management envisions a conservative approach, thus not including cytoreductive treatment.

The clinical burden related to mediator release comprises the whole spectrum from absolute absence of symptoms, to chronic disturbances with a variable impact on quality of life (fatigue, cutaneous and gastrointestinal manifestations) until severe, potentially life-threatening allergic reactions. The use of anti-mediators is generally recommended for asymptomatic and mildly symptomatic patients. The standard therapy consists of histamine receptor blockers (anti-H1 and H2 antagonists) and mast cell stabilizers (sodium cromoglycate, ketotifen), the latter ones especially used in patients suffering from gastrointestinal symptoms. Because of the risk of anaphylaxis, sometimes without an identifiable trigger, SM patients are prescribed to carry epinephrine pen self-injectors and to use the device after an appropriate training in a specialized center. When allergic reactions are due to hymenoptera stings, representing the trigger in up to 50-60% of anaphylaxes, patients must undergo life-long immunotherapy.

An important issue concerns the patients with severe mediator-related symptoms that are refractory to standard therapy: in this category, the estimation of the benefit/risk ratio for cytoreduction is particularly challenging. Few recent studies have explored the use of KIT inhibitors Masitinib (randomized, placebo-controlled trial) and Midostaurin (non-randomized, phase 2 trial) in this subset, demonstrating an improvement of symptoms with relatively safety profile. Although reasonable, the feasibility of cytoreductive treatment in this context has not been formally ascertained yet, especially in terms of long-term safety.

A clinically subtle manifestation of mediator release is osteopathy, that should be searched for at SM diagnosis by DEXA-scan. Osteoporosis should not be interpreted as a sign of disease aggressiveness, however it must be promptly identified and treated to reduce the risk of bone fractures. When resulting refractory to conventional approaches (vitamin D supplement, bisphosphonates), the use of low-dose interferon- α is considered appropriate also due to its capability to increase bone density.

Advanced variants

Although sharing systemic and organ impairment, the definition of advanced variants gathers different SM forms with variable clinical pictures and therapeutic needs. Aggressive SM (ASM) is featured by the presence of at least one C-finding, as expression of organ damage. Mast cell leukemia (MCL) is defined by massive infiltration by mast cells at

bone marrow smear ($\geq 20\%$ of total cells). Overall, MCL has dramatic outcome, even if rare MCL cases have described to show a chronic disease course, as a further proof of the variability in prognosis. The concomitant diagnosis of another hematologic malignancy leads to the diagnosis of SM with an associated hematologic neoplasm (SM-AHN). In this subset, the appraisal of the actual impact of SM on disease manifestations can be complex as well as the most appropriate therapeutic approach. In the majority of cases, the AHN component dominates and should be the major target of therapy. In some cases, especially with myeloid neoplasms and complex, high-risk molecular profile, a role for SM-directed treatment should be considered. In this subset, KIT mutant allele burden might aid to predict the extent of KIT-addiction of the disease and thus the potential efficacy of KIT inhibition, even if there is no established standard.

The general statement can only be toward an individualization of treatment, even more important in view of the rapid changes in the therapeutic scenario. Tyrosine kinase inhibitors, progressively more potent and selective, now flank conventional cytoreductive drugs (cladribine, interferon- α). The availability of controlled trials comparing such treatment modalities cannot be expected due to the rarity of advanced variants, even if the harmonization of response criteria by IWG-MRT might aid to generalize some considerations. At last, the choice of the most appropriate therapy should be rationalized by clinical picture and molecular data (particularly KIT mutational status). The most relevant therapeutic options are summarized below.

Imatinib

Imatinib has been demonstrated ineffective against the common domain mutants (D816V), but able to induce a response with certain transmembrane (F522) and juxta-membrane (V560) mutations, at doses ranging from 100 to 400 mg. Although difficult to estimate from literature, the response rate is about 30% in KITD816V-negative SM. These findings reasonably support a challenge with Imatinib in this subset (usually for at least one-two months, if clinically feasible), taking into account the well-known safety profile from other experiences (i.e. chronic myeloid leukemia). The drug is currently approved for the treatment of adult SM patients without KITD816V or with unknown mutational status.

Cladribine

Cladribine (2-chloro-deoxy-adenosine) has been used in both ISM and advanced variants and the available data are derived from retrospective series, the largest ones from Mayo Clinic and French group. It has been delivered both intravenously and subcutaneously at 0.13-0.17 mg/kg per days for a median number of 3 cycles. The overall response rate was about 40-50% in advanced forms, placing the drug as a valid therapeutic option, especially when a rapid debulking is needed, or as salvage therapy. Main concerns derived from the known immunosuppressive effect.

Interferon- α

As in the field of myeloproliferative neoplasms, Interferon- α has shown activity in SM across all clinical variants with improvement in mediator-related syndrome and in some cases reduction in mast cell burden. It is conventionally employed at 1-3 million units subcutaneously 2-3 times per week and potentially dose-escalated depending on response and tolerability. Late responses have been described, and therapy is generally continued as long as a benefit is observed. The major flaw is the high rate of withdrawal because of scarce tolerance, an issue that might improve with the increasing availability of pegylated formulation in onco-hematological setting.

Midostaurin

Midostaurin is a multi-kinase inhibitor targeting mutant and wild type KIT as well as other kinases such as FLT3, PDGFRA, VEGFR. The results of a phase 2, non-randomized clinical trial on 89 patients led to drug approval by regulatory agencies in 2017 for the treatment of adult patients with advanced SM. Midostaurin was given at 100 mg twice daily and showed an overall response rate of 60% per modified Valent and Cheson criteria. Responses were obtained regardless of KIT mutational status or the presence of concomitant AHN. Midostaurin was well tolerated: beyond hematological toxicity, expected for the clinical context, nausea and vomiting were the most frequent adverse events.

They were generally manageable with prophylactic antiemetics and assumption with food. A recent update of data after a 10-year follow-up was published: median overall survival was 40 months in the whole cohort and 18.5 months in MCL subset. No relevant long-term toxicities were observed. Midostaurin is thus an effective therapy both as first-line approach, as well as salvage treatment after other cytoreductive therapies.

Allogeneic transplant

Data about the results of allogeneic transplant (HSCT) in SM have been published as case reports or retrospective series, the largest included 57 patients transplanted in the United States and Europe. As expected, the cases were enriched for SM-AHN subset, that represented the main reason for allocation to allogeneic HSCT. In other advanced variants, the overall outcome was poor and a diagnosis of MCL was the stronger unfavorable factor for survival. Treatment-related mortality mirrored what seen in other hematologic malignancies.

Given the lack of prospective data, current indications derived from consensus opinion. From a clinical standpoint, the allocation to HSCT is easily sustainable in SM-AHN whenever indicated by AHN component, and in MCL. The decision is much more challenging for patients with advanced SM achieving in-depth responses to Midostaurin or another selective KIT inhibitor, since no robust data can favor TKI continuation versus switch to HSCT. Future guidelines incorporating more accurate risk stratification upon molecular genetics might help clinicians to rationalize this crucial clinical decision.

Investigational agents

Avapritinib/BLU-285

The drug is a kinase inhibitor featured by high selectivity for KIT mutants and limited off-target activity. The interim analysis from phase 1 study (Explorer trial; NCT02561988) showed a promising overall response rate of 83%, observed across all SM subtypes, with relatively good tolerability and short time to evidence of a clinical response. A phase 2 study (NCT03580655) is currently enrolling advanced SM cases.

DCC-2618

It is a potent inhibitor of KIT exon 17 mutants that is currently investigated within a phase 1, open-label trial (NCT02571036).

Eosinophilic syndromes

General considerations

The correlation between the extent of eosinophilia and the occurrence of organ damage is uncertain and there are not clear evidences supporting the initiation of therapy merely depending on absolute eosinophil count. The decision to treat or to observe should be based on a careful and comprehensive assessment, which is usually available at the time of diagnostic workout completion. Of note, especially with overriding clinical manifestations prompting immediate initiation of therapy, adequate sampling for genetic abnormalities must precede treatment starting: the rapid response to steroids could lead to false negative results and missing of potentially effective therapies.

Primary underlying disease definition and the presence of organ damage represent the basis for clinical management: a diagnosis of myeloid malignancy bearing specific rearrangements sustains initiation of treatment depending on availability of effective therapies. At the opposite, CEL NOS mandates for initiation of therapy because of poor prognosis and risk of clonal progression.

A watch-and-wait approach is acceptable for asymptomatic patients with absolute eosinophil count $\geq 1.500/\text{microliter}$ and no evidence of myeloid neoplasm and end-organ manifestations, provided they are closely monitored for early organ damage (echocardiography, serum troponin level, pulmonary function testing).

The therapeutic issues are discussed separately below for the specific disease entities as defined by 2016 update of WHO Classification (Table 1).

Table 1. Summary of therapeutic options in eosinophilic disorders.

Clinical subset	Treatment
Myeloid/lymphoid neoplasms with PDGFRA rearrangement	Imatinib 100 mg daily (400 mg with PDGFRA variants other than FIP1L1-PDGFRFA)
Myeloid neoplasms with PDGFRB rearrangement	Imatinib 400 mg daily
Myeloid/lymphoid neoplasms with FGFR1 rearrangement	Intensive chemotherapy (i.e. Hyper-CVAD) followed by allogeneic HSCT <i>Alternative/salvage therapy:</i> Selective FGFR1-inhibitor (Pemigatinib) within clinical trial
Myeloid/lymphoid neoplasms with PCM1-JAK2 (provisional entity)	Ruxolitinib; allogeneic HSCT
Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS)	Steroids, hydroxyurea Consider challenge with Imatinib Allogeneic HSCT if eligible candidate
Lymphocytic variant	Steroids Steroid-sparing agents (Interferon- α , anti-IL-5 monoclonal antibodies) <i>Alternative/salvage therapy:</i> Cyclosporine, Methotrexate, Alemtuzumab
Hyper-eosinophilic syndrome (HES)	Steroids Steroid-sparing agents (Interferon- α , anti-IL-5 monoclonal antibodies) Consider challenge with Imatinib Interferon- α

Myeloid/lymphoid neoplasms with PDGFRA rearrangement

The efficacy of tyrosine kinase inhibitor Imatinib in FIP1L1/PDGFRFA-positive myeloid neoplasms has been demonstrated in several studies. The recommended starting dose is 100 mg daily, usually providing rapid and deep responses. Steroids should be added in the first days of therapy with evidence of cardiac involvement (elevated serum troponin or abnormalities at echocardiogram) to prevent myocardial necrosis, rarely described with Imatinib in this patient population. Once durable and in-depth molecular remission was achieved, some studies have reported about drug discontinuation. The withdrawal of TK inhibition led to relapse in some patients, with the majority of them achieving again a molecular response at re-exposure. Other patients maintained long-term remission for more than two-years after suspension. The current general recommendation favors ongoing treatment until the role for discontinuation is more systematically addressed in the context of clinical trials. Very few cases of acquired imatinib resistance have been reported, mainly due to T674I mutation within the ATP-binding domain of PDGFR, and alternative available TKIs have demonstrated limited clinical activity in the few published reports.

In the presence of PDGFRA variants other than FIP1L1-PDGFRFA, a higher dosage of Imatinib has been usually adopted with good response rate.

Myeloid neoplasms with PDGFRB rearrangement

In patients with rearrangements of PDGFRB, the available literature indicates that imatinib should be given as first-line therapy at doses of 400 mg daily. This treatment is able to induce long-term responses with median survival rates exceeding 90% at 10 years.

Myeloid/lymphoid neoplasms with FGFR1 rearrangement

The clinical onset of hematological neoplasms bearing FGFR1 rearrangements is typically featured by different simultaneous hematological phenotypes, such as T- and B- lymphoproliferative disorders and myeloproliferation with eosinophilia, as the result of stem cell clonal involvement. The aggressive clinical presentation usually prompts immediate cytoreductive treatment, that on turn may be responsible of a delayed diagnosis. Intensive chemotherapy with regimens such as Hyper-CVAD followed by early allogeneic HSCT in eligible patients is recommended.

The TKI Ponatinib has demonstrated activity in patients with FGFR1-positive neoplasms but responses are generally partial and short-term.

Novel therapeutic opportunities are offered by selective and potent inhibitors of FGFR1: Pemigatinib is currently investigated within a phase 2, open-label clinical trial (NCT03011372) in subjects with FGFR1-rearranged neoplasms.

Myeloid/lymphoid neoplasms with PCM1-JAK2 (provisional entity)

Sparse reports describe the JAK1/2 inhibitor Ruxolitinib as able to induce complete hematologic remissions and cytogenetic responses in

patients bearing this fusion gene. Due to the obvious lack of data and variability in response duration, allogeneic transplant should be considered for suitable cases.

Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS)

According to WHO, CEL-NOS is characterized by presence of blasts, evidence of clonality by cytogenetics and signs of myeloproliferation.

In selected cases, patients with CEL-NOS may benefit from Imatinib, and a challenge might be attempted in order to assess potential sensitivity given the favorable toxicity profile. However, hematologic responses are more often partial and short-lived. Hydroxyurea and steroids can be used to control disease manifestations.

The overall prognosis is poor, evolution to acute leukemia is relatively frequent and allogeneic HSCT should be considered in selected cases, even if its role is not well-established due to the lack of systematic prospective studies.

Lymphocytic variant

Clonal T cells producing excess of IL-5 are the underlying cause of lymphocytic variant.

Corticosteroids is considered the standard first-line therapy. However, after initial response, eosinophilia tends to occur again, and disease control often requires long-term administration of therapy and the use of steroid-sparing drugs (as Interferon). The pathogenetic mechanism sustains the application of therapeutic approaches targeting T-cell clones: cyclosporine and anti-CD52 alemtuzumab have been described as effective in isolated case reports or small retrospective series.

Hyper eosinophilic syndrome (HES)

Once all other causes of hyper eosinophilia are excluded and a diagnosis of HES is formulated, steroids are considered the first-line therapy. When features of myeloproliferation and/or dysplasia are prominent, a challenge with Imatinib is a reasonable try since clinical responses have been reported up to 50% in this category.

In the lack of defined standards, the conventional steroid schedule adopted from other clinical contexts, generally provides Prednisone (PDN) 1 mg/kg for 15 days followed by slow dose-tapering. Intravenous administration can be considered with suspected absorption impairment. Especially with life-threatening manifestations (i.e. cardiac involvement) and need for rapid initiation of treatment, empiric ivermectin therapy should be given to prevent potentially fatal Strongyloides hyperinfection syndrome. When long-term treatment (PDN >10 mg daily) is required for disease control, steroid-sparing therapies should be used, among which hydroxyurea is the most adopted.

A phase 3 trial is currently investigating Mepolizumab in HES (300 mg subcutaneously monthly), after it has demonstrated to be an effective steroid-sparing agent in other clinical contexts. Additional monoclonal antibodies, anti-IL-5 Reslizumab and anti-IL-5-receptor Benralizumab, have been tested in smaller studies and in pediatric context, respectively, showing promising results.

Interferon-alfa can produce hematological responses and substitute steroids when significant contra-indications are present, or it can be associated to steroids as well.

References

- Pardanani A. Systemic mastocytosis in adults: 2019 update on diagnosis, risk stratification and management. *Am J Hematol.* 2019 Mar;94(3):363-377.
- Ustun C, Gotlib J, Popat U, et al. Consensus Opinion on Allogeneic Hematopoietic Cell Transplantation in Advanced Systemic Mastocytosis. *Biol Blood Marrow Transplant.* 2016 Aug;22(8):1348-1356.
- Valent P, Akin C, Gleixner KV, et al. Multidisciplinary Challenges in Mastocytosis and How to Address with Personalized Medicine Approaches. *Int J Mol Sci.* 2019 Jun 18;20(12).
- Gotlib J, Gerds AT, Bose P, et al. Systemic Mastocytosis, Version 2.019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw.* 2018 Dec;16(12):1500-1537.
- Shomali W, Gotlib J. The new tool "KIT" in advanced systemic mastocytosis. *Hematology Am Soc Hematol Educ Program.* 2018 Nov 30;2018(1):127-136.
- Gotlib J, Pardanani A, Akin C, et al. International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) & European Competence Network on Mastocytosis (ECNM) consensus response criteria in advanced systemic mastocytosis. *Blood.* 2013 Mar 28;121(13):2393-401.
- Lim KH, Pardanani A, Butterfield JH, et al. Cytoreductive therapy in 108 adults with systemic mastocytosis: Outcome analysis and response prediction during treatment with interferon-alpha, hydroxyurea, imatinib mesylate or 2-chlorodeoxyadenosine.

- Am J Hematol.* 2009 Dec;84(12):790-4.
- Gotlib J, Kluin-Nelemans HC, George TI, et al. Efficacy and Safety of Midostaurin in Advanced Systemic Mastocytosis. *N Engl J Med.* 2016 Jun 30;374(26):2530-41.
- Gotlib J. World Health Organization-defined eosinophilic disorders: 2017 update on diagnosis, risk stratification, and management. *Am J Hematol.* 2017 Nov;92(11):1243-1259.
- Klion A. Hyper eosinophilic syndrome: approach to treatment in the era of precision medicine. *Hematology Am Soc Hematol Educ Program.* 2018 Nov 30;2018(1):326-331.

QUALITY OF LIFE AND LONG TERM FOLLOW UP AFTER HLA IDENTICAL PERIPHERAL STEM CELL TRANSPLANT FOR ACUTE LEUKEMIA

F. Bonifazi

Institute of Hematology "Seràgnoli"; University Hospital S: Orsola-Malpighi, Bologna, Italy

Allogeneic hematopoietic stem cell transplant (HSCT) is a curative option for patients affected by acute leukemia. GVHD is the major complication of allogeneic HSCT.^{1,2} For this reason, several efforts have been made to increase the prevention of GVHD maintaining the anti-leukemia effect.³ One of the most used strategy, at least in Europe,⁴ is the addition of antilymocyte globulin (ATLG) to calcineurin inhibitors and antimetabolites. ATLG is a globulin obtained by the immunization of rabbits against the Jurkat cell line, a cell line derived from acute lymphoblastic leukemia. Several randomized trials demonstrated the efficacy of ATLG in GVHD prevention in the setting of unrelated transplants.^{5,7} In the setting of HLA identical sibling transplants, instead, there is only one study,⁸ focused on acute leukemias in remission, demonstrating the efficacy of ATLG at 30 mg /kg as total dose to prevent GVHD. The incidence of acute GVHD was 35% vs 25% while chronic GVHD incidence was reduced from 68% to 32% in the control and experimental arm, respectively. In particular, the incidence of extensive chronic GVHD was significantly reduced from 52% to 8%) as well as the severity of organ involvement.

The incidence of NRM and relapse incidence were not different in the two arms and no survival difference was found.

All these data were then confirmed in a long term follow up (5.9 years from transplant).⁹

The original study foresaw a quality of life (QoL) evaluation by means of two EORTC questionnaires: QLQ-C30 developed for cancer patients and QLQ-29 for patients undergoing HSCT.

The questionnaires were administered pre transplant, at 3, 6, 12, and 24 month after transplant.

45% of questionnaires were finally returned; there were no relationship between QoL forms return an age, arm of randomization, and GVHD; the strongest factor associated with form return was the center.

The analysis was performed by mixed models for repeated measures and linear mixed models for comparison across the overall trajectory and for point to point comparison; the assumption of missing at random was verified by a sensitivity analysis.

The analysis found that global health status was higher in the ATLG arm (p=0.02) and the treatment group difference increased over time up to 10.5 points at 24 months. Better QoL was also found in four of the five functional scales of QLQ-30 when we compared the overall course from transplant to 24 months, while only physical and social function (p=0.014) remained significantly better in a point-to-point comparison at 24 months after allogeneic HSCT (9). Further domains resulted statistically better in the ATLG were impact of family and gastrointestinal effects, both for QLQ-29.

Despite a significant difference in GVHD incidence and severity and better QoL, patients coming back at work after transplant did not differ significantly in the two arms, underlying that cultural abihits and personal motivation can be very important.

The study demonstrated that the addition of ATLG in the setting of a myeloablative allogeneic HSCT from HLA identical sibling in patients suffering from acute leukemia in remission decreases significantly the incidence and severity of chronic GVHD without negative effect on relapse, NRM and survival. Although no significant survival gain was obtained, the addition of ATLG improves significantly QoL after transplant and should be discussed during pre-transplant patients' counselling.

