Special Conference

EACR AACR SIC

Anticancer Drug Action and Drug Resistance: from Cancer Biology to the Clinic

20-23 JUNE 2015

FLORENCE ITALY

CONFERENCE SECRETARIAT

c/o ECCO – the European CanCer Organisation
Avenue E. Mounier 83, B-1200 Brussels
EAS2015@ecco-org.eu

CONFERENCE VENUE

Firenze Fiera
(Florence Conference & Exhibition Centre)
Piazza Adua 1
50123 Firenze, Italy
Tel. +39 055 497 21
www.firenzefiera.it/en

EACR European Association for Cancer Research

AACR American Association for Cancer Research
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SOCIETA' ITALIANA DI CANCEROLOGIA

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www.ecco-org.eu/EAS2015

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PROCEEDINGS BOOK

EACR-AACR-SIC 2015

ORGANISING COMMITTEE

- Richard Marais (UK) - Conference Co-Chair
- Pasi Jänne (US) - Conference Co-Chair
- Riccardo Dolcetti (IT) - Conference Co-Chair
- Paola Chiarugi (IT)
- Silvia Giordano (IT)
- Moshe Oren (IL)
- Daniel Peeper (NL)
- Neil Shah (US)
- Alice Shaw (US)
- Robert Kenney (UK)
- Jeffrey M. Ruben (US)

EXECUTIVE SCIENTIFIC COMMITTEE

- Richard Marais (UK) - Conference Co-Chair
- Pasi Jänne (US) - Conference Co-Chair
- Riccardo Dolcetti (IT) - Conference Co-Chair
- Paola Chiarugi (IT)
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- Moshe Oren (IL)
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- Neil Shah (US)
- Alice Shaw (US)

EACR-AACR-SIC Special Conference 2015

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Acknowledgements

EACR, AACR and SIC would like to thank the following organisations for their generous support of the Conference:

SPONSOR

Roche

PROFESSIONAL EDUCATIONAL GRANTS

AstraZeneca
Pfizer Oncology

EACR, AACR and SIC express sincere thanks for the generous support of the organisations sponsoring Symposia, Keynote and Award Lectures.

EACR, AACR and SIC also wish to thank the following companies and organisations for their support of the Conference by taking part in the exhibition:

- Agilent Technologies
- ANGLE plc
- Crown BioSciences
- Ephoran Multi Imaging Solutions
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- Illumina
- LGC Standards
- Medical and Biological Laboratories
- Merck Millipore
- NanoString Technologies
- Silicon Biosystems
- TEMA RICERCA

Official Media Partner

We would like to acknowledge the collaboration and support of our official media partner:

Elsevier

Letter of Welcome

Dear Colleagues,

We are very pleased to welcome you to the EACR-AACR-SIC Special Conference: Anticancer Drug Action and Drug Resistance: from Cancer Biology to the Clinic.

EACR, AACR and SIC (Italian Cancer Society) have joined forces to organise this strong multidisciplinary meeting of cancer researchers and practising clinical experts as well as those focused on the development of new targeted therapeutics.

Inhibitors targeting mutant proteins have shown unprecedented impact on the rate and duration of clinical responses. Despite these results, most cancers treated with single agents rapidly acquire resistance through a variety of mechanisms, severely limiting clinical benefit.

This conference has presentations ranging across the identification of novel drug targets; non-oncogene addiction and synthetic lethality; discovery of drug resistance mechanisms; the development of combinatorial therapies; and related translational topics, across a wide spectrum of cancer types.