References

1. Arai S, Arora M, Wang T, Spellman SR, He W, Couriel DR, et al. Graft-vs-Host Disease Working Committee of the CIBMTR. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant.* 2015; 21:266-274.
2. Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. *J Clin Oncol.* 2011; 29:2230-2239.
3. Servais S, Menten-Dedoyart C, Beguin Y, Seidel L, Gothot A, Daulne C, et al. Impact of Pre-Transplant Anti-T Cell Globulin (ATG) on Immune Recovery after Myeloablative Allogeneic Peripheral Blood Stem Cell Transplantation. *PLoS One.* 2015; 10: e0130026.
4. Ruutu T, van Biezen A, Hertenstein B, Henseler A, Garderet L, Passweg J, et al. Prophylaxis and treatment of GVHD after allogeneic haematopoietic SCT: a survey of centre strategies by the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant.* 2012; 47:1459-1464.
5. Finke J, Bethge WA, Schmoor C, Otttinger H, Stelljes M, Volin L, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol.* 2009; 10:855-864.
6. Soiffer RJ, Kim HT, McGuirk J, Horwitz ME, Johnston L, Patnaik MM, et al. Prospective, Randomized, Double-Blind, Phase III Clinical Trial of Anti-T-Lymphocyte Globulin to Assess Impact on Chronic Graft-Versus-Host Disease-Free Survival in Patients Undergoing HLA-Matched Unrelated Myeloablative Hematopoietic Cell Transplantation. *J Clin Oncol.* 2017; 35:4003-4011.
7. Locatelli F, Bernardo ME, Bertaina A, Rognoni C, Comoli P, Rovelli A, et al. Efficacy of two different doses of rabbit anti-T-lymphocyte globulin to prevent graft-versus-host disease in children with haematological malignancies transplanted from an unrelated donor: a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2017; 18:1126-1136.
8. Kroger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. *N Engl J Med.* 2016; 374:43-53.
9. Onifazi F, Solano C, Wolschke C, Sessa M, Patriarca F, Zallio F, et al. Acute GVHD prophylaxis plus ATLG after myeloablative allogeneic haematopoietic peripheral blood stem-cell transplantation from HLA-identical siblings in patients with acute myeloid leukaemia in remission: final results of quality of life and long-term outcome analysis of a phase 3 randomised study. *Lancet Haematol.* 2019; 6:e89-e99.

MOLECULAR MECHANISMS OF ACUTE MYELOID LEUKEMIA RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

L. Vago^{1,2}

¹Unit of Immunogenetics, Leukemia Genomics and Immunobiology, IRCCS San Raffaele Scientific Institute, Milano; ²Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milano, Italy

Over the last decades, the clinical results of allogeneic hematopoietic cell transplantation (allo-HCT) for acute myeloid leukemia (AML) and other hematological malignancies have improved considerably, mostly due to a significant reduction of infectious and GvHD-related mortality. Nevertheless, relapse continues to represent a frequent occurrence, with largely unsatisfactory salvage options.

Recent studies have provided key information on the biology of post-transplantation relapses, explaining AML recurrence in an evolutionary perspective: the changes in disease clonal structure and immunogenicity that are often documented at relapse are in fact proposed to represent end-results of a process of selection, allowing the outgrowth of variants that are resistant to the combination of chemotherapy and immunotherapy that is characteristic of allo-HCT.

During the presentation, we will present an overview on the mechanisms at the basis of relapse, including clonal evolution, genomic and non-genomic changes in the human leukocyte antigen (HLA) asset or enforcement of immune checkpoints.

Finally, we will discuss how understanding and untangling the interactions at the basis of relapse may provide key knowledge for the selection of personalized therapeutic approaches.

CYTOMORPHOLOGY BEFORE BONE MARROW ASPIRATE

A. Tichelli,¹ G. Zini²

¹Hematology, University Hospital – Basel, Switzerland; ²Fondazione Policlinico Universitario A. Gemelli IRCCS – Roma, Italia Istituto di Ematologia, Università Cattolica del Sacro Cuore

Hematology represents the second most important clinical laboratory testing activity in terms of number and relevance in the diagnostic pathway, after clinical chemistry. Actually blood cell count and differential

are fully automatized and are inclusive of numbers related to the classical hematological parameters and to the new indices, together with cell distributions, curves and cytograms. A robust automated flagging system allows the identification of those samples presenting with quantitative and/or qualitative abnormalities: for these samples, the smear review at microscope does represents the second level test that is mandatory for the final peripheral blood report. Today, in most laboratories the number of microscopic analysis of a blood film has greatly decreased and counts for about 10 to 15 percent of all peripheral blood analysis. In the diagnostic workup of hematological disorders microscopy still remain a diagnostic tool according to the revised WHO 2016 classification of haematological neoplasms.¹ Qualitative and quantitative consensus criteria are included into these guidelines: application of these rules guarantee worldwide uniform definitions and terminology of hematological diseases needed to facilitate patient management all over the world. WHO recommendation on peripheral blood evaluation include the manual differential on 200 cells at diagnosis together with red blood cell (RBC) and platelet quantitative and qualitative evaluation. Peripheral blood morphology represents the first diagnostic screening in the majority of hematological disorders. Moreover a prompt morphologic diagnosis in hematologic emergencies, such as in acute promyelocytic leukemia, Burkitt lymphoma, thrombotic thrombocytopenic purpura or sickle cell crisis, is critical to save patients' lives. Finally, published data on the Global Burden of Disease study show that the incidence of non-Hodgkin lymphoma (NHL) and of leukemia increased 45%, and 26% respectively from 2006 to 2016, largely due to population growth and aging.² Based on these considerations, the need of an accurate and speedy microscopic identification of abnormal circulating cells is evident, allowing an early diagnosis, with a high predictivity in both, myeloid and lymphoid neoplasms.

Microscopy is the sole diagnostic tool to detect dysplasia at level of single cells: percentage of dysplastic cells for each myeloid lineage is part of the diagnostic rules for myelodysplasia. The WHO morphological dysplastic features to be considered for each lineage, are listed in Table 1: the International Working Group on Morphology of myelodysplastic syndromes (MDS) has published further recommendation for an harmonized cell dysplasia identification.³⁻⁵

Table 1. Dysplastic features according to WHO Recommendations.

Dyserythropoiesis	Dysgranulopoiesis	Dysmegakaryopoiesis
Nuclear budding	Small or unusual large size	Micromegakaryocytes
Internuclear bridging	Nuclear hypolobation (pseudo-Pelger-Huët)	Nuclear hypolobation
Karyorrhexis	Irregular hypersegmentation	Multinucleation
Multinuclearity	Decreased granules	
Nuclear hyperlobation	Pseudo Chediak-Higashi granules	
Megaloblastic changes	Auer rods	
Ring sideroblasts		
Vacuolization		
Periodic-acid Schiff positivity		

From the FAB 1976 to the WHO 2016 the blast percentage remains a major factor for diagnosis, disease subclassification and prognosis. The definition of a blast cell is still based on the definition proposed by the FAB group: myeloblasts, monoblasts and megakaryoblasts should be included into the blast count. The WHO has added the group of blast-equivalent referring to specific immature cells in specific contexts. Erythroblasts should be considered blast equivalent only in the Pure acute erythroid leukemia, promonocytes are considered monoblast equivalent when the requisite percentage is tallied for the diagnosis of acute monoblastic, acute monocytic and acute myelomonocytic leukemia, while in acute promyelocytic leukemia abnormal promyelocytes are considered as blast equivalent.

Morphology represents the first diagnostic screening in the majority of hematological disorders, clonal and non clonal. The majority of lymphomas presenting at the onset with peripheral blood circulating cells show characteristic lymphoma cell features highly predictive of the final diagnosis. In the anemia diagnostic work-up, abnormalities in RBC size, shape, stain and/or distribution as well as presence of inclusions and/or circulating nucleated red blood cells should be accurately detected and evaluated. In Table 2 some examples of red blood cell

anomalies according to their diagnostic specificity are listed. Platelet morphological evaluation too can drive a consistent diagnostic suspect.

All the above mentioned abnormalities should be evaluated in the context of the whole automated report. Consensus recommendation for cell identification, grading and nomenclature have been published by Leukemianet⁶ and the ICSH.⁷

Table 2. Some examples of red blood cell anomalies according to their diagnostic specificity. Sometime combination of two or three anomalies provide a higher diagnostic specificity.

Morphologic feature	Specificity	Possible significance
Anisocytosis, poikilocytosis	low	Unspecific change in anemia
Polychromasia	intermediate	Regenerative anemia
Microspherocytes and polychromasia	high	AIHA with warm antibodies
Target cells	low	Liver disease, many type of hemoglobinopathies, iron deficient anemia, artefact
Target cells with microcytosis	intermediate	Thalassemia > iron deficiency
Schistocytes with thrombocytopenia	high	Microangiopathic hemolytic anemia
Howell Jolly bodies	high	Splenectomy, hyposplenism
Agglutination of red blood cells	high	AIHC with cold antibodies (cold agglutinine disease)
Teardrops	intermediate	Myelofibrosis, hemolytic anemia, thalassemia major or intermedia, MDS
Tear drops with erythroblasts and/or a bare megakaryocytic nucleus	high	Myelofibrosis
Rouleaux formation of RBC	high	Hypergammaglobulinemia (monoclonal or polyclonal)
Pappenheimer bodies	intermediate	Iron overload
Hypochromic Erythrocytes with Pappenheimer bodies	high	Sideroblastic anemia, MDS-RS
Hemoglobin C or SC crystals	pathognomonic	Homozygous hemoglobin C disease, Hemoglobin SC disease

References

- Daniel A. Arber, Attilio Orazi, Robert Hasserjian, Jürgen Thiele, Michael J. Borowitz, Michelle M. Le Beau, Clara D. Bloomfield, Mario Cazzola and James W. Vardiman. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016 127:2391-2405.
- Global Burden of Disease Cancer Collaboration. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2018 Nov 1;4(11):1553-1568.
- Goasguen JE, Bennett JM, Bain BJ, Brunning R, Vallespi MT, Tomonaga M, Zini G, Renault A (The International Working Group on Morphology of MDS). Dyserythropoiesis in the diagnosis of the myelodysplastic syndromes and other myeloid neoplasms: problem areas. *Br J Haematol.* 2018 Aug;182(4):526-533.
- Goasguen JE, Bennett JM, Bain BJ, Brunning RD, Vallespi MT, Tomonaga M, Zini G, Renault A (International Working Group on Morphology of MDS IWGM-MDS). Quality control initiative on the evaluation of the dysmegakaryopoiesis in myeloid neoplasms: Difficulties in the assessment of dysplasia. *Leuk Res.* 2016 Jun;45:75-81.
- Goasguen JE, Bennett JM, Bain BJ, Brunning R, Vallespi MT, Tomonaga M, Zini G, Renault A; International Working Group on Morphology of MDS (IWGM-MDS). Proposal for refining the definition of dysgranulopoiesis in acute myeloid leukemia and myelodysplastic syndromes. *Leuk Res.* 2014 Apr;38(4):447-53.
- Zini G, Bain B, Bettelheim P, Cortez J, d'Onofrio G, Faber E, Haferlach T, Kacirkova P, Lewandowski K, Matutes E, Maynadié M, Meletis J, Petersen BL, Porwit A, Terpos E, Tichelli A, Vallespi T, Woessner S, Bennett J, Bene MC. A European consensus report on blood cell identification: terminology utilized and morphological diagnosis concordance among 28 experts from 17 countries within the European LeukemiaNet network WP10, on behalf of the ELN Morphology Faculty. *Br J Haematol.* 2010 Nov;151(4):359-64.
- Palmer L, Briggs C, McFadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin SJ. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol.* 2015 Jun;37(3):287-303.

CLINICAL APPLICATIONS OF PRECISION MEDICINE IN MULTIPLE MYELOMA

N. Bolli^{1,2}

¹Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy; ²Section of Hematology, Department of Clinical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Introduction

Personalized treatment is an attractive strategy that promises increased efficacy with reduced side effects in cancer. The feasibility of such an approach has been greatly boosted by next-generation sequencing (NGS) techniques, which can return detailed information on the genome and on the transcriptome of each patient's tumor, thus highlighting biomarkers of response or druggable targets that may differ from case to case. However, while the number of cancers sequenced is growing exponentially, much fewer cases are amenable to a molecularly-guided treatment outside of clinical trials to date. Here, I will highlight current advances promoted by NGS in multiple myeloma (MM) and challenges that application of precision medicine is facing in this field.

Molecular pathogenesis of MM and associated gammopathies

Multiple myeloma (MM) is a post-germinal center B-cell neoplasm characterized by the accumulation of clonal plasma cells in the bone marrow, the production of a monoclonal antibody detectable in serum, and end organ damage. The disease is thought to initiate in the germinal center. Here, initiating events are multiple trisomies (hyperdiploid karyotype) or translocations involving recurrent oncogenes and the immunoglobulin heavy chain locus.¹ All cases are preceded by asymptomatic stages, either clinically evident or not. Gene mutations are frequent in MM at diagnosis (NDMM), mostly in genes related to the MAPK pathway, the NF-κB pathway, the DNA damage response/TP53 pathway.^{2,3} However, the mutational spectrum of MM is very heterogeneous. In a study of 843 patients, of 54 significantly mutated genes only *KRAS*, *NRAS*, *IGLL5* and *DIS3* were mutated in more than 10% of patients. Furthermore, within a single patient, many mutations are only present in a fraction of cells (i.e. they are subclonal) or may present in lesions from some anatomical locations but not others. Consequently, gene mutations are regarded to as late events that contribute to MM heterogeneity and impact disease progression more than its initiation, along with additional aneuploidies and *MYC* translocations.¹

In asymptomatic stages, namely monoclonal gammopathy of unknown significance (MGUS) and smoldering MM (SMM) the genomic makeup is more poorly characterized. Studies show fewer aneuploidies, translocations and gene mutations. Historically, FISH showed fewer trisomies in hyperdiploid MGUS as compared to MM, suggesting this is a multi-step process that may be incomplete at this stage. However, NGS analysis has reported that asymptomatic stages are also characterized by substantial heterogeneity. Furthermore, analysis of sample series where available has shown variable degrees of spontaneous evolution of genes mutations, cytogenetic lesions and mutational signatures, implying some level of genomic instability.⁴⁻⁶

In relapsed/refractory (RRMM) stages, studies have been attempted to identify gene mutations that may confer chemoresistance. In this respect, the Cereblon E3 ubiquitin ligase complex -target of immunomodulatory genes, IMiDs- and proteasome subunit genes -target of proteasome inhibitors- have been scrutinized with underwhelming results. Given the low incidence of mutations in these candidate genes, other mechanisms are more likely to be responsible of chemoresistance. As a matter of fact, RRMM again shows genomic evolution prompted by treatment, together with an enrichment of high-risk features.²

Clinical approach to prognostication in monoclonal gammopathies

Collectively, the above evidence points at MM as a disease prone to evolution, both spontaneously and after treatment, and both in space and over time. This is not surprising given the well-known natural history of the disease, but recent advances in NGS technologies have provided us with an unprecedented depth of analysis of the cell-intrinsic features associated with the natural history of the disease. Despite these advances, diagnostic criteria distinguish MGUS from SMM based on surrogate measures of disease burden, and SMM from MM based on the presence of end-organ damage or myeloma-defining events.⁷

SMM is a clinically-defined entity where an excess of clonal plasma cells is found in the bone marrow, but no end-organ damage or myeloma-defining events are present. The current approach in SMM is watch-and-wait. However, evidence in favor of early treatment is growing, at least for high-risk cases. In fact, SMM is a clinical diagnosis of cases that range from indolent ones that behave similar to MGUS to aggressive ones that progress quickly to MM. Several risk factors have been proposed to stratify patients based on the risk of progression. Some are based on laboratory values, others on imaging, but few on intrinsic characteristics of tumor cells: among those, high-risk cytogenetic lesions, gene expression profiling and abnormal immunophenotype.⁸ However, only rarely such complex techniques are performed in routine diagnosis of SMM. Consequently, the most commonly used risk model for SMM progression relies on % bone marrow plasma cells and levels of the monoclonal protein and free light chains, i.e. clinical and laboratory markers that are indirect measures of the size of the plasma cell clone and provide imperfect prediction.

At diagnosis, MM is staged in three groups based on the R-ISS that relies on surrogates of disease burden (albumin, beta-2 microglobulin, LDH) and FISH for three high-risk cytogenetic features (t(4;14), t(14;16), del(17p)).⁹ This information was historically only relevant for the patient in terms of management of expectations, as no risk-adapted treatment in myeloma was available. However, the landscape is rapidly changing and it is likely that a more refined, molecularly-guided prognostication will be required soon.

Translational advances promoted by NGS in monoclonal gammopathies

Smoldering myeloma

DNA and RNA-based NGS studies have shown that asymptomatic stages carry a globally lower number of mutations than NDMM, which parallels early observations of fewer aneuploidies with FISH. Clonal heterogeneity was also observed, implying spontaneous evolution of SMM through acquisition of new genetic lesions conferring a proliferative/survival advantage. This was particularly true at the level of single-cell RNA, where some cases labelled as MGUS instead revealed plasma cells with a clearly malignant transcriptome.⁶ In progressive cases, analysis of serial samples highlighted two routes of evolution into symptomatic disease: i) one where the bulk of disease evolved from minor or entirely new subclones; ii) another where clinical progression was not associated with any genomic change, and was generally quicker.⁵ From a biological point of view, the former are true smoldering cases that need to acquire new lesions to shift towards an aggressive phenotype. In the latter case two scenarios are instead possible: SMM can evolve due to loss of immunosurveillance, or -more likely given the short timeframe- these where actual aggressive myelomas from the moment of diagnosis that just need more time to accumulate enough tumor burden and/or end-organ damage to meet clinical criteria for progression. Furthermore, the sequencing of the whole-genome in ultra-high risk SMM cases allowed the analysis of two important aspects thanks to the analysis of intronic lesions. Firstly, complex genomic rearrangements -intra- or inter-chromosomal catastrophic events with loss, gain and exchange of genomic material typical of aggressive cancers- where often present, albeit at a lower cancer cell fraction, showing that ultra-high risk SMM already carries features of genomic instability observed in NDMM. Secondly, mutational processes -the physiological or aberrant biochemical reactions leading to the acquisition of DNA mutations- had a very distinct temporal pattern of action. Early mutations from pre-cancer and initiation phases arise from the activity of the DNA deaminase AID or from processes associated with cell ageing. Late mutations on the contrary, i.e. the ones arising at the time of disease progression, are more often caused by a cancer-associated mutational process driven by aberrant activity of the APOBEC family of DNA deaminases.

Limitations of the described studies are several, ranging from low number of samples, to contamination from normal cells in some cases, to an inevitable bias towards higher-risk SMM cases. However, data are promising enough to believe that NGS will in the future unravel the actual determinants of disease progression in SMM and that will have a profound impact on clinical management of asymptomatic patients.

Newly diagnosed myeloma

In NDMM, NGS studies have contributed to a better understanding

of disease pathogenesis in terms of pathways involved and overall clonal dynamics. However, differently from other neoplasms, these results have not proven to be practice-changing. Instead, the main translational advances pertain to disease prognostication. Currently, the R-ISS captures a mix of surrogate measures of disease burden and aggressiveness and a limited set of cell-intrinsic genomic lesions of prognostic significance. However, three main aspects are to take into account when thinking of the coming years: i) not all high-risk features are captured by the R-ISS; ii) novel treatments may significantly influence the list of high-risk features; iii) treatment may become risk-adapted if not target-based soon.

Coupled with the availability of large public datasets amenable to analysis, NGS can allow analysis of several risk factors that go beyond the ones captured by the ISS, and in some instances even beyond what described by FISH and SNP arrays in the past years. Perhaps the best known are those included in the definition of “double hit” MM, i.e. amplification (but not a single gain) of chr1q and bi-allelic inactivation of *TP53* by mutations of one allele and deletion of the other. These lesions confer poor prognosis independently of the R-ISS. Other markers have a less clear impact, and among those are deletions in chr(1p) and hypodiploidy, the latter being harder to study since it requires conventional karyotyping if NGS is not available. In contrast, single gene mutations seem to have very little prognostic value in most cases.² The one exception is the Myeloma XI UK trial, where *EGR1* and *IRF4* mutations conferred good prognosis, and *ZFX4* a bad one. By analysis of the whole mutational spectrum, NGS has highlighted further prognostically-relevant information that go beyond single-gene mutations. Firstly, hypermutated samples carry worse prognosis.² This concept can be extended to the analysis of cytogenetic lesions, where several studies have now highlighted that prognosis is inversely proportional to their number, often independently of their type. Lastly, initial reports have highlighted that cases with high contribution from APOBEC mutational signature have worse prognosis independently from R-ISS, their number of mutations and cytogenetic subgroup.