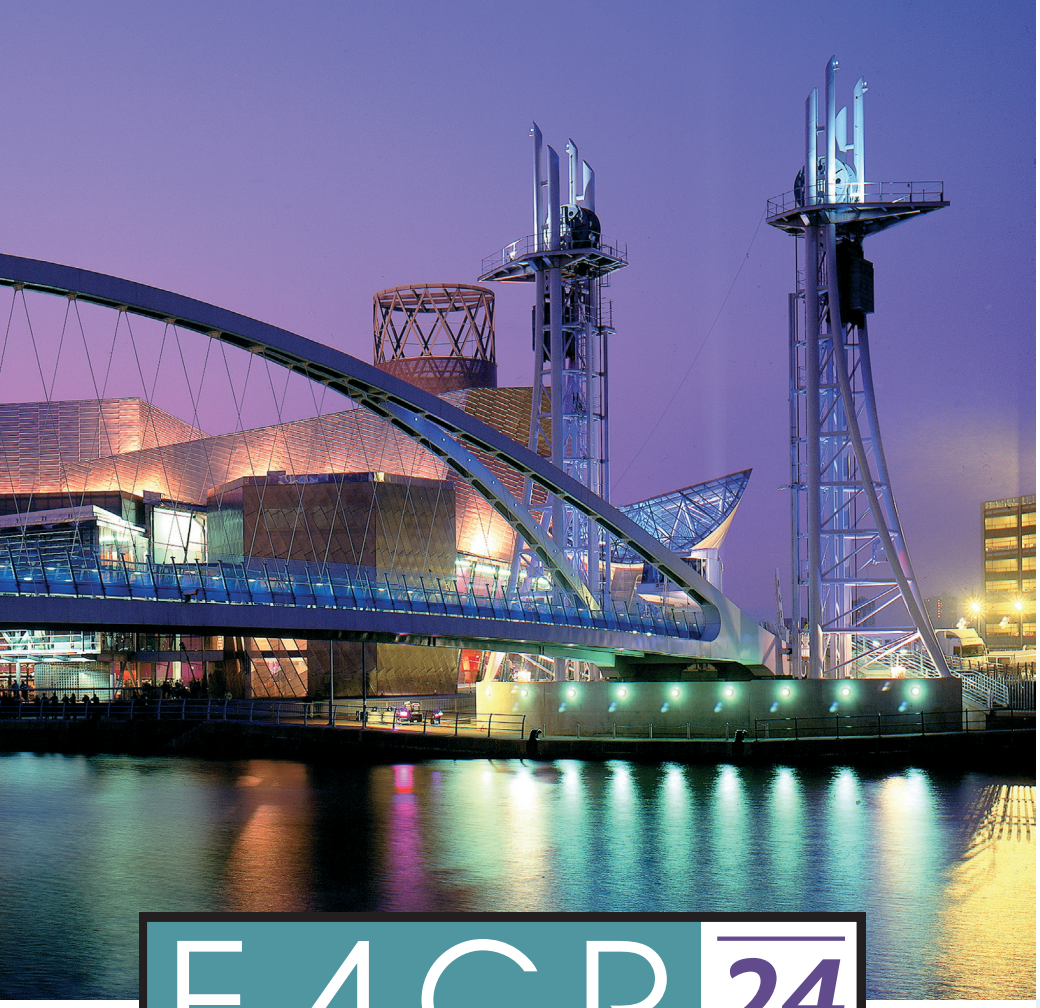
We trust you will share our excitement about the unique scientific programme of this meeting and the impressive attendance of experts from many countries around the world.

We thank you for choosing to take an active part in this ground-breaking conference and contribute your own invaluable expertise and unique perspective to its work.

Richard Marais,
Conference Co-Chair (EACR)

Pasi Jänne,
Conference Co-Chair (AACR)

Riccardo Dolcetti,
Conference Co-Chair (SIC)



EACR 24

9 – 12 July 2016 | Manchester, UK

24th Biennial Congress of the

European Association for Cancer Research

From Basic Research to Precision Medicine

EARLY SPEAKER CONFIRMATIONS

Visit us at the EACR booth to see the full list of confirmed speakers so far and to find out more about the Congress including bursaries.

2015-2016
SCIENTIFIC CONFERENCES

Presenting the most significant research on cancer etiology, prevention, diagnosis, and treatment

CRI-CIMT-EATI-AACR The Inaugural International Cancer Immunotherapy Conference: Translating Science into Survival
September 16-19, 2015 • New York, NY

Chromatin and Epigenetics in Cancer
Co-Chairpersons: Peter A. Jones, Sharon Y. R. Dent, and Charles W. M. Roberts
September 24-27, 2015 • Atlanta, GA

Advances in Breast Cancer Research
Co-Chairpersons: Matthew J. Ellis, Charles M. Perou, and Jane E. Visvader
October 17-20, 2015 • Bellevue, WA

Advances in Ovarian Cancer Research: Exploiting Vulnerabilities
Co-Chairpersons: Kathleen R. Cho, Douglas A. Levine, and Benjamin G. Neel
October 17-20, 2015 • Orlando, FL

Fourth AACR International Conference on Frontiers in Basic Cancer Research
Chairperson: M. Celeste Simon
Co-Chairpersons: James P. Allison, John E. Dick, Nathanael S. Gray, and Victor E. Velculescu
October 23-26, 2015 • Philadelphia, PA

The Basic Science of Sarcomas
Co-Chairpersons: Robert G. Maki, Angelo Paolo Dei Tos, Jonathan A. Fletcher, Lee J. Helman, and Brian A. Van Tine
November 3-4, 2015 • Salt Lake City, UT

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Scientific Committee Co-Chairpersons: Levi A. Garraway, Lee J. Helman, and Jean-Charles Soria
November 5-9, 2015 • Boston, MA

Advances in Pediatric Cancer Research: From Mechanisms and Models to Treatment and Survivorship
Co-Chairpersons: Scott A. Armstrong, Charles G. Mullighan, Kevin M. Shannon, and Kimberly Stegmaier
November 9-12, 2015 • Fort Lauderdale, FL

New Horizons in Cancer Research: Bringing Cancer Discoveries to Patients Shanghai 2015
Co-Chairpersons: Lewis C. Cantley and Carlos L. Arteaga
November 12-15, 2015 • Shanghai, China

Eighth AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Co-Chairpersons: John M. Carethers, Marcia R. Cruz-Correa, Mary Jackson Scroggins, Edith A. Perez, Beti Thompson, and Cheryl L. Willman
November 13-16, 2015 • Atlanta, GA

Developmental Biology and Cancer
Co-Chairpersons: Hans Clevers, Stuart Orkin, and Suzanne Baker
November 30-December 3, 2015 • Boston, MA

Tumor Metastasis
Co-Chairpersons: Bruce R. Zetter, Melody A. Swartz, and Jeffrey W. Pollard
November 30-December 3, 2015 • Austin, TX

CSHA/AACR Joint Meeting: Big Data, Computation, and Systems Biology in Cancer
Conference Organizers: Andrea Califano, William C. Hahn, Satoru Miyano, and Xuegong Zhang
December 1-5, 2015 • Suzhou, China

EORTC-NCI-EMA-AACR International Conference on Biomarkers in Cancer Drug Development
Co-Chairpersons: Denis A. Lacombe and John W. Martens
December 3-4, 2015 • Brussels, Belgium

Noncoding RNAs and Cancer
Co-Chairpersons: Howard Y. Chang, Jeannie T. Lee, and Joshua Mendell
December 4-7, 2015 • Boston, MA

San Antonio Breast Cancer Symposium
Co-Directors: Carlos L. Arteaga, Virginia Kaklamani, and C. Kent Osborne
December 8-12, 2015 • San Antonio, TX

AACR-IASLC Joint Conference on Lung Cancer
Co-Chairpersons: Alice T. Shaw and Karen L. Kelly
January 4-7, 2016 • San Diego, CA

The Function of Tumor Microenvironment in Cancer Progression
Co-Chairpersons: Raghu Kalluri, Robert A. Weinberg, Douglas Hanahan, and Morag Park
January 7-10, 2016 • San Diego, CA

Patient-Derived Cancer Models: Present and Future Applications from Basic Science to the Clinic
Co-Chairpersons: Manuel Hidalgo, Hans Clevers, S. Gail Eckhardt, and Joan Seoane
February 11-14, 2016 • New Orleans, LA

10th AACR-JCA Joint Conference Breakthroughs in Cancer Research: From Biology to Therapeutics
Co-Chairpersons: Frank McCormick and Tetsuo Noda
February 16-20, 2016 • Maui, HI

The Cancer Cell Cycle: Tumor Progression and Therapeutic Response
Co-Chairpersons: Julien Sage, J. Alan Diehl, and Karen E. Knudsen
February 27-March 2, 2016 • Orlando, FL

AACR Annual Meeting 2016
Program Committee Chairperson: Scott Armstrong
April 16-20, 2016 • New Orleans, LA

Learn more and register at www.AACR.org/Calendar

AACR American Association for Cancer Research

FINDING CURES TOGETHER℠

SOCIETA' ITALIANA DI CANCEROLOGIA

58TH ANNUAL MEETING OF THE ITALIAN CANCER SOCIETY

VERONA, 7-10 SEPTEMBER 2016

REVOLUTIONARY ROAD

ACCELERATING CONVERSION OF CANCER BIOLOGY INTO PERSONALIZED CLINICAL ONCOLOGY

Dear colleagues,

We are pleased to invite you to the 58th Annual Meeting of the Italian Cancer Society (SIC), which will be held in Verona, 7-10 September 2016.