Overall, survival is influenced by an increasing genomic complexity more than the presence/absence of a handful of genetic lesions. This implies that a much larger number of lesions need analyzing to accurately prognosticate NDMM. Unsurprisingly, novel risk scores are emerging that take into account a larger number of cytogenetic lesions to improve prognostication in NDMM, to the point where FISH-based tests will not be able to keep up with clinical requirements.

However, high-risk features are relative to the treatments available. Examples include the t(4;14) and the *TRAF3* gene deletion, both cases that seems to respond well to first-line bortezomib. On the contrary, the unique prognostic effect of gene mutations within the Myeloma XI UK trial likely stems from the use of IMiDs first line in the UK trial and PIs elsewhere, again underscoring the treatment-specific nature of risk factors. Likely, with increasing availability of treatments and large datasets of clinically-annotated myeloma genomes, this list is going to expand realizing the much valued paradigm of precision medicine through the identification of correlates of drug response. Again, this will mandate that extended genotyping is performed at diagnosis for every patient.

Furthermore, the treatment landscape of NDMM is rapidly changing thanks to the introduction of novel treatments and their combinations. Risk-adapted treatment is not standard but is being already embraced by some groups. Examples include performing or not autologous stem cell transplantation (ASCT) at remission in first line for standard-risk patients; and the use of tandem ASCT in first line, which has shown improved survival in patients with high-risk features and is widely used in Europe in this setting.

Last, the introduction of minimal residual disease (MRD) monitoring in people achieving deep responses will become relevant. Performed either with flow-cytometry techniques or NGS, the requirement is that sensitivity be at least 1×10^{-5} , for which a large number of cells are needed. MRD-negative status seems to predict longer-term survival regardless of the treatment administered and of the risk score at diagnosis.

Relapsed/Refractory myeloma

Much less is known about the genomics of relapsed-refractory myeloma. Initial studies suggest that cases retain a significant heterogeneity, with subclones showing expansion or reduction based on the type of treatment, increased number of mutations, copy-number abnormalities, complex rearrangements and contribution from novel mu-

tational signatures.² Targeted sequencing studies have highlighted increasing prevalence of mutations conferring resistance to IMiDs (particularly in CRBN, IKZF1, IKZF3) and PIs (PSMB5, PSMB8, PSMB9, PSMD1, and PSMG2). However, mutated cases are still a great minority and mutations are often subclonal, suggesting that while functionally relevant, the clinical impact of these mutations and their utility to guide further treatment will need validation. On the contrary, the evidence that new mutations and cytogenetic lesions can be acquired at relapse suggests the utility to repeat genotyping at this stage. Future studies will be required to assess whether the predictive and/or prognostic value of genomic alterations described in NDMM is conserved in advanced stages.

Future perspectives of personalized medicine applied to monoclonal gammopathies

NGS has shown how MM genome is characterized by conspicuous heterogeneity and a subclonal structure that gains complexity as the disease evolves. The hypothesis is that a precise characterization of this complexity in each patient offers a better possibility to predict, if not prevent, disease evolution and thus improve clinical management. On the contrary, risk scores used in current practice rely on clinical and laboratory markers and on a handful of cytogenetic lesions assessed by FISH that are not enough to capture the described complexity and measure MM aggressiveness. NGS thus has the potential to change how we approach plasma cell dyscrasias in the clinic, i.e. moving from surrogate measures of tumor burden to actual quantification of disease extension coupled with detailed biological analysis of the subclones present in each case.

Nevertheless, 8 years since the first NGS study in MM has been published, clinical practice has been relatively slow in embracing NGS. Likely, MM intrinsic heterogeneity and the variety of treatment options have hampered the rapid identification of novel prognostic and predictive markers, and there is no consensus so far as to whether, and how, NGS should be used to re-define high-risk disease. Also, NGS is seen as a slow, expensive and non-standardized technique. Furthermore, NGS in MM requires cumbersome sample pre-processing with CD138 cell purification.

However, envisioning the future of the approach to MM in the next 5-10 years is a worthy exercise, particularly when many centers are already performing large-scale NGS studies in all patients at diagnosis and this is likely to become a new standard in the future. Arguably, NGS is already a cheaper and less labor-intensive nowadays as compared to the three FISH tests required by the R-ISS, but this will become more evident once genotyping of an extended set of genomic lesions will be required. In fact, NGS can represent a one-stop solution for a genome-wide analysis of aneuploidies, translocations and gene mutations. In SMM, genomic and/or transcriptomic cell-intrinsic features may become more compelling than measurement of levels of the monoclonal protein in predicting progressive cases. In particular, the number of mutations, the presence of complex chromosomal rearrangements, the mutational signature profile are all attractive candidates to integrate current FISH markers and identify higher-risk cases. Once RNA-sequencing technologies will become more widespread and standardized, a gene-expression signature applied to single cells may highlight the presence of an aggressive subclone within an otherwise indolent disease. While these studies will be performed in purified bone marrow cells, thus hampering the possibility of serial examination, cell-free DNA may become an attractive, alternative source of tumor DNA amenable to serial sampling. As of today, sensitivity issues limit its application, but in the future techniques such as digital-droplet PCR may prove effective for this purpose.

In NDMM, NGS analysis will inform on current higher risk cytogenetic markers as per IMWG recommendations. But more importantly, genome-wide copy-number analysis will inform on genome-wide aneuploidies that may change the prognosis of the patient. The simultaneous analysis of gene mutations will provide an added value for prognostication, particularly for *TP53* mutations. Clearly, the quantitative nature of NGS data will also provide an estimate of the number of cells affected by each lesion, which has clinical correlates. Also, a comprehensive mutational analysis of each case will highlight cases that are hypermutated and those with an increased contribution from APOBEC, again markers of high-risk disease. After treatment, NGS analysis of the clonal IGH rearrangement in the bone marrow will provide crucial information on MRD, which is of prognostic value today and may in-

fluence post-remission treatment in the future. In fact, clinical trials are already ongoing today where different treatment arms are proposed based on the risk score at diagnosis and on the achievement of MRD-negative status, so that the future may bring innovative strategies to personalize treatment in MM.

At relapse, NGS may provide important correlates of drug response. This has implications for treatment with ixazomib in high-risk second line cases in Italy, and for off-label venetoclax in t(11;14) cases. Furthermore, additional predictors of venetoclax response are represented by relative levels of expression of *BCL2* and *MCL1*, something that RNA-seq can readily identify. In the future, the identification of “druggable” lesions, i.e. those for which a targeted treatment is available may represent a further option for refractory patients. Of note, single-agent targeting of actionable gene mutations has been attempted with MEK inhibitors in *KRAS* and *NRAS* mutated cases, and with vemurafenib in *BRAF* mutated cases. Results so far have been somehow disappointing, and this likely stems from the great heterogeneity and tendency to branching evolution of RRMM cases. Again, NGS can inform on the cancer cell fraction of each case and this may be used to predict cases that are more likely to respond to treatment.

Last, it is important to underline how much of the work has so far concentrated on genomic-transcriptomic features of the clonal plasma cells. However, as technologies improve it will be extremely relevant to also analyze the proteome of the tumor, as well as the microenvironment and immune environment that are relevant both in SMM and in NDMM treated with immunotherapies.

References

- Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer*. 2012;12:335–48.
- Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nature Communications*. 2014;5:2997.
- Walker BA, Mavrommatis K, Wardell CE, Ashby TC, Bauer M, Davies FE et al. Identification of novel mutational drivers reveals oncogene dependencies in multiple myeloma. *Blood*. American Society of Hematology; 2018;:blood-2018-03-840132–24.
- Mikulasova A, Wardell CE, Murison A, Boyle EM, Jackson GH, Smetana J et al. The spectrum of somatic mutations in monoclonal gammopathy of undetermined significance indicates a less complex genomic landscape than that in multiple myeloma. *Haematologica*. 2017;102:1617–25.
- Bolli N, Maura F, Minvielle S, Gloznik D, Szalat R, Fullam A et al. Genomic patterns of progression in smoldering multiple myeloma. *Nature Communications*. 2018;9:3363.
- Ledergor G, Weiner A, Zada M, Wang S-Y, Cohen YC, Gatt ME et al. Single cell dissection of plasma cell heterogeneity in symptomatic and asymptomatic myeloma. *Nat Med*. 2018;24:1867–76.
- Rajkumar SV, Dimopoulos MA, Palumbo A, Bladé J, Merlini G, Mateos M-V et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15:e538–48.
- Rajkumar S. Smoldering multiple myeloma. *Blood*. 2015;125:3069–75.
- Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosiñol L et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. *J Clin Oncol*. 2015;33:2863–9.

NEW DRUGS IN ACUTE MYELOID LEUKEMIA

F. Ferrara

Division of Hematology, Cardarelli Hospital, Naples, Italy

In the recent years, the possibility of using new molecular techniques, in particular next-generation sequencing, has provided new insights in the mechanisms of development and clinical progression of acute myeloid leukemia (AML). Furthermore, a clonal hierarchy has been established according to mutations which occur early and dominate the process of leukemogenesis.¹ As a consequence, after more than 4 decades, in which the combination of an anthracycline, usually daunorubicin, given for 3 days with continuous infusion of cytarabine for 7 days (3+7) has been the standard induction regimen for patients with AML, targeted therapy against mutant driver proteins as well as agents whose mechanisms of action and efficacy may not be dependent on mutational complexity have been developed and are now available into clinical practice,^{2,4} as summarized in Table 1. In this review, drugs that may emerge as important for the treatment of AML in the next years will be discussed on the basis of clinical relevance for newly diagnosed or relapsed/refractory patients.

Table 1. New drugs recently approved for the treatment of AML

Drug	Target	Indications
Midostaurin	FLT3/ITD	De novo AML with FLT3/ITD mutation
Gilteritinib	FLT3/ITD FLT3/TKD	Refractory/relapsed AML with FLT3/ITD or FLT3/TKD mutation
Glasdegib	Hedgehog pathway	newly-diagnosed AML 75 years or older or not eligible to intensive induction therapy
Venetoclax	BCL-2	newly-diagnosed AML 75 years or older or not eligible to intensive induction therapy in combination with HMA
Ivosidenib	IDH1	Newly diagnosed AML patients 75 years or older or not eligible to ICT or refractory/relapsed patients with IDH1 mutation
Enasidenib	IDH2	Refractory/relapsed AML patients with IDH1 mutation
CPX-351	No specific	Newly-diagnosed therapy-related AML or AML with myelodysplasia-related changes.
Gemtuzumab-ozogamycin	CD33	CD33 positive naïve AML patients with favorable or intermediate karyotype

Isocitrate dehydrogenase inhibitors

Mutations in IDH1 or IDH2 are detected in approximately 20% of patients with AML and are associated with DNA hypermethylation, aberrant gene expression, cell proliferation and abnormal differentiation. While the prognostic impact of IDH mutations are unclear and are correlated with the location of the mutation and other co-occurring genomic abnormalities, for relapsing AML patients harboring a mutation in IDH 1 or 2 (IDH1/2), potential treatment options have undergone a paradigm shift away from intensive cytotoxic chemotherapy to targeted therapy with selective inhibitors, such as enasidenib (ENA) for IDH2 or ivosidenib (IVO) for IDH1, both recently approved by FDA.⁵ Response rate including complete remission (CR) or complete remission with incomplete hematologic recovery (CRi) have been achieved in more than 20% of patients and median survival is around 9 months. These results seem to compare favorably with those described with salvage intensive chemotherapy which results in significantly lower response rate and survival. In addition, the possibility of combining aggressive or attenuated chemotherapy with either ENA or IVO is currently object of investigation in ongoing clinical trials. Of interest, differentiation syndrome (DS) with IVO and ENA has been described in 30-40% of patients with relapsed or refractory AML, also in absence of leukocytosis and careful recognition of signs and symptoms of DS are expected to lead to earlier diagnosis and treatment, decreasing complications and mortality. Worthy of note, on May 2019 IVO received also FDA approval for newly diagnosed patients with IDH2 mutation.

Venetoclax

For AML patients who are candidate to treatment with hypomethylating agents (HMA) or low dose cytarabine (LDARA-C), recently published data clearly demonstrate a marked superiority of the combination with venetoclax (VEN), a potent, selective, oral inhibitor of BCL-2. The BCL-2 protein plays an important role in the survival and persistence of AML blasts, as it is a key regulator of the mitochondrial apoptotic pathway. BCL-2 maintains myeloblast survival by sequestering pro-apoptotic BAX, resulting in mitochondrial dependence on BCL-2. Following treatment with VEN + HMA, in naïve patients CR + CRi rates exceeds 70%; worthy of note, patients with poor-risk cytogenetics and those ≥ 75 years achieved CR + CRi rates of 60% and 65%, respectively, which are inconceivable with intensive chemotherapy (ICT) or HMA alone.⁶ Impressive results have been also reported in terms of median survival leading to FDA approval, even though con-

tinued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials, which are ongoing. Overall, toxicity is acceptable, more frequent adverse event being nausea, diarrhea, constipation, fatigue, hypokalemia, decreased appetite and febrile neutropenia. As a consequence, it is expected that in the daily practice most patients who are candidate to HMA, will be given the combination with VEN. VEN + HMA should be also considered in older patients potentially fit for ICT, carrying unfavorable cytogenetics or tp53 mutation.

FLT3 inhibitors

Mutations in the FLT3 gene is one of the most frequent, and clinically relevant class of AML mutations, approximately found in 30% of patients. FLT3 mutations include 2 categories: internal tandem duplications (FLT3/ITD mutations) in or near the juxtamembrane domain of the receptor and point mutations resulting in single amino acid substitutions occurring within the activation loop of the tyrosine kinase domain (FLT3/TKD mutations). The incidence of FLT3/ITD mutations varies according to age and clinical risk group, being less common in pediatric AML and in AML arising from an antecedent myelodysplastic syndrome (MDS). Midostaurin (Mido) is an oral, multi-targeted kinase inhibitor recently approved for the treatment of AML in newly-diagnosed adults with FLT3 mutation on the basis of results from "RATIFY" trial, a global, randomized, placebo-controlled international phase 3 trial aimed to determine whether the addition of midostaurin to induction and consolidation, followed by 1 year of maintenance, would improve the OS of patients with FLT3-mutated AML aged 18 to 59 years. The results demonstrated that arm randomized to receive midostaurin had a higher median OS, compared with the placebo arm. The 4-year OS rates were 51.4% in the midostaurin arm vs 44.3% in the placebo arm; of note, the rate of CR was not statistically different (59% vs 53%), respectively, suggesting that the clinical benefit was mainly related to better quality of CR. While these results have established a new standard of care, research is in progress because mutations in the target receptor and activation of alternative signaling pathways cooperate in inducing resistance to first generation FLT3 inhibitors; different second generation inhibitors such as crenolanib, quizartinib and gilteritinib (recently approved by FDA and EMA as single agent for refractory or relapsed AML patients), have been proven to be effective. In particular, results from the phase III ADMIRAL trial showed the median survival was 9.3 months with gilteritinib compared with 5.6 months in those who received salvage chemotherapy, leading to a 36% reduction in the risk of death. Additional results also demonstrated showed that the CR/CRi with hematologic recovery (CRh) rate more than doubled with gilteritinib versus chemotherapy at 34% and 15%, respectively. Patients on gilteritinib also had higher rates of undergoing allogeneic hematopoietic stem cell transplant (HSCT) than those on salvage therapy, at 26% versus 15%, respectively.

Similarly, in the open-label trial Quantum-R, 367 patients who had received standard therapy with or without allogeneic hematopoietic stem cell transplantation were randomly assigned 2:1 to receive quizartinib 60 mg once daily (n = 245) or salvage chemotherapy (n = 122). Investigator's choice of chemotherapy consisted of: low-dose cytarabine; mitoxantrone, etoposide, and cytarabine; or granulocyte colony-stimulating factor, fludarabine, cytarabine, and idarubicin. The primary endpoint was overall survival in the intention-to-treat population. Median follow-up was 23.5 months. Median overall survival was 6.2 months in the quizartinib group vs 4.7 months in the salvage chemotherapy group. Estimated 12-month survival was 27% vs 20%. Adverse events led to death in 13% of the quizartinib group and 10% of the chemotherapy group. All second generation FLT3 inhibitors are currently being investigated in combination with ICT, epigenetic therapies, proteasome inhibitors, downstream kinase inhibitors, phosphatase activators and other drugs which alter AML signaling.⁷

Glasdegib

Glasdegib (G), an oral, small molecule inhibitor of the Hedgehog pathway, implicated in the maintenance of leukemia stem cell populations in several model systems, represents a further therapeutic option for AML in older patients or uneligible for ICT. Approval by FDA of G in combination with LDARAC was based on the results on a multicenter, open-label, randomized study including 115 patients with newly-diagnosed AML 75 years or older or not eligible to intensive induction

therapy.^{5,6} Patients were randomized 2:1 to receive glasdegib, 100 mg daily, with LDAC 20 mg subcutaneously twice daily on days 1 to 10 of a 28-day cycle (N=77) or LDAC alone (N=38) in 28-day cycles until disease progression or unacceptable toxicity (51). With a median follow-up of 20 months, median survival was 8.3 months for the G + LDAC arm and 4.3 months for the LDAC alone arm and (p=0.0002). CR rate was 17% for the experimental arm vs. 1 % in the control group (p=0.05). The recommended G dose is 100 mg orally, once daily.⁸

CPX-351

CPX-351 is a liposomal combination of DNR and ARA-C in a 1:5 molar ratio, recently approved for newly-diagnosed therapy-related AML (t-AML) or AML with myelodysplasia-related changes (AML-MRC). A randomized, multicenter, open-label controlled trial comparing the liposomal formulation to a standard 3 + 7 in 309 patients 60-75 years of age with newly-diagnosed t-AML or AML-MRC demonstrated an improvement in OS (9.6 months vs. 5.9 months for the "control arm") and increased CR rate (48 vs. 32 %). The toxicity profile of CPX-351 was similar to that seen with standard "7+3" with the exception of more prolonged neutropenia and thrombocytopenia on the experimental arm. Notwithstanding, in the CPX-351 arm the 60 days mortality was lower for and more patients were eligible to HSCT. This is the first FDA-approved treatment specifically for patients with t-AML or AML-MRC; worthy of note, the label of the drug actually also includes younger patients.⁹

Immunotherapy of AML

HSCT has been the most successful immunotherapy for AML over the past years, especially with the advances made in using alternative donors. Unfortunately, older and less fit patients are poor candidates for allogeneic HSCT due to still significant toxicity and a high relapse rate, mainly in poor risk patients.

An alternative way to use immunotherapies in AML has been to use monoclonal antibodies (MoAbs), that target particular antigens. CD33, CD123 and C-type lectin-like molecule (CLL1) are leukemia-specific antigens, which are expressed on the majority of myeloid blasts and leukemic stem cells but not expressed (or expressed at lower levels) in normal tissues.

Gemtuzumab ozogamycin (GO), a CD33-directed antibody drug conjugate linked to the cytotoxic antibiotic calicheamicin, received approval for the treatment of newly-diagnosed CD33-positive AML in adults. Approval of GO combination treatment was based on a randomized trial of 271 patients aged 55-70 years with newly diagnosed AML treated with daunorubicin and cytarabine with or without 3 mg/m² fractionated GO on days 1, 4, and 7, which resulted in an event-free survival (EFS) of 13.6 months for GO + daunorubicin and cytarabine and 8.8 months for daunorubicin and cytarabine alone. In addition, data from five randomised controlled trials (3325 patients) were analyzed, with overall survival as the primary endpoint. While the addition of GO did not increase the proportion of patients achieving CR with or without complete peripheral count recovery, it significantly reduced the risk of relapse. At 6 years, the survival benefit was especially apparent in patients with favorable cytogenetic characteristics, but was also observed in those with intermediate ones. On the contrary, patients with adverse cytogenetic characteristics did not benefit. On this basis, it is expected that patients with CD33 positive AML and fit for ICT with CD33 positive AML, accounting for more than 80 % and including core binding factor (CBF) AML, *i.e.* AML with (t[8;21] or inv[16]/t[16;16]) and AML with intermediate karyotype will be given 3 + 7 + GO with the exception of those harboring the FMS-like tyrosine kinase 3 (FLT3) mutations in whom Midostaurin would be preferred.

T cell-recruiting antibody constructs, chimeric antigen receptor (CAR) cells, checkpoint inhibitors, and dendritic cell vaccination are currently object of clinical investigation, with encouraging but still preliminary results.¹⁰

Conclusions

AML still remains a challenge to both patients and clinicians; however, we have now a number of therapeutic targets, potentially leading to personalized therapy. As induction therapy is concerned, it is clear that the classical 3 + 7 combination within few years will represent an undertreatment for most newly diagnosed patients (Figure 1). In all young adults ICT will be given in combination with other agents with

the aim of improving CR rate and in order to bridge high risk patients to HSCT with undetectable MRD. In older patients, apart from CPX-351 for those with t-AML or AML-MRC, the combination of DNR and ARA-C would be hopefully substituted by VEN plus HMA or new still unexplored combinations, which are expected to favorably compare with 3+7 in terms of either survival or quality of life.