The most recent findings in basic, translational and clinical cancer research will be presented as lectures, oral presentations and poster discussions.

The presence of outstanding national and international experts, sharing their latest results in basic and discovery-driven translational cancer research, will provide an exciting environment for both senior and young investigators, who will benefit from this excellent opportunity to establish collaborations with other investigators and young SIC members.

The participation of the younger colleagues will be supported by the “Piero Trivella” Awards for the best poster presentations, by the “Elena Cappannini” Award for the best 2015 publication, and by Pezcoller Foundation scholarship.

Traditionally, the lecture in memory of Prof. Giorgio Prodi will be given by an internationally recognized Italian investigator.

The Meeting will take place at the “Polo Zanotto” Congress Center at the University of Verona. This location is very close to the city centre, at walking distance from the most famous historical and cultural attractions in Verona, such as Piazza Erbe, the Giulietta’s balcony and the “Arena”.

We look forward to welcoming you all in Verona,

Davide Melisi and Giampaolo Tortora

Accreditation Information

Italian Continuing Medical Education (CME): 1783-130228

Accreditation Statement: We applied for CME accreditation (Italian Physicians only) for the following disciplinary areas: Medical Surgeon *(Disciplines: Haematology, Medical Genetics, Pathological Anatomy, Pharmacology and Clinical Toxicology, Clinical Pathology, Internal Medicine, Clinical Biochemistry, Oncology)*; Biologist, Pharmacist, Chemist *(Discipline: Analytical Chemistry)*; Physicist *(Discipline: Health Physics)*; Veterinary Surgeon. **Credit Designation Statement:** No. 4,4 Italian Ministry of Health CME *(Continuing Medical Education)* credits have been assigned with a participation of 22 hours. **Claiming CME Credit:** Participants who wish to claim Italian CME accreditation, need to be registered to the conference. At the conference venue in Florence they should show their badge at the **Italian CME desk** (staffed by Adria Congrex) where they will get access to the CME form and will need to sign the attendance sheet. **Questions about CME:** For any further questions kindly contact the Italian CME desk at the venue.

AACME Accreditation Information - Continuing Medical Education (CME)

ACCREDITATION STATEMENT

The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education activities for physicians.

CREDIT DESIGNATION STATEMENT

AACR has designated this live activity for a maximum of 24.0 *AMA PRA Category 1 Credit(s)™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Credit certification for individual sessions may vary, dependent upon compliance with the ACCME Accreditation Criteria. The final number of credits may vary from the maximum number indicated above.

CLAIMING (CME) CREDIT

Physicians and other health care professionals seeking *AMA PRA Category 1 Credit(s)™* for this live continuing medical education activity must complete the CME Request for Credit Survey. Certificates will only be issued to those who complete the survey. The Request for Credit Survey is available by accessing the link below.

ecco-org.eu/EAS2015.

STATEMENT OF EDUCATIONAL NEED, TARGET AUDIENCE, AND LEARNING OBJECTIVES

With the availability of new technologies, including genome-wide knockdown and knockout libraries, proteomics, metabolomics and next-generation sequencing, the gap between basic research, clinical research, and drug development is decreasing. This conference will not only present the latest achievements of multidisciplinary research dealing with drug action and resistance but also highlight remaining key challenges that require the concerted efforts of an array of specialists to be solved. A clinically relevant and well-studied example is BRAF mutant melanoma. Although its treatment has improved recently, no curative therapy is available. Clinically validated driver genes include the mutant oncoproteins BRAF. The discovery of the common BRAFV600E mutation in melanoma has spurred the development of targeted therapies with significant clinical benefits. Vemurafenib, a drug targeting BRAFV600E, has become the first targeted therapy for patients diagnosed with mutant BRAF metastatic melanoma. However, although this compound or other drugs targeting other components of the MAPK pathway initially reduce tumor burden, eventually all melanomas become resistant and patients succumb to the disease. Drug resistance is caused by a plethora of mechanisms, both MAP kinase-dependent and –independent, making it virtually impossible to design an effective therapy from which all patients would benefit. There are currently ongoing clinical trials in which melanoma patients are treated simultaneously with BRAF and MEK inhibitor. Although responses are promising, resistance can eventually still occur. Therefore, it is important to identify novel therapeutic targets that can be used in the treatment of melanoma patients in combination with existing therapies. The perspectives and challenges of targeted melanoma treatment have become a showcase and are representative of targeted therapy across a wide tumor spectrum. Finally, there have been unprecedented improvements in immunotherapies, opening the avenue to develop combinatorial targeted and immune treatment.