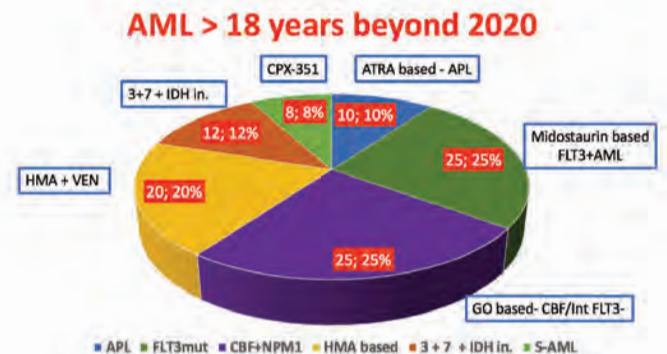


Figure 1. In the years to come no patient will receive classical 3 + 7.

References

- Kaysner S, Levis MJ. Clinical implications of molecular markers in acute myeloid leukemia. *Eur J Haematol.* 2019 Jan;102(1):20-35.
- Talati C, Sweet K. Recently approved therapies in acute myeloid leukemia: A complex treatment landscape. *Leuk Res.* 2018;73:58-66
- Pollyea DA. Which novel agents for acute myeloid leukemia are likely to change practice? *Best Pract Res Clin Haematol.* 2018;31(4):391-395
- Ferrara F, Vitagliano O. Induction therapy in acute myeloid leukemia: Is it time to put aside standard 3 + 7? *Hematol Oncol.* 2019 Apr 2. doi: 10.1002/hon.2615. [Epub ahead of print]
- DiNardo CD, Stein EM. SOHO State of the Art Update and Next Questions: IDH Therapeutic Targeting in AML. *Clin Lymphoma Myeloma Leuk.* 2018 Dec;18(12):769-772
- Guerra VA, DiNardo C, Konopleva M. Venetoclax-based therapies for acute myeloid leukemia. *Best Pract Res Clin Haematol.* 2019 Jun;32(2):145-153.
- Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia.* 2019 Feb;33(2):299-312.
- Hoy SM. Glasdegib: First Global Approval. *Drugs.* 2019 Feb;79(2):207-213.
- Talati C, Lancet JE. CPX-351: changing the landscape of treatment for patients with secondary acute myeloid leukemia. *Future Oncol.* 2018 May;14(12):1147-1154
- Liu Y, Bewersdorf JP, Stahl M, Zeidan AM. Immunotherapy in acute myeloid leukemia and myelodysplastic syndromes: The dawn of a new era? *Blood Rev.* 2019 Mar;34:67-83

MANAGEMENT OF THE ASPLENIA

M. Casale¹, M. Balocco², V.M. Pinto², G.L. Forni²

¹Ematologia e Oncologia Pediatrica, Dipartimento della Donna, del Bambino e di Chirurgia Generale e Specialistica, Azienda Ospedaliera Universitaria- Università degli Studi di Napoli Luigi Vanvitelli, Napoli; ²Centro della Microcitemia e delle Anemie Congenite, Ospedale Galliera, Genova, Italy

Splenectomy or functional asplenia are among the major risk factors recognized among the haematological disorders as causes of death or long-term disability due to largely preventable invasive bacterial infections.

The spleen functions are clearly explained by the three anatomical and functional compartments of the organ. The red pulp is an open and slow circulation area dedicated to the filtration of blood, in which antigens and other surface molecules are removed, as well as aged erythrocytes and bacteria. The slow and prolonged interaction of blood cells and bloodborne pathogens allows phagocytosis and cell activation. The white pulp is responsible for adaptive antigen-specific immunity, containing lymphocytes and macrophages that form sheaths and nodules around the arterioles. The marginal zone lies in the midst of these two zones and performs functions of innate and not antigen specific immunity, and contains above all B memory cells. The bacteria circulating in the blood can be directly recognized and removed by splenic macrophages but more often they must be opsonized, that is covered

by complement proteins and other opsonizing molecules that allow a rapid and efficient interaction with the phagocytic cells. Once opsonized the bacteria are efficiently eliminated by splenic and hepatic macrophages. Bacteria provided with a capsule that avoids the binding with the complement and other opsonizing proteins are poorly opsonized, and must necessarily be eliminated from the spleen thanks to IgM memory, pentameric antibodies that facilitate the binding of the complement and opsonizing proteins to encapsulated bacteria and facilitate their removal. IgM memory are produced by a unique B-cell population found only in the marginal zone of the spleen. This is why splenectomy or dysfunctional spleen mainly damages innate and natural immunity against capsulated bacteria, especially in the first years of life, in which there is a physiological and transient IgM memory deficit.¹

So, the spleen represents a particular immunological environment fundamental for the removal of polysaccharide antigens and for the optimal antibody response especially in the first years of life. However, the risk of serious infections in asplenic patients persists throughout life, as mortal infectious episodes have been described even decades after splenectomy; therefore it is evident that prevention and immediate and aggressive treatment of infections are the cornerstone of the prevention of life threatening events.² The main challenge in the management of asplenia-related complications lies in the high heterogeneity of underlying diseases and the need for a wide training of caregivers in different clinical settings. Asplenia is a complication of several congenital or acquired hematologic diseases,^{3,4} or hemoglobinopathies⁵⁻⁷ often requiring splenectomy (Table 1).

Table 1. Asplenia Associated Diseases.

Congenital hemolytic anemias
Thalassemic syndromes
Sickle Cell Disease
Chronic platelet diseases
Autoimmune hemolytic anemias
Immunodeficiencies
Lymphoproliferative diseases
Asplenic syndromes
Post traumatic

Sickle cell anemia or sickle cell disease is a condition characterized by functional asplenia already from the first months of life, caused by splenic infarcts due to the pathological interaction of HbS with the splenic tissue.⁷⁻⁹ Patients with sickle cell disease should always be considered at high risk for serious infections, even if they have splenomegaly.⁷ In some cases splenectomy can worsen the clinical course of some diseases and should be considered as an evolutionary and pejorative risk factor of an acute event.⁸ While in some diseases, such as thalassemias and sickle cell anemia, chronic anemia persists despite splenectomy and the patient continues the specialized follow-up for the underlying disease,⁵⁻⁹ under other conditions, such as hereditary spherocytosis, the anemia characterized by a greater hemolytic component, is resolved by splenectomy³ and patients are lost after a few years to the specialist follow-up and it is probable that they will not carry out the vaccination warnings and all the procedures foreseen for the management of their infectious risk that remains throughout life [3]. Furthermore, it has been observed that in children with isolated congenital asplenia or associated malformations, rapidly evolving serious infections are the first cause of mortality.¹ Reactive thrombocytosis develops characteristically a few days after splenectomy and tends to shrink in the first few months. For patients with platelet counts in excess of 1,000,000/ μ L, aspirin 65 mg daily may be considered to minimize the development of the stroke or thrombosis. In general, no treatment is indicated to directly reduce the platelet count.

It was shown that complications related to asplenia are preventable through proper training and information for doctors and patients.² For this reason it is essential that all health care providers, ranging from haematologists, general practitioner, emergency physicians, paediatricians, are aware of the recommended procedures for the management of infectious risk in asplenic and splenectomized patients, in order to reduce mortality and long-term complications related to asplenia.

In Europe very few multicenter studies are reported and in Italy specific data on mortality and long-term complications in asplenic patients are not available, making it particularly difficult to identify critical issues

in clinical management, in resource planning and corrective actions. For these reasons, the Italian Society of Thalassemia and Hemoglobinopathies (SITE), with the endorsement of the Italian Association of Pediatric Hematology and Oncology (AIEOP), supported and promoted the production of the first national recommendations on the management of asplenia-related complications, with the aim of providing a practical guide updated and adapted to the Italian medical-assistance context for the management of the asplenic patients.¹⁰

The document has been designed to guarantee a quick and easy to use consultation, providing an immediate reference to the topic of interest (Figure 1).

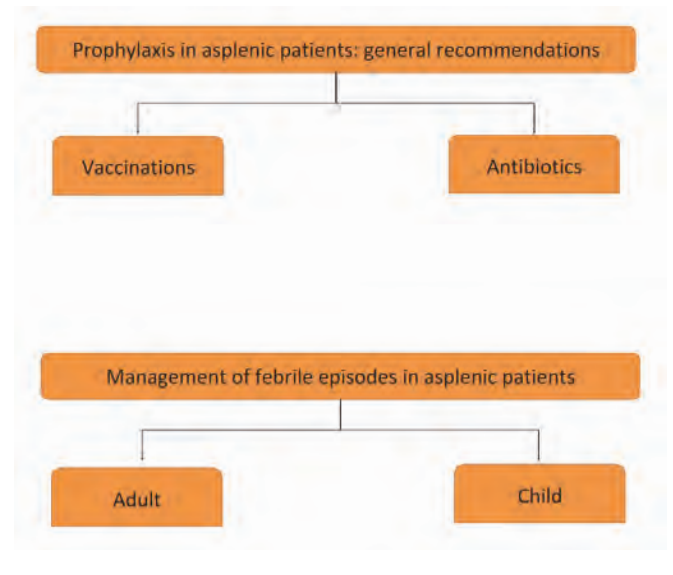


Figure 1. Algorithm by SITE for the management of infective risk in splenectomized patients. Modified http://site-italia.org/download.php?file=Management_Asplenici.pdf].

The recommendations concern two fundamental fields: the prevention of invasive infections through specific vaccination schedules and indications for antibiotic prophylaxis, and the immediate management of fever and infectious events. From the home page, by clicking on the box related to the topic of interest, the reader is directly referred to the associated recommendations. The document is available online on the website of the Italian Society of Thalassemia and Hemoglobinopathies (http://site-italia.org/download.php?file=Management_Asplenici.pdf).¹⁰

The Italian Network of Asplenia is the result of coordinated and systematic project involving over 40 Italian centers and represents the first network of health care providers and experts dealing with the prevention and treatment of complications related to asplenia. A database has been created and currently contains a considerable number of data regarding infectious and thrombotic complications, antibiotic and vaccination prophylaxis and mortality from over 1,400 asplenic patients. The Italian Network of Asplenia aims to detect critical issues and identify resolution mechanisms, promoting prevention and health in a group of patients at high risk of serious but largely preventable infections. A report containing information about the management of asplenia can be downloaded and printed in order to provide patients or caregivers with a clear and reliable guide. In addition, the database includes a system that signals any missed vaccines or booster doses to be performed. It is also possible to print only the card relating to the vaccines to be delivered to the patient or to insert in the folder, as a memorandum of the vaccines performed and to be performed. The fundamental objective is to create an efficient and secure network of information flow, and the haematologist plays a crucial role in order to keep the attention on the asplenia-related complications. Finally, it has been shown that the inclusion of patients with special needs in a single and coordinated database improves management, standardizing it on the highest level of assistance, increases knowledge, and encourages the development of new lines of research. It is possible to participate in the activities of the Italian Asplenia Network by making a request through the SITE website (http://www.site-italia.org/2018/network_asplenia.php).

Conclusions

Asplenic patients have a high risk of serious, life-threatening infections. It is necessary to implement all vaccination prophylaxis measures and to treat any febrile event in an aggressive and timely manner. The hematologist who follows splenectomized patients plays a crucial role in the correct information flow to patients, general practitioners, emergency physicians.

References

- Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. *Lancet*. 2011 Jul 2;378(9785):86-97.
- Rubin LG, Schaffner W. Clinical practice. Care of the asplenic patient. *N Engl J Med*. 2014 Jul 24;371(4):349-56.
- Casale M, Perrotta S. Splenectomy for hereditary spherocytosis: complete, partial or not at all? *Expert Rev Hematol*. 2011 Dec;4(6):627-35.
- Ladogana S, Maruzzi M, Samperi P, Condorelli A, Casale M, Giordano P, Notarangelo LD, Farruggia P, Giona F, Nocerino A, Fasoli S, Casciana ML, Miano M, Tucci F, Casini T, Saracco P, Barcellini W, Zanella A, Perrotta S, Russo G; AIHA Committee of the Associazione Italiana di Ematologia ed Oncologia Pediatrica. Second-line therapy in paediatric warm autoimmune haemolytic anaemia. Guidelines from the Associazione Italiana Onco-Ematologia Pediatrica (AIEOP). *Blood Transfus*. 2018 Jul;16(4):352-357.
- Casale M, Cinque P, Ricchi P, Costantini S, Spasiano A, Prossomariti L, Minelli S, Frega V, Filosa A. Effect of splenectomy on iron balance in patients with -thalassaemia major: a long-term follow-up. *Eur J Haematol*. 2013 Jul;91(1):69-73.
- Pinto VM, Poggi M, Russo R, Giusti A, Forni GL. Management of the aging beta thalassaemia transfusion-dependent population - The Italian experience. *Blood Rev*. 2019 Aug 7;100594. doi: 10.1016/j.blre.2019.100594. [Epub ahead of print] Review
- Russo G, De Franceschi L, Colombatti R, Rigano P, Perrotta S, Voi V, Palazzi G, Fidone C, Quota A, Graziadei G, Pietrangelo A, Pinto V, Ruffo GB, Sorrentino F, Venturelli D, Casale M, Ferrara F, Sainati L, Cappellini MD, Piga A, Maggio A, Forni GL. Current challenges in the management of patients with sickle cell disease - A report of the Italian experience. *Orphanet J Rare Dis*. 2019 May 30;14(1):120.
- De Franceschi L, Lux C, Piel FB, et al. Access to emergency department for acute events and identification of sickle cell disease in refugees. *Blood*. 2019 Feb 11. pii: blood-2018-09-876508.
- Pinto VM, Balocco M, Quintino S, Forni GL. Sickle Cell Disease: a review for the internist. *Intern Emerg Med*, 2019 Aug 5. doi: 10.1007/s11739-019-02160-x. Epub ahead of print Review.
- Casale M, Russo G, Sainati L, Colombatti R, Palazzi G, Forni GL, Perrotta S. La gestione del rischio infettivo nei bambini asplenic: raccomandazioni dal Network Italiano Asplenia. *Quaderni acp*. 2019; 26(4): 158-161.

EMOFILIA E TERAPIA GENICA: PRESENTE E FUTURO

E. Peyvandi

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy

Abstract

Gene therapy is rapidly becoming a new therapeutic strategy for haemophilia A and B treatment. In the 1990s, studies in animal models showed that adeno-associated vectors (AAV) exhibited an efficient expression of factor IX (FIX).¹⁻³ In the first clinical trial in patients with haemophilia B, therapeutic levels of FIX were documented but the expression remained only for few weeks.⁴ Subsequently, improvements in vector design, such as the use of different AAV serotypes, the development of the self-complementary vector, the engineering of the transgene with codon optimization and liver specific expression cassette resulted in circulating FIX level between 2 and 5% for long-lasting period.⁵⁻⁷ Recently, a natural gain of function FIX variant (Padua) inserted in the F9 cDNA improved the expression of FIX achieving a level of more than 30% resulting in cessation of infusions and in a greatly reduction of bleeding events.⁸ Encouraging clinical progresses have been also obtained from trials of gene therapy for haemophilia A. Transgene expression persisted for three years with circulating FVIII activity levels of 37% in patients treated with AAV vector containing a codon-opti-

mized F8 cDNA.⁹

Patients with a severe phenotype (FVIII or FIX level <1%) have been shown to change their phenotype to mild or even normal. Gene therapy is becoming an exciting choice in the field of haemophilia treatment. Clinical trials have also demonstrated that one single intravenous infusion of AAV vector containing F8 or F9 cDNA can achieve high protein expression levels for a long period of time, resulting in the absence of spontaneous or traumatic bleeds and allowing the cessation of prophylaxis regimens. Therefore, the effectiveness of gene therapy, accelerated by scientific progress and clinical successes, justifies continued optimism and increasing efforts toward making these therapies the future of haemophilia management.

However, there are still a number of critical issues that need to be addressed. The increase in liver enzymes, reported in both clinical trials for haemophilia A and B, will need to be better understood and overcome, and the safety profile of different AAV serotypes and the effect of the vector manufacturing process are further areas for study. Moreover, the potential genotoxicity derived from integrating gene delivery vectors should not be underestimated.

The rapid progress and results achieved in gene therapy of haemophilia A and B have not left enough time for patients and clinicians to understand deeply and completely the consequences of such data in term of safety and economic impact on the National Health System. The scientific community, along with regulatory and patients organizations, needs to evaluate clearly the pros and cons of progressing from severe phenotype with a level of <1% to moderate, or even normal phenotype. In addition, it is extremely important to understand for how long the expression of AAV vectors could remain after a single infusion and what could happen to those patients who lose the expression.

New trials are emerging with the use of powerful and flexible vehicles such as lentiviruses which shown a high efficiency of gene transfer and a capacity for stable transgene integration in the genome of target cells.¹⁰ Finally, genome editing technologies are evolving very quickly. These approaches could also offer a precise tool for correcting the genome and can overcome many of the drawbacks of strategies that rely on viral vectors. Future research hopefully will shed more light on these questions.

References

- Herzog RW, Hagstrom JN, Kung SH, et al. Stable gene transfer and expression of human blood coagulation factor IX after intramuscular injection of recombinant adeno-associated virus. *Proc Natl Acad Sci USA* 1997;94:5804-5809.
- Wang L, Takabe K, Bidlingmaier SM, III CR, Verma IM. Sustained correction of bleeding disorder in hemophilia B mice by gene therapy. *Proc Natl Acad Sci USA* 1999;96:3906-3910.
- Chao H, Mao L, Bruce AT, Walsh CE. Sustained expression of human factor VIII in mice using a parvovirus-based vector. *Blood* 2000;95:1594-1599.
- Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 2006;12: 342-347.
- Nathwani AC, Tuddenham EG, Rangarajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* 2011;365:2357-2365.
- Nathwani AC, Reiss UM, Tuddenham EG, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med* 2014;371:1994-2004.
- Miesbach W, Meijer K, Coppens M, et al. Gene therapy with adeno-associated virus vector 5-human factor IX in adults with hemophilia B. *Blood* 2018;131:1022-1031.
- George LA, Sullivan SK, Giermasz A, et al. Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant. *N Engl J Med* 2017;377:2215-2227.
- Rangarajan S, Walsh L, Lester W, et al. AAV5-Factor VIII Gene Transfer in Severe Hemophilia A. *N Engl J Med* 2017;377:2519-2530.
- Milani M, Annoni A, Moalli F, et al. Phagocytosis-shielded lentiviral vectors improve liver gene therapy in nonhuman primates. *Sci Transl Med*. 2019;11: pii: eaav7325.

Author Index

- Abbaszadeh, Z. 4, 28
 Abbati, G. 135
 Abbeante, M. 164
 Abbenante, M.C. 36, 39, 46, 134, 152
 Abboud, C. 142
 Abruzzese, E. 3, 16, 22, 23, 24, 35, 51, 73, 74, 140, 141, 142
 Accorsi Buttini, E. 42, 59, 145
 Accorsi, E. 29, 85
 Accurso, V. 123, 124, 125, 126
 Advani, A. 94
 Agboyi, L. 9, 10
 Agliastro, G. 159
 Aguenouz, M. 119
 Aguzzi, C. 140
 Aguzzi, R. 25
 Alati, C. 5, 12, 95, 98, 172
 Albano, F. 11, 12, 15, 21, 22, 23, 35, 39, 106, 107, 108, 135, 140, 141, 146
 Alberio, F. 122, 169, 172
 Albertazzi, L. 100, 105
 Alfieri, P. 98, 155, 165
 Alfinito, F. 147
 Alfonso, V. 13
 Algarotti, A. 19, 20, 82
 Algeri, M. 4
 Alibrandi, A. 119, 164
 Allegra, A.G. 119, 164
 Alma, E. 112
 Alonci, A. 164
 Altini, M. 97, 116
 Alunni Fegatelli, D. 140
 Amadori, S. 11, 12, 52, 53, 54, 149
 Ambrosiani, L. 122, 169, 172
 Amendola, A. 110, 139, 143, 145, 170
 Ammirati, L. 64, 166
 Anaclerico, B. 73, 74
 Andreani, M. 4, 20
 Andreoli, M. 85, 97
 Andriani, A. 53, 73, 74, 98, 103, 117, 165, 173
 Anelli, L. 15, 106, 135, 146
 Angelillo, P. 112, 151
 Angelini, D.F. 13, 136
 Angelini, M. 170
 Angelopoulou, M.K. 27
 Angelucci, E. 19, 45, 83, 85, 93, 133
 Anghileri, M. 141, 144
 Angrilli, F. 128
 Anichini, M. 168
 Annechini, G. 53
 Annibali, O. 11, 17, 46, 62, 86, 117, 129
 Annunziata, M. 22, 35, 95
 Annunziato, F. 14
 Ansell, S.M. 78
 Ansuinelli, M. 43, 108, 143
 Antolino, A. 148, 167
 Antolino, G. 117
 Antonelli, L. 86
 Antonioli, E. 65, 66, 69, 71, 117, 157
 Anzalone, N. 6
 Apicella, V. 35
 Aprile, L. 51, 92
 Arapaki, M. 27
 Arcaini, L. 7, 46, 111, 155
 Arcari, A. 47, 62, 111, 112, 169
 Arcese, W. 12, 13, 20, 21, 42, 52, 90, 95
 Arcuti, E. 107, 108
 Arena, F. 85, 97
 Arena, V. 1, 37
 Argnani, L. 8, 27, 37, 60, 61, 62, 77
 Arleo, M.A. 123
 Arlett, L. 31
 Armiento, D. 5, 86
 Arpinati, M. 84, 133
 Artale, S. 141, 144
 Asimakopoulos, J. 27
 Assanelli, A. 44, 80, 131
 Attal, M. 6
 Attardi, E. 65
 Attingenti, E. 157
 Attolico, I. 22, 107, 108, 135, 143, 145, 146
 Attrotto, C. 42, 52
 Audisio, E. 13, 29, 88, 92
 Auteri, G. 3, 16, 32, 41, 56, 121
 Autore, F. 82, 84, 102, 103, 104, 105, 133, 158
 Avanzato, C. 39
 Avvisati, G. 5, 11, 54, 86, 129
 Aydin, S. 29, 88

 Ba, I. 36
 Baccarani, M. 23, 51, 140, 141, 146
 Bacci, F. 60
 Bacigalupo, A. 19, 45, 82, 83, 84, 88, 133, 158
 Badolato, R. 167
 Baer, M.R. 2
 Bagot, M. 46
 Bahceci, E. 2
 Bakiri, M. 27
 Baldacci, E. 5, 56, 57, 123
 Baldazzi, C. 46, 92, 93, 134, 137
 Baldini, L. 38, 48, 49, 64, 121, 137, 143
 Balducci, S. 39, 107
 Ballanti, S. 2, 69, 157
 Ballerini, F. 79, 85, 90, 137, 156
 Balliu, M. 14
 Balocco, M. 218
 Balzarini, P. 114
 Balzarotti, M. 7, 47, 62, 115
 Bandiera, L. 114
 Bandini, L. 46, 152
 Banella, C. 136
 Banzi, R. 97
 Baratè, C. 22, 23, 35, 39, 143
 Barbato, A. 64, 67
 Barberi, W. 81
 Barbieri, M. 38
 Barbui, A.M. 114
 Barbui, T. 121
 Barcellin, W. 26
 Barcellini, W. 25, 32, 56
 Bardi, A. 178
 Bardy-Bouxin, N. 142

 Baretta, S. 63
 Bari, A. 97
 Barilà, G. 17, 65, 66, 166
 Barosi, G. 15
 Barozzi, P. 31
 Barsotti, S. 39
 Bartalucci, N. 12, 14
 Bartoletti, D. 3, 16, 41, 56
 Bartolini, M. 53
 Bartolomei, F. 33, 121
 Bartoncini, S. 28
 Baruzzo, G. 144
 Basilico, C. 35
 Bassan, R. 13, 34, 35, 94, 150, 155
 Bassani, B. 89
 Basset, M. 11, 49
 Bassi, S. 169
 Battista, M.L. 131
 Battistini, L. 13, 136
 Battistini, R. 155
 Bavaro, L. 23, 35, 39, 106
 Beggiano, E. 41
 Beghin, A. 167
 Beksac, M. 49
 Bellavia, S. 72
 Bellesi, S. 84, 88, 112, 128
 Bellofiore, C. 70, 79, 119
 Belotti, A. 69, 157
 Benedetti, E. 45, 68, 107, 133
 Benedetti, F. 20, 96
 Benevolo, G. 3, 10, 16, 41, 73
 Benvenuti, P. 40
 Berentsen, S. 26
 Bergamaschi, M. 3, 16, 41, 51, 54, 102, 140
 Berman, E. 2
 Bernard, S. 47
 Bernardi, M. 13, 44, 80, 131, 151
 Bernardi, S. 42, 51, 85, 120, 145
 Bernardini, A. 2
 Bernasconi, D.P. 33
 Bernasconi, P. 20, 21
 Berno, T. 49, 64, 65, 161, 166
 Bernuzzi, P. 169
 Bertaglia, I. 81, 86
 Bertaina, A. 45
 Bertamini, L. 134, 152
 Berti, V. 29
 Bertocchi, E. 147
 Bertolami, W. 68
 Bertoldero, G. 112
 Bertozzi, I. 56, 72, 122
 Bertuglia, G. 117
 Bertulli, A. 85
 Bettelli, F. 31
 Betti, C. 151
 Betti, S. 33, 121
 Betulla, A. 178
 Biagetti, G. 56, 122
 Bianchetti, N. 27
 Bianchi, A. 129
 Bianchi, B. 46

- Bianchi, M. 26, 33
 Bianchino, G. 143
 Bianco, R. 153, 170, 171, 174
 Binotto, G. 3, 16, 22, 23, 51
 Bisso, N. 156
 Bitetti, C. 118, 139
 Bizzarri, V. 100
 Bizzoni, L. 123
 Blade, J. 9
 Blanchard, J. 9
 Blau, I. 9
 Bo, A. 79
 Boccadoro, M. 2, 6, 9, 10, 49, 64, 69
 Bocchia, M. 22, 24, 52, 61, 128, 141, 146, 151, 160, 161
 Boccomini, C. 79
 Bochicchio, M.T. 51, 91, 92, 134
 Bodet-Milin, C. 6
 Boffa, I. 4, 28
 Boggio, E. 32
 Bogoni, G. 56, 72, 122
 Boissel, N. 36
 Bolis, S. 111
 Bolli, N. 49, 214
 Bonadonna, P. 40, 206
 Bonalumi, A. 157
 Bonanni, M. 88
 Bonanno, O. 105
 Boncompagni, R. 66, 69
 Bonferroni, M. 130, 156
 Bonfichi, M. 27, 114
 Bonifacio, M. 3, 16, 22, 23, 34, 35, 36, 39, 40, 51, 73, 92, 93, 106, 140, 141, 206
 Bonifazi, F. 20, 21, 45, 58, 84, 212
 Bonini, C. 44
 Bonomini, S. 81, 86
 Borghero, C. 20, 45
 Borin, L. 90, 141, 144
 Borlenghi, E. 11, 13, 54, 58, 59, 88, 89
 Borleri, G. 82
 Borsi, E. 9, 10, 50
 Bosi, A. 12, 13, 57, 65, 66, 69, 71, 112, 113, 168
 Bosi, C. 3, 16, 169
 Bossi, A. 121
 Bossi, E. 63
 Bossi, L.E. 141, 144
 Bottelli, C. 58
 Botto, B. 28, 78, 79
 Bovetti, K. 4, 28
 Bozzoli, V. 7
 Bragagnolo, M. 101
 Bramanti, S. 82
 Branca, A. 64, 65, 161, 166
 Brandts, C.H. 11
 Breccia, M. 3, 11, 16, 22, 41, 43, 54, 73, 74, 89, 108, 123, 140, 141, 143, 146, 150, 190
 Bregante, S. 45, 133
 Bresciani, P. 21
 Briani, C. 150
 Bringhen, S. 10, 64, 157
 Brioschi, F. 121
 Brioschi, F.A. 63
 Brocca, M.C. 125, 171
 Broccoli, A. 8, 37, 60, 61, 62, 77
 Brodsky, R.A. 4, 25
 Brogi, A. 43
 Brossart, P. 11
 Browett, P. 4
 Brummendorf, T.H. 142
 Brundu, E. 109
 Brunetti, C. 133
 Brunetti, G.A. 53, 150
 Bruno, A. 153, 170, 171, 174
 Bruno, B. 20, 21
 Bruno, S. 51, 91, 135
 Brunori, M. 157
 Brusceggin, A. 49
 Bruzzese, A. 108, 143
 Bruzzese, M. 119
 Bruzzone, M. 102
 Buccisano, F. 12, 13, 42, 52, 89, 90, 95
 Bucelli, C. 24, 51, 121, 143, 144
 Buda, G. 66, 68, 69
 Budelli, S. 84
 Buklijas, K. 13, 20
 Bulla, A. 79
 Buquicchio, C. 153, 173
 Busca, A. 5, 21, 30, 31, 88
 Businaro, C. 109
 Buttignol, S. 50
 Büttner, R. 72
 Buzzatti, E. 42, 90, 95
 Buzzichelli, A. 65, 71
 Cabibbo, S. 148, 167
 Cabras, A. 27
 Cabras, M.G. 46, 48, 115
 Caccamo, N. 117
 Cacciola, E. 125
 Cacciola, R. 125
 Cafforio, L. 1, 18, 35
 Cafro, A. 2
 Cagnetta, A. 90, 102, 137
 Caillot, D. 6
 Cairoli, R. 11, 12, 43, 114, 141, 144
 Caivano, A. 135, 138
 Calabrese, M. 97
 Calabresi, L. 14
 Calabretto, G. 17, 65
 Calabrò, M. 116, 159
 Calafiore, V. 12, 67
 Caliendo, I. 110
 Califano, C. 95, 110, 120, 157
 Calimeri, T. 6, 47, 112
 Calistri, D. 92, 93, 135
 Calistri, E. 23, 141
 Calore, E. 31
 Cambò, B. 81, 86, 92
 Cambria, D. 64, 67, 75, 79, 119
 Camera, A. 4, 28, 95
 Cametti, G. 165
 Camiolo, G. 64, 67, 75, 79
 Campagna, A. 43
 Campagna, G. 152
 Campanelli, R. 15
 Camperchioli, A. 154, 162, 163, 166
 Campiotti, L. 13
 Canale, F.A. 79, 85
 Cancelli, V. 97
 Candoni, A. 12, 21, 30, 31, 75, 89, 92, 131, 138
 Canepa, L. 66
 Cangini, D. 92, 93, 97, 116, 126, 147, 163
 Cantonetti, M. 17, 117
 Cantoni, S. 56, 57
 Cantore, N. 54
 Cantù, S. 63
 Canzonieri, C. 80
 Canzonieri, V. 114
 Caocci, G. 22, 54, 100, 109, 140, 141
 Capalbo, S. 68, 116, 145, 157, 160
 Capelli, C. 19
 Capelli, D. 12, 31, 75, 170
 Capelli, L. 135
 Capochiani, E. 157
 Capodanno, I. 54, 98, 140, 147
 Caporale, R. 66, 71
 Capozzi, F. 51
 Cappelli, A. 60
 Cappelli, L.V. 1, 18
 Cappellini, M.D. 143
 Cappello, E. 38
 Cappuccio, I. 76, 124, 152
 Capra, A. 2, 10, 69
 Caprioli, C. 13, 20
 Caprioli, V. 116
 Caprioli, E. 153
 Caracciolo, D. 28
 Caracciolo, F. 68, 107
 Caramella, D. 68
 Caramella, M. 141
 Caratozzolo, I. 10, 50, 66, 117
 Caravita di Toritto, T. 117
 Caravita, T. 10, 64, 69
 Carbone, A. 165
 Carbone, C. 5
 Carbone, F. 118
 Cardani, S. 132
 Carducci, B. 26
 Carella, A.M. 20, 21
 Carella, M. 8, 37, 61, 62, 77
 Carli, A. 137
 Carli, G. 32, 56, 73
 Carlier, P. 79
 Carlier, T. 6
 Carlino, D. 163
 Carlisi, M. 117, 159
 Carlo-Stella, C. 78
 Carlomagno, S. 4
 Carloni, S. 135
 Carluccio, P. 54, 108, 133, 135
 Carluccio, V. 153, 173
 Carminati, E. 137
 Carmosino, I. 41, 43, 53, 108, 123, 140, 143
 Carniti, C. 48
 Carobene, A. 171
 Carpenedo, M. 32, 33, 40, 56, 57
 Carrabba, M.G. 44, 80, 131
 Carrai, V. 56, 112
 Carraro, M.C. 141, 144
 Carraro, S. 65
 Carrer, A. 40
 Cartoni, C. 53, 149
 Carubbi, C. 15
 Caruso, S. 4, 28
 Casadei, B. 8, 37, 61, 62, 77
 Casale, M. 218

- Casaluci, G.M. 158
 Casavecchia, G. 145
 Cascavilla, N. 5, 10, 68, 93, 137, 140, 145, 157, 164
 Cascella, S. 145
 Casciani, P. 90, 95
 Casirati, G. 44
 Cassaday, R. 94
 Cassin, R. 38, 43, 100, 102
 Cassiodoro, G. 153, 170, 171, 174
 Castagna, A. 151
 Castagna, L. 82
 Castagnetti, F. 22, 23, 51, 140, 141, 146
 Castagnola, C. 31
 Castellani, G. 51, 93
 Castelli, F. 29
 Castellino, A. 130, 156
 Castellino, C. 48, 130, 156
 Catalano, G. 136
 Catalano, L. 71, 97, 116, 118, 148, 159
 Catani, L. 56
 Catellani, H. 31
 Cattaneo, C. 5, 29, 30, 31, 59, 88
 Cattaneo, D. 3, 6, 16, 22, 24, 121, 143
 Cattina, F. 42, 85, 120, 145
 Cavagna, E. 97
 Cavagna, R. 13
 Cavallari, C. 81
 Cavallari, M. 178
 Cavalli, F. 115
 Cavalli, M. 18, 35
 Cavallin, M. 52
 Cavallo, F. 28, 46, 48
 Cavattoni, I. 13
 Cavazzini, F. 3, 16, 41, 141, 146, 178
 Cavo, M. 2, 3, 6, 8, 9, 10, 16, 23, 35, 36, 37, 39, 41, 46, 49, 50, 56, 58, 61, 62, 64, 66, 77, 84, 87, 89, 106, 117, 134, 136, 137, 141, 146, 152
 Cea, M. 2, 90, 102, 137
 Cecchetti, C. 6, 47, 112
 Ceccolini, M. 116, 118, 126
 Cecconi, N. 68, 155
 Cedrone, M. 73, 74, 146
 Cefalo, M.G. 5
 Ceglie, S. 127, 168, 170
 Celino, C. 113
 Celli, M. 31
 Cellini, C. 10, 64, 69
 Cembrola, B. 4
 Cencini, E. 46, 61, 111, 128, 160, 161
 Cerchione, C. 51, 71, 92, 93, 97, 116, 118, 126, 147, 148, 157, 163
 Cerciello, G. 147
 Ceretto, C. 165
 Cerno, M. 20, 50, 131, 132, 153
 Cerqui, E. 58, 59, 88
 Cerrano, M. 92
 Cerrato, C. 2
 Cerretti, R. 52
 Cervetti, G. 68, 155
 Cesaretti, M. 46
 Cesaro, S. 5
 Cesini, L. 93, 102
 Charlier, B. 76
 Chatzidimitriou, C. 27
 Chauvie, S. 6
 Chen, Y. 1
 Chiappella, A. 7, 28, 47, 48, 79, 115
 Chiara, A. 6
 Chiarenza, A. 18, 79, 100
 Chiaretti, S. 34, 35, 93, 94
 Chiarini, M. 88
 Chiatamone Ranieri, S. 123
 Chierichini, A. 12, 30
 Chirumbolo, G. 84
 Chitarelli, I. 139, 145
 Chiurazzi, F. 59
 Chiusolo, P. 19, 20, 33, 45, 82, 83, 84, 88, 96, 121, 133, 158
 Chou, W. 2
 Ciabatti, E. 39, 107, 155
 Ciacci, G. 165
 Ciancia, R. 48
 Ciancio, A. 118, 139
 Ciarrocchi, A. 29
 Ciavarella, S. 99, 113, 127, 158
 Cibien, F. 150
 Ciccone, G. 48, 88, 115, 140
 Ciccone, M. 172, 178
 Ciccone, N. 92
 Cicconi, L. 11, 13
 Ciceri, F. 2, 6, 13, 20, 21, 39, 44, 45, 80, 112, 131, 151
 Ciciarello, M. 87, 89
 Cifarelli, L. 9, 10
 Cigana, C. 50, 131, 132, 138, 153
 Cilloni, D. 73, 189
 Ciminello, A. 33, 121
 Cimino, G. 46, 53, 73, 74, 103, 117
 Cimmin, C. 159
 Cimminiello, M. 110, 139, 143, 145, 170
 Cimmino, C. 76, 116, 124
 Cinti, P. 81
 Ciofini, S. 24, 151
 Ciolli, S. 100, 104
 Ciotti, G. 150
 Circhetta, E. 86
 Citiso, S. 68
 Clappier, E. 36
 Clavio, M. 79, 85, 90, 137, 156
 Clerici, D. 44, 151
 Coccaro, N. 15, 106
 Coccini, V. 40
 Cocito, F. 111
 Codeluppi, K. 3, 16, 41, 57
 Colaci, E. 31
 Colafigli, G. 22, 123, 140
 Colasante, C. 31
 Colasante, F. 50, 131, 132
 Colombo, A.A. 21
 Colombo, M. 89
 Colombo, N. 137
 Colonna, P. 145
 Coluzzi, S. 110, 139, 143, 145
 Colvin, R. 1
 Conconi, A. 69, 115
 Congiu, A.G. 48, 85, 161
 Consani, G. 68
 Conserva, M.R. 15, 106
 Consoli, U. 32, 157
 Consolini, S. 155
 Consonni, D. 25, 143
 Conte, E. 103
 Conte, G.M. 6
 Conti, C. 52
 Conticello, C. 49, 64, 67, 69, 70, 119, 157
 Contini, E. 12
 Contini, P. 137
 Contrino, A.D. 123, 124, 125, 126
 Coppetelli, U. 117
 Coppi, M.R. 51, 107, 125, 171
 Coppolecchia, L. 100, 105
 Corbingi, A. 103, 105, 133
 Cornelisse, P. 49
 Corradi, G. 87, 89
 Corradini, P. 13, 27, 48, 63, 77
 Corrente, F. 103, 128
 Cortelazzo, S. 13
 Cortelezzi, A. 11, 13, 21
 Cortes, J.E. 2
 Corti, A. 6
 Corti, C. 44, 80, 131, 151
 Corti, M.E. 47
 Cortinovis, I. 121
 Corvatta, L. 67, 70
 Corvini, F. 85
 Corvò, R. 85
 Coscia, M. 18, 100, 102, 104
 Così, E. 56
 Costanza, G. 125
 Cottone, F. 51, 53, 54, 55, 96, 147
 Coviello, E. 79, 102
 Cox, M.C. 7, 47
 Cozza, S. 145
 Craviotto, L. 81, 86
 Crea, E. 18, 32, 101
 Crescenzi, G.A. 129
 Crippa, C. 49, 59, 64
 Crisà, E. 140
 Criscuolo, C. 157
 Criscuolo, M. 5, 17, 30, 39, 40, 75, 88
 Cristiano, G. 46, 87, 137, 152
 Crò, F. 109
 Croci, G.A. 121
 Cruciani, M. 165
 Crucitti, L. 43, 47, 114
 Crugnola, M. 3, 16, 23, 51, 54, 73
 Cuccaro, A. 112, 128
 Cucci, R. 94
 Cufari, P. 98
 Culurgioni, F. 100, 104, 156, 168, 171
 Cumbo, C. 15, 106
 Cuneo, A. 3, 12, 16, 18, 41, 100, 102, 104, 135, 150, 172, 178
 Cupelli, L. 96
 Curci, P. 68, 130, 157
 Curnis, F. 6
 Curti, A. 35, 36, 39, 46, 87, 89, 92, 106, 134, 136, 137, 152
 Cusenza, V.Y. 100
 Cutrona, G. 18
 Cutuli, M. 154, 162, 163, 166
 Czuczman, M. 7
 D'Adda, M. 3, 51, 58, 120, 141, 144, 146
 D'Addio, A. 41
 D'Addosio, A. 73, 74

- D'Agostino, M. 117
 D'Alò, F. 112, 128
 D'Arco, A.M. 11, 147
 D'Ardia, S. 29, 54, 88
 D'Arena, G. 62, 104, 139
 D'Auria, F. 62
 D'Elia, M. 137, 164
 D'Innocenzo, S. 128
 Dabusti, M. 172, 178
 Daffini, R. 29, 58
 Dal Molin, A. 144
 Dal Piaz, F. 76
 Dalla Palma, B. 81, 86
 Damiani, D. 23, 52
 Daniele, A. 99
 Danise, P. 147
 Daprile, C. 58
 Daraia, B. 118, 139
 Dargenio, M. 5, 75, 92, 137, 163, 164
 Dazzi, F. 157
 De Angelis, B. 4, 28
 De Angelis, G. 52
 De Bardi, M. 13
 De Bellis, E. 42, 52, 89, 90, 95
 De Benedittis, D. 108, 123
 De Biasi, E. 23
 De Canal, G. 141, 144
 De Candia, M.S. 107
 De Celis, I.A. 7
 De Cicco, G. 50
 De Fabritiis, P. 12, 53
 De Falco, G. 95
 De Gregorio, F. 22
 De Lorenzo, D. 112
 De Luca, M.L. 123, 135, 138, 143
 De Luca, V. 28
 De Luigi, M.C. 79
 De Marchi, R. 50, 131, 132, 138, 153
 De Martino, F. 151
 De Matteis, G. 106
 De Matteis, S. 93, 135
 De Muro, M. 73, 74
 De Novellis, D. 59
 De Novi, L.A. 18, 35
 De Padua, L. 103, 165
 De Paoli, L. 13, 100
 De Polo, S. 134, 152
 De Propriis, M.S. 1, 18, 37
 De Risi, C. 68
 De Rosa, L. 117
 De Sabbata, G. 2
 De Santis, G. 153, 173
 De Santis, S. 23, 39, 106
 De Stefano, V. 5, 26, 33, 56, 57, 73, 74, 84, 112, 117, 121, 128, 157, 184
 De Summa, S. 99, 113, 127
 De Vito, R. 28, 167
 De Vivo, A. 58
 DeAngelo, D. 94
 Deary, B. 1
 Decembrino, N. 5
 Defina, M. 92, 151
 Defrancesco, I. 46, 111
 Del Bufalo, F. 4
 Del Fabro, V. 67, 69, 70, 119
 Del Giudice, I. 1, 18, 37
 Del Poeta, G. 100, 103, 104
 Del Principe, M.I. 5, 12, 30, 31, 52, 90, 92, 95, 103
 Del Vecchio, L. 62
 Delaini, F. 13, 77, 82, 114
 Delia, M. 5, 30, 92, 106, 108, 133, 135
 Dell'Acqua, A. 118
 Della Pepa, R. 76, 116, 129, 152
 Della Starza, I. 18, 35, 94
 Dellacasa, C. 88
 Delle Cave, G. 76, 116, 124, 159
 Delledonne, M. 39
 Deodato, M. 17, 46, 102, 192
 DePadua, L. 173
 Depau, C. 132
 Dereli Eke, E. 42
 Derudas, D. 64, 157
 Desogus, E. 156
 Devizzi, L. 63
 Di Battista, V. 116, 126, 147, 163
 Di Blasi, R. 30
 Di Bona, E. 11, 140
 Di Camillo, B. 141, 144
 Di Cecca, S. 4, 28
 Di Cerbo, M. 86
 Di Folca, S. 152
 Di Francesco, A. 19
 Di Giorgio, M. 67, 69
 Di Giorgio, R.M. 119
 Di Giovanni, A. 33, 84
 Di Giusto, S. 52
 Di Grazia, C. 19, 45, 83, 133
 Di Landro, F. 33, 121
 Di Leo, S. 147
 Di Marco, R. 154, 162, 163, 166
 Di Martino, F. 24
 Di Mauro, L. 135
 Di Noi, M.L. 107, 108, 130, 146
 Di Paolo, G. 167
 Di Pietro, C. 41, 56
 Di Raimondo, F. 3, 16, 41, 64, 67, 69, 70, 75, 79, 93, 119, 157
 Di Renzo, N. 11, 54, 68, 96, 137, 140, 145, 157, 163, 164
 Di Renzo, N. 18
 Di Rocco, A. 7, 62, 112, 113
 Di Stasi, A. 2
 Dianzani, U. 32
 Dilorenzo, R. 133
 Dimopoulos, M.A. 49
 Dimopoulou, M.N. 27
 Dimou, M. 27
 Dionisi, F. 173
 Diral, E. 21, 41
 DiRenzo, N. 160
 Discepoli, G. 67, 170
 Ditonno, P. 145
 Divella, R. 99
 Diverio, D. 22, 140, 143
 Divona, M. 11, 13, 52
 Divona, M.D. 136
 do Valle, I.F. 51
 Dodero, A. 48, 63
 Dohner, H. 11
 Dohner, K. 11
 Dombret, H. 36
 Dominietto, A. 19, 21, 45, 85
 Donatelli, F. 31
 Donati, B. 29
 Dotta, L. 167
 Dozza, L. 6, 9, 10, 49, 50, 64, 117
 Dragonetti, G. 30, 88
 Driessen, C. 49
 Dubbini, M.V. 30, 138
 Dubbini, V. 131, 138
 Dunbar, A. 120
 Dunlop, A. 25
 Durzu, T. 132
 Efficace, F. 11, 51, 53, 54, 55, 96, 147
 Ehninger, G. 11
 Elba, S. 130, 156
 Elena, C. 22, 39, 40, 141, 144, 204
 Elia, L. 34, 92, 94
 Elidi, L. 13
 Elli, E. 3, 41
 Elli, E.M. 16, 40, 121
 Elliot, H. 1
 Erba, P.A. 68
 Ergoli, M. 110
 Esposito, F. 42, 90, 95
 Evangelista, A. 7, 48, 79, 88, 155
 Fabbiano, F. 2, 11, 12, 34, 35, 54, 94
 Fabbri, A. 7, 61, 112, 128, 160, 161
 Fabbri, M. 113
 Fabbri, N. 8, 37, 61, 62, 77
 Fabbriatore, C. 110
 Fabiani, E. 42
 Fabris, F. 72, 122
 Facchetti, F. 114
 Facchin, G. 31, 138
 Facco, M. 17, 37, 65, 98, 101, 102
 Facco, N. 78
 Fagioli, F. 20
 Falco, M. 4
 Falcone, A.P. 51, 68
 Falconi, G. 42
 Falcucci, P. 103
 Falini, B. 13, 17
 Fallanca, F. 6
 Falzetti, F. 140
 Fama, A. 29
 Fanci, R. 30
 Fanelli, F. 152
 Fanelli, F. 76, 124
 Fani, A. 65
 Fanin, R. 21, 23, 31, 32, 50, 52, 75, 89, 131, 132, 138, 153
 Fanti, S. 6, 60
 Faraone, I. 138
 Farcomeni, A. 113
 Farina, F. 30, 44, 131, 151
 Farina, L. 20, 63, 111
 Farina, M. 42, 51, 58, 85, 97, 120, 145
 Fathi, A.T. 2
 Fatica, S. 58
 Fattizzo, B. 25, 26
 Fava, A. 28
 Fazi, P. 5, 11, 12, 18, 53, 57, 93, 96, 147

- Fazio, F. 11
 Fazzi, R. 68
 Fedele, R. 133
 Federici, I. 32
 Federico, V. 137, 163, 164
 Feltri, M. 122, 169, 172
 Fenoglio, D. 79
 Ferrara, F. 11, 34, 92, 93, 95, 118, 129, 157, 216
 Ferrara, K. 59, 71, 116
 Ferrari, A. 36, 46, 51, 71, 91, 92, 93, 105
 Ferrari, G. 92, 93
 Ferrari, M.L. 19, 51
 Ferrari, S. 58, 114
 Ferrarini, I. 150
 Ferrario, A. 111
 Ferreri, A.J.M. 6, 47, 62, 112, 115
 Ferrero, D. 13, 35, 141
 Ferrero, S. 7, 28, 46
 Ferretti, A. 123
 Ferrigno, I. 24, 61
 Fiaccabrino, S. 14
 Fianchi, L. 13, 54, 75, 88, 89
 Fiedler, W. 11
 Filardi, N. 110, 139, 143, 145, 170
 Fili, C. 75, 138
 Filippelli, A. 76
 Filippi, A.R. 28
 Fina, M.P. 163
 Finazzi, M.C. 19, 81
 Finco, G. 195
 Finelli, C. 58
 Fiorentini, A. 32, 170
 Fisogni, S. 114
 Flenghi, L. 155
 Flinn, I. 7
 Florena, A.M. 72, 124
 Floris, R. 168, 171
 Foà, R. 1, 11, 12, 18, 21, 22, 34, 35, 37, 43, 53, 81, 92, 93, 94, 96, 98, 100, 101, 102, 104, 108, 117, 140, 141, 143, 146
 Fogli, M. 58
 Fogliatto, L.M. 7
 Foglietta, M. 130, 156
 Foli, A. 11, 49
 Follini, E. 81, 86
 Fontana, D. 63
 Fontana, M. 91
 Fontana, M.C. 39, 51, 93, 134, 152
 Fontana, R. 104, 153, 170, 171, 174
 Fonzi, E. 51, 92, 93
 Foppoli, M. 6
 Forese, P. 163
 Forghieri, F. 31, 75, 92
 Forni, G.L. 198, 218
 Foroni, C. 42, 85, 145
 Forte, V. 95
 Fortunato, A. 145
 Fowler, N.H. 7
 Fozza, C. 11, 22, 93
 Frabetti, F. 163
 Fraboni, D. 52
 Fracchiolla N.S. 5, 12, 34, 89, 92
 Fragasso, A. 118, 139
 Frairia, C. 11, 29, 54, 88
 Franceschini, E. 31
 Frangi, C. 43
 Franzino, M. 88
 Frappampina, R. 135, 146
 Frazzetto, S. 119
 Frazzi, R. 100
 Frediani, R. 165
 Fresa, A. 103
 Frezzato, F. 37, 78, 98, 101, 102
 Frezzato, M. 112
 Frigeni, M. 46
 Frigeri, F. 157
 Frisoli, S. 138
 Frison, L. 23
 Frongia, S. 98
 Fronti, E. 169
 Frustaci, A. 17, 104
 Frustaci, A.M. 100, 192
 Fumagalli, M. 90
 Furfaro, G. 28
 Fusco, A. 50
 Fusillo, M. 106
 Fustier, P. 7
 Gabrielli, G. 8, 37, 61, 62, 77
 Gagliardi, A. 157
 Gagliardi, F. 6
 Gaidano, G. 5, 10, 18, 54
 Galassi, L. 31
 Galasso, R. 139
 Galbo, F. 159
 Galieni, P. 2
 Galimberti, S. 22, 23, 24, 35, 39, 68, 107, 140, 143, 155
 Galitzia, A. 100
 Gallamini, A. 6
 Galli, E. 17, 168
 Galli, M. 9, 10, 64, 157
 Gallina, A.M. 20
 Gambacorti-Passerini, C. 40, 63, 90, 142
 Gambella, M. 102
 Gamberi, B. 6, 49, 66, 71, 157
 Gandolfi, L. 3, 42, 51, 85, 97, 145
 Gangemi, D. 98, 157, 165
 Ganser, A. 11
 Gardellini, A. 122, 172
 Gardellini, A. 169
 Garderet, L. 6
 Garozzo, G. 148, 167
 Garvey, K.B. 32, 127, 168, 170
 Garzia, M. 5, 30
 Gasbarrini, A. 60
 Gasparini, V.R. 17
 Gasperi, M. 154, 162, 163, 166
 Gastino, A. 28
 Gaudio, F. 130
 Gay, F. 2, 6, 49
 Gazzera, G. 10
 Geffner, M. 4
 Gentile, L. 164
 Gentile, M. 2, 18, 62, 100, 104, 111, 157
 Gentili, S. 67, 70
 Gentilini Cacciola, E. 125
 Gerardi, C. 97
 Germagnoli, L. 109
 Germano, C. 68, 153, 173
 Geromin, A. 52, 131, 132
 Geskin, L. 46
 Gesuiti, P. 81
 Gesullo, F. 14
 Ghelli Luserna Di Rorà, A. 36, 135
 Ghelli, A. 92, 93
 Gherlinzoni, F. 150
 Ghetti, M. 51, 91, 93, 135
 Ghiggi, C. 85
 Ghirotto, L. 98, 147
 Giai, V. 51
 Giallongo, C. 64, 67, 70, 75, 79, 119
 Giammarco, S. 19, 45, 83, 84, 133, 158
 Gianelli, U. 121
 Gianfaldoni, G. 12, 13
 Giannantonio, C. 26
 Giannarelli, D. 38, 100
 Giannini, B.M. 92, 93
 Giannini, M.B. 97, 126, 147, 163
 Giannone, A.G. 72
 Giannotta, A. 58, 68
 Giardini, I. 32
 Giatra, C. 27
 Giesinger, J. 53
 Giglio, F. 21, 44, 151
 Giles, F. 142
 Gilioli, A. 31
 Gini, G. 7, 47, 62, 111, 114
 Gioia, G. 110
 Giona, F. 14
 Giordano Resti, A. 47
 Giordano, A. 47, 76, 116, 118, 124, 130
 Giordano, A.M. 100
 Giordano, C. 59, 76, 77, 118, 129, 152
 Giordano, G. 154, 162, 163, 166
 Giorgi, M. 28
 Giovanardi, F. 147
 Giraldo-Castellano, P. 142
 Girasoli, M. 125, 161, 171, 173
 Girelli, G. 81
 Girgenti, M. 138
 Girlanda, P. 164
 Girlanda, S. 44, 80
 Girmenia, C. 53, 100, 149, 150
 Giudice, V. 76
 Giuliani, G. 18
 Giuliani, N. 10, 69, 157
 Giusti, D. 31
 Giustini, V. 88
 Gjertsen, B. 142
 Gobbi, G. 15
 Gobbi, M. 79, 85, 90, 137, 156
 Godio, L. 165
 Goldaniga, M. 111
 Goldschmidt, H. 9
 Golfieri, R. 60
 Gomez, H. 115
 Gospodarowicz, M.K. 115
 Goteri, G. 114, 170
 Gottardi, D. 18
 Gottardi, M. 92
 Gotti, M. 155
 Govi, S. 47
 Gozzer, M. 81
 Gozzetti, A. 2, 18, 117
 Gozzini, A. 22, 23, 24, 140

- Gramegna, D. 29, 58, 88, 141
 Grassi, A. 19, 20, 81, 82
 Grassi, S. 39, 107
 Grasso, M. 69, 130, 156
 Gravetti, A. 97
 Greco, A. 68, 111
 Greco, G. 68
 Greco, R. 44, 131, 151
 Gregorj, C. 86
 Greve, B. 95, 98
 Greve, M.B. 172
 Gribben, J. 7
 Grifoni, A. 129
 Grifoni, F.I. 40
 Griguolo, D. 3, 16, 23, 138
 Grillo, G. 21
 Grimaldi, F. 95
 Grisariu, S. 133
 Gritti, G. 114
 Gualand, F. 19
 Gualandi, F. 45, 83
 Guaragna, G. 125, 126, 137, 161, 171, 173
 Guariglia, R. 153, 170, 171, 174
 Guarini, A. 1, 18, 35, 37, 68, 96, 97, 99, 113, 127, 137, 140, 145, 158, 160
 Guella, A. 140
 Guercio, M. 4, 28
 Guerrero, G. 13, 136
 Guerrini, F. 39, 107, 143
 Guggiari, E. 44
 Guglielmelli, P. 12, 14, 73
 Gugliotta, G. 22, 23, 141, 146
 Gugliotta, L. 5
 Guidetti, A. 27, 63, 77
 Guidi, S. 20
 Guidotti, F. 122, 169, 172
 Guidotti, S. 169
 Gulino, A. 113
 Guolo F. 79
 Guolo, F. 85, 90, 137, 156
 Gurnari, C. 42, 90, 95
 Gurrieri, C. 161, 166

 Habermann, T.M. 78
 Hájek, R. 49
 Hanel, M. 11
 Hernández-Rivas, J.M. 92, 93
 Hertenstein, B. 11
 Hill, A. 25, 26
 Hillmen, P. 25
 Hochhaus, A. 142
 Hohaus, S. 7, 112, 128
 Hong, X. 7
 Hongeng, S. 1
 Hosono, N. 2
 Huang, M. 4

 Iaccarino, L. 13
 Iaccarino, S. 92
 Iaria, G. 95
 Ibatici, A. 18, 85, 102, 104
 Ilari, C. 1, 18
 Ilariucci, F. 18, 48, 100, 105
 Imbergamo, S. 37, 78, 92, 98, 102, 150, 166
 Imbrogno, E. 93

 Imola, M. 122, 149
 Imovilli, A. 23, 35
 Impera, L. 15, 106, 135
 Ingrosso, C. 133
 Innao, V. 119, 164
 Innocenti, I. 82, 84, 103, 104, 105, 133, 158
 Intermesoli, T. 13, 20, 54, 141, 146
 Introcaso, T. 143
 Introna, M. 19
 Inwards, D. 78
 Iodice, M. 5, 57
 Ionova, T. 98
 Iori, A.P. 20, 45, 81, 133
 Iotti, S. 31
 Iovino, G. 29, 88
 Iovino, L. 68
 Irno Consalvo, M. 12, 13, 52
 Irrera, G. 21
 Isidori, A. 3, 16
 Isola, M. 132
 Iurlo, A. 3, 16, 22, 23, 24, 51, 121, 141, 143, 144
 Izutsu, K. 7
 Izzo, B. 76
 Izzo, V. 76

 Jabbour, E. 94
 Jankovic, M. 33
 Jilma, B. 26
 Jimenez-Zepeda, V. 49
 Johnsen, H.E. 49
 Johnston, P.B. 78

 Kalambakas, S. 7
 Kantarjian, H. 94
 Karlin, L. 6
 Karmiris, T. 27
 Kasner, M. 2
 Kim, Y. 46
 Klimko, N. 31
 Konstantopoulos, K. 27
 Kontopidou, F. 27
 Korkolopoulou, P. 27
 Kovalchuk, S. 112, 155, 168
 Kraeber-Bodéré, F. 6
 Krampera, M. 3, 16, 23, 36, 40, 150
 Kulasekararaj, A. 4, 25
 Kulasekararaj, A.G. 25
 Kunkl, A. 79, 137
 Kwiatkowski, J. 1

 La Barba, G. 140
 La Manna, M.P. 117
 La Sala, E. 12, 93
 La Starza, R. 92
 La Terra, V. 148, 167
 La Verde, G. 117
 Laddaga, F.E. 130
 Laginestra, M.A. 35
 Lagreca, I. 31
 Lahuerta, J.J. 9
 Lalinga, V. 62
 Lambusta, E. 75
 Lamorte, D. 135, 138
 Lamparelli, T. 20
 LaNasa, G. 132

 Lanfranchi, A. 167
 Languille, L. 26
 Lanza, F. 12, 41
 Lapucci, C. 109
 Larocca, A. 10, 69
 Larocca, L.M. 128
 Larson, R.A. 2
 Latagliata, R. 3, 16, 22, 41, 43, 73, 74, 108, 123, 140, 143, 150
 Latargia, M.L. 141, 144
 Lattanzi, R. 81
 Laudisi, A. 102
 Laudisi, M. 172, 178
 Laureana, R. 95
 Laurenti, L. 18, 19, 45, 81, 82, 83, 84, 100, 102, 103, 104, 105, 133, 158
 Laurenzana, I. 135, 138
 Lauzzana, P. 138
 Lavorgna, S. 12, 13, 52
 Lazzari, L. 44, 151
 Lazzaro, A. 169
 Lazzarotto, D. 30, 31, 75, 89, 92, 138
 Lazzi, S. 160
 Leanza, R. 164
 Lee, H. 49
 Lee, J. 2
 Lee, J.W. 4, 25
 Leip, E. 142
 Lemoli, R. 41
 Lemoli, R.M. 3, 16, 79, 85, 90, 102, 137, 156
 Lenti, V. 97
 Lentini, M. 38
 Leo, M. 153, 173
 Leonard, J.P. 7
 Leoncin, M. 17
 Leoncini, L. 160
 Leone, A. 76, 124, 159
 Leone, J. 142
 Leonetti-Crescenzi, S. 73, 74
 Leotta, V. 67, 69, 70, 119
 Lerario, G. 153, 173
 Lessi, F. 64
 Levati, G. 25, 26, 143
 Levato, D. 38
 Levato, L. 38, 100, 104, 140, 141, 146
 Levine, R.L. 120
 Levis, M. 28
 Levis, M.J. 2
 Lewis, R.E. 152
 Li Volti, G. 64, 67
 Li Pira, G. 4
 Liaskas, A. 27
 Liberati, A.M. 10, 47, 69
 Liberati, M. 18
 Liberatore, C. 44, 131, 151
 Limongiello, M.A. 103
 Link, H. 11
 Lint, M. Van 19
 Lionetti, M. 49
 Lisi, C. 143
 Lisi, G. 167
 Lissandrini, L. 77
 Lista, C. 101
 Liu, C. 2
 Liu, X. 2
 Lleshi, A. 27

- Lo Coco, F. 11, 12, 13, 42, 52, 54, 136
 Locatelli, F. 1, 4, 28, 45
 Lohneis, P. 72
 Lokhorst, H.M. 9
 Lolli, G. 8, 37, 61, 62, 77
 Lombardi, A.M. 72
 Lombardi, M. 167
 Lombardo, A. 157
 Lonati, S. 38
 Longo, M.C. 107
 Longo, R. 105
 Lopedote, P. 6
 Lopez-Guillermo, A. 115
 Lorentino, F. 20, 44, 131, 151
 Lorenzi, L. 114
 Loseto, G. 62, 111, 113, 127
 Lubbert, M. 11
 Lucarelli, C. 113
 Lucchesi, A. 56, 71, 126, 135, 140, 154, 162, 163, 166
 Lucchini, E. 32
 Luchetti, S. 79
 Lucia, E. 140
 Luciano, L. 22, 24, 140
 Lucioni, M. 114
 Luminari, S. 29, 46, 100, 111
 Lunghi, F. 44, 51
 Lunghi, M. 34, 51, 89
 Lupo Stanghellini, M.T. 44, 80, 131, 151
 Luponio, S. 163, 164
 Luppi, M. 12, 31, 54, 93
 Lussana, F. 13, 19, 20, 21, 81, 92
- Macro, M. 6
 Maestrini, G. 150
 Maffei, R. 31
 Maggi, L. 14
 Magi, A. 12
 Magri, M. 154, 162, 163, 166
 Maiolo, E. 112, 128
 Maiorana, L. 148, 167
 Malafrente, R. 76, 112
 Malagola, M. 21, 42, 51, 85, 97, 120, 145
 Malato, A. 73
 Malato, S. 141, 144
 Malcovati, L. 187
 Malfitano, A. 114
 Mallano, S. 173
 Mammoliti, S. 20, 21
 Mancini, G. 70
 Mancini, M. 23, 39, 43, 106, 108, 123, 143
 Mancini, S. 21, 168, 170
 Mancini, V. 92, 160
 Mancino, A. 34
 Manconi, L. 79, 85, 137
 Mancuso, K. 9, 10, 49, 50, 66, 117
 Mancuso, S. 117
 Mandelli, F. 11
 Manenti, G.O. 148, 167
 Manfra, I. 81, 86, 169
 Mangerini, R. 137
 Mangiacavalli, S. 157
 Mannarella, C. 118, 139
 Mannarelli, C. 14, 113
 Mannelli, F. 12, 14, 40, 209
- Mannelli, L. 112, 113, 158
 Manni, S. 65, 78
 Mannina, D. 18, 35, 46, 47
 Mansueto, G. 62, 139
 Manzoni, M. 49
 Maracci, L. 67, 70
 Marafioti, V. 76, 124
 Maraglino, A.M.E. 75
 Marangon, M. 8, 27, 37, 61, 62, 77
 Marasca, R. 18, 31, 150
 Marasco, V. 63
 Marcacci, G. 157
 Marcenaro, E. 79
 Marcheselli, L. 46
 Marchesi, F. 5
 Marchetti, M. 18, 69, 148, 150
 Marchionni, F. 84
 Marcolin, R. 79, 85, 90, 137, 156
 Marconi, G. 36, 46, 51, 87, 89, 91, 93, 134, 137, 152
 Mariggio, E. 108, 123
 Mariggio, E. 143
 Mariglia, P. 1, 18, 37
 Marinello, S. 150
 Marino, L. 76, 171
 Mariotti, B. 52
 Markovic, U. 67
 Marks, D. 94
 Markt, S. 44, 80, 131
 Marmont, F. 13
 Marsh, J. 25
 Martelli, M. 23, 35, 39, 106, 115, 155
 Martelli, M.P. 12
 Martello, M. 9, 10, 50, 117
 Martellosio, G. 167
 Martinelli, G. 2, 12, 23, 35, 36, 39, 40, 46, 51, 71, 89, 91, 92, 93, 94, 97, 116, 118, 126, 134, 135, 146, 147, 148, 152, 163
 Martinelli, V. 76, 97, 124
 Martini, F. 68, 107, 155
 Martini, V. 13, 98
 Martino, B. 3, 16, 22, 30, 51, 54, 95, 98, 121, 140, 141, 146, 172
 Martino, E. 67
 Martino, M. 45, 133, 157
 Martorelli, C. 171
 Martorelli, M. 153, 170, 174
 Marzocchi, G. 49, 50, 64, 66
 Masala, E. 43
 Masciopinto, P. 130
 Mascolo, M. 152
 Masneri, S. 42
 Massa, M. 15
 Massaia, M. 130, 156
 Massaro, F. 57, 71
 Masselli, E. 15
 Mastaglio, S. 44, 131, 151
 Mastini, C. 90
 Mastruzo, B. 130
 Mateos, M-V. 9
 Matera, R. 137, 163, 164
 Mattei, D. 13, 130, 156
 Matturro, A. 110, 139, 143, 145, 170
 Maurillo, L. 12, 13, 42, 52, 73, 74, 89, 90, 95
 Mauro, F.R. 1, 18, 26, 37, 98, 100, 101, 102, 103, 104
- Mazza, P. 12, 68, 73, 96, 140, 160
 Mazzantini, E. 107
 Mazzara, S. 113
 Mazzeo, A. 164
 Mazzini, T. 53
 Mazziotta, F. 68
 Mazzoni, C. 168
 Mazzucconi, G. 73, 74
 Mazzucconi, M.G. 5, 56, 57
 McCulloch, R. 7
 Mecacci, B. 151
 Melandri, A. 178
 Mele, A. 68
 Mele, G. 68, 109, 132, 157
 Melillo, L. 5, 11, 12, 31, 75, 137
 Melle, F. 113, 114
 Mellqvist, U.H. 49
 Mendicino, F. 73
 Mengarelli, A. 5, 96
 Mengucci, C. 51
 Menon, K. 135
 Mercante, L. 95
 Merchionne, F. 107, 126
 Merli, A. 122, 149
 Merli, F. 29, 46, 57, 71, 98, 115, 147, 155
 Merli, M. 46, 111
 Merlini, G. 11, 49
 Messeri, M. 65, 71
 Messerotti, A. 31
 Messina, C. 44, 80, 131
 Mestice, A. 133
 Metafuni, E. 19, 45, 84, 133, 158
 Metelli, M.R. 107
 Mian, M. 155
 Mianulli, A. 149
 Mianulli, A.M. 122
 Micallef, I.N. 78
 Miccolis, R. 68, 153
 Miccolis, R.M. 173
 Michel, M. 26
 Michelato, A. 13, 20
 Micheletti, M. 167
 Michelutti, A. 52
 Micò, C. 19, 20, 81, 82
 Micucci, G. 32, 73, 127, 168, 170
 Miggiano, M.C. 23
 Miglino, M. 79, 85, 90, 102, 137, 156, 161
 Mignone, F. 35
 Milani, P. 11, 49
 Milani, R. 44, 131
 Milella, L. 138
 Milone, G. 20, 96
 Mina, R. 69
 Mineo, G. 35
 Minervini, A. 15, 106
 Minervini, C.F. 15, 106
 Minetto, P. 75, 79, 85, 90, 92, 137, 156
 Minga, P. 192
 Minoia, C. 97, 99, 113, 127, 158
 Miotti, V. 20
 Misericocchi, E. 47
 Mitra, M.E. 159
 Modorati, G. 47
 Mohamed, S. 43, 108, 123, 143
 Moia, R. 18

- Moioli, M.C. 114
Molica, M. 22, 108, 123, 140, 143
Molica, S. 2, 18, 38, 100, 102
Molinari, A.L. 18, 48, 115, 122, 149
Molisso, A. 26
Mologni, L. 63
Molteni, A. 43
Monaco, F. 10, 69
Monagheddu, C. 140
Monaldi, C. 23, 39, 106
Mondello, A. 72
Montanaro, M. 53, 73, 74, 103
Montefusco, E. 141
Montefusco, V. 2, 10, 64
Montemurro, T. 84
Montesinos, P. 2
Montillo, M. 181
Montuori, N. 76
Morabito, F. 18, 157
Morano, G. 150
Morciano, M.R. 68
Mordini, N. 19, 130, 133, 156
Morè, S. 67, 70
Moreau, P. 6
Moreira, C. 7
Morelli, F. 103, 104, 105
Morelli, M. 73
Morello, E. 42, 85, 97, 120, 145
Morello, L. 7, 47
Moretta, L. 4
Morganti, R. 68
Mori, V. 67
Morigi, A. 8, 37, 61, 62, 77
Moroni, C.F. 169
Morra, E. 141, 144
Morselli, M. 31
Morsia, E. 32, 127, 168, 170
Mortaruolo, C. 59
Moschetta, A. 113
Moseley, A. 4
Mosna, F. 92
Motta, G. 79, 114
Motta, M. 17
Mufti, G. 25
Mulas, O. 22
Munteanu, D. 165
Murru, R. 18, 100, 104
Musiu, C. 104, 156, 168, 171
Musolino, C. 2, 119, 164
Mussetti, A. 27
Mussini, C. 31
Musso, M. 45, 96
Mussolin, L. 63
Musto, P. 2, 62, 69, 70, 89, 118, 135, 138, 139, 157
Musuraca, G. 71, 92, 126, 135, 148
Muzi, C. 47

Nadali, G. 30, 31, 54
Naldi, I. 112
Nanni, C. 6, 60
Nanni, J. 46, 51, 87, 91, 134, 137, 152
Nanni, L. 8, 37, 61, 62, 77
Nanni, M. 18
Napolitano, M. 5
Napolitano, R. 51, 91, 93, 135
Nappi, D. 71, 95, 97, 116, 118, 148
Narni, F. 2, 20, 31, 64
Nasa, G. La 11, 22, 54, 100, 104, 109, 156, 168, 171
Nasillo, V. 31, 150
Nassi, L. 7, 48, 62, 97, 115, 158
Negri, A. 127
Negrini, M. 172
Nencini, V. 65, 71
Neri, A. 18, 38, 49
Neri, B. 13
Neubauer, A. 2
Neuhof, A. 94
Nichelatti, M. 43, 114
Nicolino, B. 29
Nicolosi, M. 28, 140
Niebla, A.M. 7
Nierychlewska, P. 107
Nigro, S. 43
Niro, G. 154, 162, 163, 166
Niscola, P. 54
Nitti, R. 44
Nizzoli, M.E. 17
Nogueira, N.I. 136
Nosi, D. 43
Notaro, R. 4
Noto, A. 38, 147
Novo, M. 78, 79
Nowakowski, G. 7
Nowakowski, G.S. 78
Nozza, A. 82
Nozza, F. 118
Nozzoli, C. 66, 69, 71
Nuccorini, R. 110, 118, 139, 143, 145, 170
Nunziante, F. 147
Nuvolone, M. 11, 49

O'Brien, S. 94
Ocadlikova, D. 87
Oerlemans, S. 55
Offidani, M. 10, 17, 49, 64, 67, 69, 70, 117, 157
Offner, F. 7
Oldani, E. 13, 20, 21
Olin, R. 2
Oliva, E.N. 98
Oliva, S. 145
Olivieri, M. 46, 87, 134, 152
Olivieri, A. 20, 21, 32, 67, 70, 127, 133, 168, 170
Olivieri, J. 21
Oltolini, C. 151
Omedé, P. 2
Onida, F. 19, 45
Onofrillo, D. 106, 128, 167, 171
Opinto, G. 113, 127
Oppi, S. 100
Orciuolo, E. 21, 68
Oriol, A. 9
Orlandi, E.M. 22
Orlandini, F. 155
Orlando, A. 84
Orlando, D. 4, 28
Orlando, N. 33
Orlando, V. 117
Orofino, A. 67
Orofino, M.G. 168, 171
Orsini, F. 109
Orsini, P. 15, 106
Orsucci, L. 18, 28, 46, 79
Ortenzio, P. 72
Ortiz-Romero, P. 46
Ortiz, S. 25
Oteri, R. 119
Ottaviani, E. 46, 51, 87, 134, 137
Ottaviani, M. 152
Ottone, T. 12, 13, 52

Pacilli, A. 14
Padella, A. 51, 91, 92, 93
Pagani, C. 29, 88
Pagano, L. 5, 17, 30, 31, 39, 75, 88
Palandri, F. 3, 16, 32, 41, 56, 73, 121
Palazzo, G. 68
Palladini, G. 11, 49
Pallotti, F. 109
Palm, A. 139
Palma, A. 58
Palma, T. 110
Palmieri, R. 42, 52, 90, 95
Palmieri, S. 118
Palombi, F. 155
Palumbo, A. 3, 16, 41, 54, 64, 69, 79
Palumbo, G. 53
Palumbo, G.A. 3, 16, 41, 54, 64, 67, 73, 75, 79
Panayiotidis, P. 27
Pancani, F. 12, 112, 113
Pancrazzi, A. 14
Pane, F. 59, 71, 73, 76, 95, 97, 116, 118, 124, 129, 140, 141, 146, 147, 148, 152, 159
Pangalis, G.A. 27
Pantani, L. 9, 10, 49, 50, 64, 66, 117
Paolini, A. 31
Paolini, R. 150
Paolini, S. 2, 36, 46, 58, 87, 92, 93, 134, 137, 152
Paolo, M. 154, 162, 163, 166
Paoloni, F. 5, 11, 18, 57
Papacci, P. 26
Papalinetti, G. 20
Paparò, C. 165
Papayannidis, C. 35, 36, 39, 40, 46, 51, 87, 89, 91, 92, 93, 106, 134, 137, 152
Paradiso, A. 99
Parciante, E. 15, 106, 146
Pardee, T. 2
Parente, A. 154, 162, 163, 166
Parente, R. 40
Pareto, A.E. 71, 116, 118, 148
Parisi, M. 67
Parisi, S. 46, 58, 87, 134, 137, 152
Parma, M. 21, 45
Parrinello, N. 64, 67
Parrinello, N.L. 70, 75, 79, 119
Pasanisi, A. 58, 107
Pascale, S. 106, 118, 128, 167, 171
Pascale, S.P. 110, 139, 143, 145, 170
Pasciolla, C. 137
Pasquale, R. 105
Pasquini, G. 92, 93
Passamonti, F. 73, 111
Passannante, M. 90, 137
Passi, A. 29, 58, 59, 88

- Pastore, D. 58, 68, 73, 96, 107, 125, 126, 133, 135, 137, 140, 160, 161, 171, 173
- Paterno, G. 52, 90, 95
- Patriarca, A. 26, 32
- Patriarca, F. 6, 9, 10, 20, 50, 64, 131, 132, 133, 153, 157
- Paulli, M. 114
- Pavan, L. 17, 65
- Pavesi, F. 80, 131, 151
- Pavone, V. 5, 68, 73, 96, 115, 160
- Pavoni, C. 13, 19, 20, 77, 81, 82, 114
- Pazzaglia, M. 51, 91
- Peccatori, J. 44, 80, 131, 151
- Pedroni, C. 98
- Peffault de Latour, R. 25
- Pelizzoni, F. 49
- Pellegrini, C. 8, 27, 37, 61, 62, 77
- Pellegrino, C. 26
- Pelo, E. 12
- Peluso, I. 159
- Pennese, E. 7
- Pensato, V. 136
- Pepe, S. 81
- Peracchio, C. 155
- Peragine, N. 37
- Percesepe, A. 15, 35
- Perego, A. 141, 144
- Perez, A. 123, 125, 124
- Perfetti, P. 90
- Perl, A.E. 2
- Perna, M. Di 71, 116
- Perricone, M. 51
- Perrone, G. 63
- Perrone, M.P. 81
- Perrone, S. 6, 47, 112
- Perrone, T. 7, 130, 135
- Perrot, A. 6
- Perrotta, M. 167, 171
- Perruccio, K. 5, 30, 31
- Perutelli, F. 143
- Peruzzi, B. 66, 71
- Pescosta, N. 2, 10, 64, 69
- Petracci, E. 163
- Petrella, L. 154, 162, 163, 166
- Petriccione, L. 73, 74
- Petrini, M. 39, 68, 69, 107, 155
- Petronacci, A. 67
- Petrucci, M.T. 2, 9, 11, 49, 64, 69, 117, 157
- Peyvandi, F. 220
- Pezzatti, S. 33
- Pezzullo, L. 129, 153, 170, 171, 174
- Piazza, F. 64, 65, 78, 101, 102, 150, 161, 166
- Piazza, F. Di 123, 124, 125, 126
- Piazza, R. 90
- Piazza, R.G. 63
- Picard, M. 129
- Picardi, A. 20, 21
- Picardi, M. 76, 77, 116, 152
- Piccioni, A.L. 43
- Piccirillo, N. 82, 84
- Piccolo, G. 76, 116, 118, 124, 152
- Piccolomo, A. 107, 130
- Piciocchi, A. 1, 12, 17, 18, 34, 37, 101, 104, 117
- Picone, G. 51
- Piemontese, S. 44
- Pietrantuono, G. 62, 118, 139, 143
- Pietrasanta, D. 18
- Pileri, S. 48
- Pileri, S.A. 114
- Pilo, F. 109
- Pinci, P. 26
- Pinelli, M. 167
- Pingeon, M. 76
- Pinto, A. 7, 27, 48
- Pinto, V. 198
- Pinto, V.M. 218
- Pioli, V. 31
- Pioltelli, M.L. 141
- Pioltelli, P. 20
- Piovani, C. 60
- Piras, E. 132
- Piroddi, A. 168, 171
- Pisani, F. 103
- Pisapia, G. 133
- Pitisci, A. 4
- Pizzarell, G. 149
- Pizzi, M. 78, 161
- Pizzo, S. 37, 101
- Pizzolato, M. 40
- Pizzolo, G. 92
- Pizzuti, M. 110, 118, 139, 140, 143, 145, 170
- Plati, C. 173
- Platzbecker, U. 11
- Plebani, A. 167
- Plenteda, C. 86
- Pochintesta, L. 81, 86, 169
- Podoltsev, N. 2
- Poggiu, M. 69
- Poidomani, M. 148, 167
- Poidomani, S. 67
- Poletti, A. 9, 10
- Polito, F. 119
- Pollara, C.P. 29
- Polli, V. 7, 149
- Poloni, A. 170
- Polverelli, N. 3, 16, 41, 42, 51, 85, 97, 120, 145
- Pompa, A. 49, 157
- Pontone, M. 71
- Ponzoni, M. 6, 47, 112
- Porrizzo, M. 22, 43, 108, 140, 143
- Porretto, F. 146
- Porrini, R. 74
- Porta, F. 167
- Porter, J. 1
- Potenza, L. 30, 31
- Pozzi, G. 15
- Pozzi, S. 155
- Prandi, R. 98
- Praticò, G. 95, 98, 172
- Pravato, S. 78, 98, 102
- Pregno, P. 22, 23, 28, 140, 141
- Prete, E. 68
- Prezioso, L. 81, 86, 169
- Priolo, G. 28
- Priotto, R. 130, 156
- Prota, D. 149
- Puccini, B. 29, 112, 113, 155, 158, 168
- Puggioni, P.L. 84, 112, 128
- Pugliese, G. 137
- Pugliese, N. 76, 97, 116, 118, 124, 152, 159
- Puglisi, F. 64, 67, 70, 75, 79, 119
- Pulini, S. 10
- Pulsoni, A. 17
- Pungolino, E. 141, 144
- Putzulu, R. 82
- Puzzolo, M.C. 37
- Quaglia, F.M. 172, 178
- Quaresima, M. 71, 117
- Quarta, A. 58, 107
- Quattrocchi, L. 81
- Quattrone, M. 88
- Quintana, G. 126
- Quintarelli, C. 4, 28
- Quoti Tubi, L. 78
- Rabassi, N. 138
- Racca, S. 151
- Radici, V. 131, 132, 138, 153
- Raffini, M. 46
- Ragaini, S. 46, 87, 134, 152
- Raimondo, M. 116, 118, 159
- Raiola, A. 19
- Raiola, A.M. 45, 83, 133
- Rambaldi, A. 11, 13, 19, 20, 21, 34, 35, 45, 77, 81, 82, 94, 106, 114, 133
- Rambaldi, B. 42, 85, 120
- Rana, A. 99
- Randi, M.L. 56, 72, 122
- Rapezzi, D. 130, 156
- Raponi, S. 1, 18
- Rascato, M.G. 152
- Rasko, J. 1
- Raso, S. 117, 123, 124, 125
- Raspadori, D. 52, 61
- Rassidakis, G. 27
- Rattotti, S. 46
- Rauco, A. 73
- Rauco, A.M. 74
- Ravano, E. 43
- Ravetti, J.L. 79
- Re, A. 7, 27, 47, 48, 112, 114
- Re, F. 7, 18, 46, 48, 85, 86, 120, 145
- Recchia, A.G. 157
- Recher, C. 2
- Reda, G. 18, 38, 43, 100, 102, 104, 105
- Reddicono, G. 35, 145
- Rege Cambrin, G. 51, 140, 146
- Remondini, D. 36, 51, 91, 92, 93
- Rendicono, G. 68
- Renni, R. 58
- Renso, R. 33, 40
- Renzi, D. 103
- Resta, D. 112
- Ria, R. 69, 157
- Riboni, R. 114
- Ricardi, U. 28, 115
- Ricci, F. 39, 107
- Ricci, P. 58
- Ricci, R. 81
- Ricco, A. 15, 54, 107, 108, 135
- Ricerca, B.M. 26
- Riemma, C. 100, 101
- Rigacci, L. 29, 112, 155, 161
- Righetto, R. 173
- Righi, S. 93

- Rigolin, G.M. 100, 102, 104, 150, 172, 178
 Rinaldi, E. 125, 126, 161, 171, 173
 Rios, R. 9
 Risitano, A. 4, 45
 Risitano, A.M. 21, 25
 Ristow, K.M. 78
 Riva, G. 31
 Riva, M. 43, 49, 64, 89, 150, 161, 166
 Rivoli, G. 102, 117
 Rivolti, E. 2, 5, 56, 57, 71
 Rizzello, I. 10, 50, 66, 117
 Rizzi, R. 2, 68
 Rizzi, S. 84
 Rizzo, C. 126
 Rizzo, L. 108, 123, 140, 143
 Rizzo, M. 117
 Rizzo, S. 126
 Rizzo, V. 164
 Rizzuto, G. 27
 Robustelli, V. 36, 46, 92, 93
 Rocca, U. La 81
 Rocchi, M. 98
 Rocchi, S. 9, 10, 50, 66, 117
 Rocco, S. 118, 157
 Rodà, F. 98
 Rodeghiero, F. 5, 32, 54, 56, 57
 Rojas, R. 132
 Rollig, C. 11
 Romagnoli, S. 12, 14
 Romani, C. 54, 92
 Romani, L. 46
 Romano Gargarella, L. 98
 Romano, A. 51, 64, 67, 69, 70, 73, 74, 75, 77, 79, 107, 119
 Romano, I. 168
 Ronco, F. 95
 Ronconi, F. 129
 Ronconi, S. 10, 69, 97, 116, 118, 148, 163
 Rondoni, M. 35, 39, 40, 106
 Rosamilio, R. 76, 129, 153, 170, 171, 174
 Rosati, S. 43, 98, 100, 103, 108, 143
 Rosignoli, C. 131
 Rosinol, L. 9
 Rossi, A. 77, 114, 169
 Rossi, D. 18, 49
 Rossi, E. 32, 33, 56, 73, 74, 84, 121, 157, 184
 Rossi, F. 40, 111
 Rossi, G. 13, 29, 58, 59, 88, 89, 120, 141, 144, 164
 Rossi, P. 107
 Rossi, V. 90
 Rossini, M. 178, 206
 Rosti, G. 23, 51, 140, 141, 142, 146
 Rosti, V. 15, 121
 Rotondo, E. 167, 171
 Rotondo, F. 32, 56, 122, 149, 178
 Rottinghaus, S.T. 25
 Rotunno, G. 14
 Ruffini, A. 29
 Ruggeri, M. 5, 23, 56
 Rule, S. 7, 47
 Rupoli, S. 32, 51, 127, 140, 141, 168, 170
 Rusconi, C. 7, 47, 48, 112, 114
 Russo Rossi, A. 24, 51
 Russo Rossi, A.R. 146
 Russo Rossi, A.V. 108
 Russo, A. 24, 51, 123, 124, 125, 126, 145
 Russo, D. 3, 5, 20, 42, 51, 85, 97, 120, 138, 145
 Russo, F. 86
 Russo, S. 35, 51
 Rutella, S. 87
 Ruzzenenti, M.R. 79
 Sabatelli, S. 68
 Sabattini, E. 41, 60, 93
 Saccà, V. 122, 169, 172
 Saccardi, R. 21, 66, 69
 Saccenti, E. 172, 178
 Sacchi, N. 20
 Sacchi, S. 147
 Sacchini, D. 169
 Saccona, F. 140
 Saeli, A. 148, 167
 Saglio, G. 140, 141, 146
 Salehzadeh, S. 107
 Salek, S. 98
 Salemi, D. 35
 Salih, H.R. 11
 Salmoiraghi, S. 13
 Saltarelli, F. 165, 173
 Salutari, P. 12, 34
 Salvestrini, V. 136
 Salvi, S. 92, 93
 Salvini, M. 10
 Salvucci, M. 23, 146
 Salwender, H.J. 9
 Sammarelli, G. 81, 86
 Sammartano, V. 151
 Sampaolo, M. 62
 San-Miguel, J.F. 9
 Sancetta, R. 51, 150
 Sangaletti, S. 89
 Sanpaolo, G. 68
 Santambrogio, E. 79
 Santarone, S. 21
 Santeramo, M.T. 173
 Santeramo, T.M. 153
 Santi, R. 29, 112, 113
 Santini, V. 43, 54
 Santodirocco, M. 135
 Santoni, A. 61, 128
 Santopietro, M. 14
 Santoro, A. 35, 48, 92, 93, 94
 Santoro, C. 5, 32, 56, 57, 73, 74, 123
 Santoro, L. 92
 Santoro, M. 117, 123, 124, 125, 126, 153, 159
 Santoro, M.S. 173
 Sapienza, G. 67
 Saporiti, G. 20, 133
 Sardo, M. 123, 124, 125
 Sardo, S. 195
 Sarlo, C. 86, 95
 Sarris, A.H. 115
 Sartor, C. 36, 46, 58, 87, 134, 137, 152
 Sartori, R. 23, 47
 Sassi, M. 81, 86
 Sassone, M. 6, 47, 112
 Sau, A. 5, 106, 128, 167, 171
 Sauer, M. 1
 Saussele, S. 142
 Savino, E. 99
 Savoia, M. 159
 Sborgia, M. 11, 54
 Scaffidi, L. 3, 22, 23, 35, 39, 40, 206
 Scalzulli, E. 22, 43, 108, 123, 140, 143, 190
 Scapinello, G. 64, 111, 161, 166
 Scappini, B. 12, 112, 113
 Scarano, E. 6
 Scarciolla, O. 118
 Scardocci, A. 86
 Scarfò, L. 104
 Scarisbrick, J. 46
 Scarpellini, P. 151
 Scarsini, P. 157
 Scattolin, A.M. 13
 Scattoni, A. 113, 127
 Scheinberg, P. 7
 Scheliga, A. 7
 Schena, D. 40, 206
 Schiattone, L. 104, 160
 Schiavo, D. 50, 138
 Schiavotto, C. 77
 Schifano, C. 81, 86
 Schifone, C. 146
 Schiller, G. 1
 Schinco, P. 57
 Schinina, G. 70, 119
 Schinina, G. 79
 Schlenk, R.F. 11
 Schmitz, N. 11
 Schrezenmeier, H. 25
 Sciacovelli, A. 99, 158
 Sciarra, R. 7, 47
 Scimè, R. 96
 Scirè, E. 126
 Sciumè, M. 40, 89, 121
 Scomazzon, E. 78, 98, 102
 Scortechini, A.R. 32, 127, 168, 170
 Scortechini, I. 70
 Segreto, R. 81, 86
 Selleri, C. 45, 73, 76, 153, 170, 171, 174
 Semenzato, G. 3, 16, 17, 23, 37, 64, 65, 78, 98, 101, 102, 150, 161, 166
 Serana, F. 167
 Serio, A. 79
 Serio, B. 76, 95, 153, 170, 171, 174
 Serio, F. 151
 Serpenti, F. 44, 80
 Serra, M. 58
 Serreli, V. 109, 132
 Servadio, M. 55, 147
 Sessa, M. 20, 21, 84
 Severin, F. 37, 78, 98, 101
 Severino, A. 152
 Sgherza, N. 3, 16, 22, 24, 58, 107
 Shafii, B.M. 81
 Sharma, G.G. 63, 90
 Siakantaris, M.P. 27
 Sibilla, S. 68
 Sica, A. 7
 Sica, S. 21, 33, 35, 45, 82, 83, 84, 88, 133, 140, 158
 Sicuranza, A. 22, 24, 61, 128
 Signori, L. 112, 113
 Signorini, L. 29
 Silvestris, I. 38
 Simeon, V. 70

- Simeone, E. 138
 Simonetti, F. 157
 Simonetti, G. 51, 71, 89, 91, 92, 93, 135
 Simula, M.P. 22, 132
 Sinibaldi, M. 4, 28
 Siniscalchi, A. 117, 157
 Siragusa, S. 73, 117, 123, 124, 125, 126
 Sireci, F. 147
 Sirianni, S. 52
 Sivori, S. 4
 Skrypets, T. 158
 Smith, B.D. 142
 Soci, F. 57, 71
 Soddu, S. 18, 93
 Solfrizzi, M.P. 58, 107
 Solli, V. 9, 10
 Sommer, K. 53, 55, 96
 Soncini, E. 167
 Sonia, R. 126
 Sonneveld, P. 6, 9, 49, 69
 Sorà, F. 19, 35, 45, 82, 83, 84, 88, 133, 141, 146, 158
 Sorasio, R. 21, 156
 Soresina, A. 167
 Soriasio, R. 130
 Soriente, I. 147
 Soscia, R. 35
 Soulier, J. 36
 Soverini, S. 23, 35, 39, 106, 141, 146
 Spadano, A. 2, 64, 106, 128, 167, 171
 Spadano, R. 145
 Spadea, A. 5, 73, 74
 Spagnoli, A. 89
 Sparano, F. 54
 Specchia, G. 15, 18, 20, 58, 68, 96, 106, 107, 108, 130, 133, 135, 137, 140, 142, 145, 146, 157, 160, 164
 Spedini, P. 141, 144
 Sperotto, A. 50, 131, 132, 153
 Spina, A. 107, 125, 126, 171, 173, 178
 Spina, F. 141, 144
 Spina, M. 46, 97, 114
 Spina, V. 49
 Spinelli, M. 33
 Spinelli, O. 13, 20, 35, 94, 106
 Spinosa, G. 145
 Spiriti, A. Aloe 43
 Spirito, F. 74
 Spolzino, A. 17, 81, 86
 Sportoletti, P. 18, 100, 104
 Stabile, D. 170
 Staderini, M. 65, 66, 69, 71
 Stagno, F. 22, 24, 51, 140, 141, 146
 Stanzani, M. 152
 Statuto, T. 62
 Stefanelli, A. 157
 Stefani, P.M. 7, 47
 Stefanoni, P. 20, 114
 Stefoni, V. 8, 27, 37, 48, 61, 62, 77
 Steidl, C. 63, 90
 Stelitano, C. 18, 98, 100, 112
 Stella, R. 23, 131, 138
 Stelljes, M. 94
 Stocchi, F. 22
 Stocchi, R. 50, 157
 Stock, W. 94
 Storti, G. 12
 Storti, S. 115
 Strada, P. 79
 Strino, L. 110
 Stromberg, U. 142
 Stuart, R. 2
 Stulle, M. 23, 35
 Sucato, G. 159
 Sutto, E. 16, 56
 Svegliati Baroni, G. 32
 Szer, J. 25
 Tabacchi, E., 60
 Tacchetti, P. 6, 9, 10, 50, 64, 66, 117
 Tafuri, A. 12, 103
 Tafuri, M. 86, 129
 Tajana, M. 13
 Talami, A. 46, 87, 134, 152
 Tamagnini, E. 98, 155, 165
 Tammiso, E. 178
 Tanasi, I. 23, 36, 40
 Tani, M. 48, 115
 Tao, G. 1
 Tarantini, F. 107, 146
 Tarantini, G. 68, 153, 160, 173
 Tarantino, G. 32, 125, 159
 Tarantino, V. 46
 Targhetta, C. 132
 Tatò, D. 153
 Taurino, D. 19, 81, 82
 Tavarozzi, R. 107
 Tavazzi, D. 143
 Tebaldi, M. 92, 93, 206
 Tecchio, C. 7, 21
 Tedeschi, A. 46, 100, 102, 104, 181, 192
 Tedone, E. 79, 90, 137
 Tendas, A. 53
 Tenti, E. 23
 Teofili, L. 26, 33, 84
 Teramo, A. 17, 65
 Termini, R. 9, 10
 Terragna, C. 9, 10, 36, 37, 50, 64
 Terranova, C. 164
 Terrazzi, E. 133
 Terruzzi, E. 13
 Testoni, N. 9, 46, 50, 66, 92, 93, 117, 134, 137, 152
 Thieblemont, C. 47
 Thiede, C. 11
 Thompson, A. 1
 Thrasher, A. 1
 Thuret, I. 1
 Tiacchi, E. 17
 Tibullo, D. 64, 67, 70, 75, 79, 119
 Tichelli, A. 213
 Tieghi, A. 3, 12, 16, 41
 Tiribelli, M. 3, 16, 23, 51, 52, 140, 141, 146
 Tirindelli, M.C. 86
 Tisi, M. 7
 Tisi, M.C. 62
 Tocci, D. 24
 Todaro, G. 81, 86
 Todiere, A. 137
 Todisco, E. 13, 89
 Toffoletti, E. 153
 Toffoletti, E. 52
 Tognazzi, L. 71
 Tognolo, L. 178
 Tolomelli, G. 84, 122, 149
 Tomarchio, V. 86, 129
 Tomasi, P. 178
 Tomassetti, S. 122, 149
 Tomasso, A. 103, 105
 Tomei, G. 173
 Tommasi, S. 113
 Tommaso, R. De 51, 91
 Torresan Delamain, M. 46
 Torsello, G. 79
 Tosi, M. 35
 Tosi, P. 10, 39, 117, 122, 149
 Tota, G. 15, 106
 Trabacchi, E. 23, 146, 169
 Tralongo, P. 72
 Tramutoli, A. 110
 Trappolini, S. 67, 92
 Trasarti, S. 123
 Trastulli, F. 129
 Travaglini, S. 13
 Trawinska, M. 3, 16, 22, 73, 74
 Tregambe, D. 167
 Trentin, L. 17, 18, 37, 77, 78, 98, 100, 101, 102, 104, 105, 150, 161, 166
 Triantafyllou, P. 27
 Triggiani, M. 40
 Trimarco, V. 37, 65
 Tringali, S. 21
 Trino, S. 135, 138
 Tripepi, G. 157
 Tripodo, C. 113
 Trneny, M. 7
 Troia, R. 49
 Trojani, A. 141, 144
 Tucci, A. 115
 Tucker, D. 47
 Turra, A. 42, 85, 97, 120, 145
 Turrini, M. 122, 141, 144, 169, 172
 Tuzzo, D. 29
 Uccello, G. 157
 Uda, S. 100, 104, 156
 Urbano, M.A. 107, 146
 Urbino, I. 29, 88
 Urso, A. 172, 178
 Usai, M. 156
 Usala, E. 51, 146
 Ustun, C. 2
 Vacca, A. 21, 132, 133
 Vaccaro, E. 153
 Vadakekolathu, J. 87
 Vagge, S. 85
 Vago, L. 20, 131, 213
 Valent, P. 39
 Valenti, V. 169
 Valentini, C. 90
 Valentini, C.G. 26, 33
 Valeri, F. 32
 Vallerini, D. 31
 Valli, R. 29
 Vallisa, D. 81, 86, 169

- Valotto, L. 61
 Valvano, L. 62
 van de Poll-Franse, L. 55
 van der Holt, B. 49
 Van Lint, M.T. 45
 Vanazzi, A. 115
 Vandendries, E. 94
 Vannucchi, A.M. 12, 14, 40
 Varaldo, R. 45, 83
 Varettoni, M. 18, 46, 105
 Variami, E. 27
 Vassallo, F. 79
 Vassilakopoulos, T.P. 27
 Vecchio, V. 125
 Vedovato, S. 17, 65
 Vedruccio, F. 154, 162, 163, 166
 Vegliante, M.C. 113, 127
 Velardi, A. 21
 Venditti, A. 12, 13, 42, 52, 89, 90, 95
 Vento, G. 26
 Venturelli, D. 200
 Venturi, C. 46
 Venturini, B. 149
 Venza, P. 159
 Verga, L. 30, 31, 63
 Vergine, C. 68
 Vergoni, F. 112, 113
 Veroli, A. Di 52, 73, 74, 90
 Verri, M. 129
 Versari, A. 6
 Vertone, D. 110, 139, 143, 145, 170
 Vezzaro, T. 72
 Vianelli, N. 3, 5, 16, 30, 32, 41, 56, 57
 Vianello, F. 75, 161
 Vicentini, S. 28
 Vicenzetto, C. 17
 Vigna, E. 157
 Vignetti, M. 1, 11, 12, 18, 32, 34, 35, 53, 54, 94, 96, 123, 147
 Villa, M.R. 95, 104
 Villani, G. 76, 153, 170, 171, 174
 Villani, L. 15
 Villani, O. 62, 139, 143
 Villivà, N. 73, 74
 Vincelli, D. 5, 49, 121, 157
 Vincelli, I.D. 5, 49
 Vincenzi, B. 86
 Vinti, L. 4, 28
 Viola, N. 170
 Viqueira, A. 142
 Visceglie, D. 58
 Visco, C. 7, 46, 47, 155
 Viscovo, M. 116
 Visentin, A. 17, 37, 78, 98, 100, 101, 102, 105, 150, 161, 166
 Vita, G. 62
 Vitagliano, O. 47, 129
 Vitale, A. 34, 35, 92, 93
 Vitale, C. 100, 102
 Vitale, M. 15
 Vitali, S. 36, 92, 93
 Vitiello, S. 76, 124, 159
 Vitolo, R. 76
 Vitolo, U. 7, 28, 29, 47, 78, 79, 88, 115
 Vitucci, A. 58
 Viviani, S. 27, 77
 Vollono, L. 159
 Volpetti, S. 32, 48, 112
 Volta, E. 178
 Vona, G. 118
 Voso, M.T. 11, 12, 13, 42, 52, 90, 136
 Votino, C. 155
 Vozella, F. 117
 Wagner, S. 87
 Wang, T. 94
 Wattad, M. 11
 Watts, J. 142
 Weisel, K. 9
 Wester, R. 49
 Witzig, T.E. 78
 Wu, C. 7
 Wu, K.L. 49
 Xue, E. 44
 Xumerle, L. 39
 Yannikos, T. 27
 Yokoyama, H. 2
 Yoon, S. 2
 Young, J.R. 78
 Za, T. 33, 84, 117, 121
 Zacheo, I. 95, 118
 Zagaria, A. 15, 106, 146
 Zaja, F. 32, 57, 112
 Zaldini, P. 105
 Zallio, F. 20, 21, 45, 133
 Zamagni, E. 6, 9, 10, 49, 50, 64, 66, 117, 157
 Zambello, R. 2, 17, 64, 65, 66, 69, 112, 157, 161, 166
 Zamprogna, G. 181, 192
 Zangaglio, C. 42, 51, 85, 120, 145
 Zancanella, M. 122, 169, 172
 Zanelli, M. 29
 Zanghì, P. 13
 Zangrilli, I. 128
 Zanni, M. 48
 Zannier, M.E. 31, 75, 89, 138
 Zanotti, R. 39, 40, 77, 106, 206
 Zappalà, D. 38
 Zappasodi, P. 5
 Zappasoldi, P. 92
 Zappone, E. 151
 Zhang, H. 7
 Ziccheddu, B. 49
 Zilioli, V.R. 7
 Zini, G. 82, 213
 Zinzani, P.L. 8, 17, 18, 27, 37, 46, 60, 61, 62, 77, 175
 Zito, A. F. 113
 Zoli, V. 155
 Zollner, T. 42, 85, 97, 120, 145
 Zonta, A. 130
 Zuanelli Brambilla, C. 24, 151
 Zucano, S. 107
 Zucca, E. 115
 Zweegman, S. 9, 49