After participating in this CME activity, physicians should be able to:

1. Assess genetic, proteomic, metabolic and functional oncogenomic shRNA and CRISPR screenings for identification of novel drug targets.
2. Analyse the discovery of drug resistance mechanisms.
3. Interpret the development of combinatorial therapies, including immuno- and targeted therapies, across a wide spectrum of cancer types.
4. Articulate non-oncogene addiction and synthetic lethality.

DISCLOSURE STATEMENT

It is the policy of the AACR that the information presented at AACR CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, AACR will provide information that Scientific Programme Committee members and speakers have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity. The disclosure information follows on pages 8 & 9..

ACKNOWLEDGMENT OF FINANCIAL OR OTHER SUPPORT

This activity is supported by professional educational grants from AstraZeneca and Pfizer. Any others will be disclosed at the activity.

QUESTIONS ABOUT CME?

Please review the CME related FAQ section on the conference website or contact the Office of CME at +1 (215) 440-9300 or *cme@aacr.org*.

DISCLOSURE OF FINANCIAL RELATIONSHIPS

In compliance with the standards set by the Accreditation Council for Continuing Medical Education (ACCME), it is the policy of the American Association for Cancer Research (AACR) that the information presented at CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, the AACR has provided information that programme committee members, speakers, and abstract presenters have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity.

Relationships are abbreviated as follows:

E Employee of listed company
G Grant/research support recipient
A Advisor or review panel member
C Consultant
S Stock Shareholder
SB Speakers' Bureau
H Honoraria
O Other

LAST NAME	FIRST NAME	COMPANY	RELATIONSHIPS	TYPE	ROLE
Allavena	Paola	**Istituto Clinico Humanitas**	No Relationships		Speaker
Allison	James	**UT MD Anderson Cancer Center**	Bristol Meyers-Squibb, Jounce Therapeutics	**C,O**	Speaker
Ambrogio	Chiara	**Spanish National Cancer Research Centre**	No Relationships		Speaker
Apicella	Maria	**University of Turin**	No Relationships		Speaker
Arthur	Ronald	**American Association for Cancer Research**	No Relationships		Programme Committee
Barbacid	Mariano	**Spanish National Cancer Research Centre**	No Relationships		Speaker
Bardelli	Alberto	**IRCC - University of Turin, Medical School**	Horizon Discovery, Trovagene, Biocartis	**A,S**	Speaker
Barila	Daniela	**University Tor Vergata**	No Relationships		Speaker
Baselga	José	**Memorial Sloan Kettering Cancer Center**	No Relationships		Speaker
Berns	Anton	**Netherlands Cancer Institute**	No Relationships		Speaker
Brodt	Pnina	**McGill University Health Centre**	No Relationships		Speaker
Brown	Melissa	**The University of Queensland**	No Relationships		Speaker
Bruna	Alejandra	**Cancer Research UK**	No Relationships		Speaker
Carmeliet	Peter	**Catholic University of Leuven**	No Relationships		Speaker
Chiarugi	Paola	**University of Florence**	No Relationships		Programme Committee, Speaker
Clarke	Paul	**Institute of Cancer Research**	Genentech	**O**	Speaker
Colombo	Mario	**Fondazione IRCCS Instituto Nazionale Tumori**	No Relationships		Speaker
Coussens	Lisa	**OHSU Knight Cancer Institute**	No Relationships		Speaker
D'Incalci	Maurizio	**IRCCS**	OncoEthix	**A**	Speaker
Defilippi	Paola	**University of Turin**	No Relationships		Speaker
Di Agostino	Silvia	**Regina Elena National Cancer Institute**	No Relationships		Speaker
Di Nicolantonio	Federica	**FPRC Onlus**	No Relationships		Speaker
Dive	Caroline	**CRUK Manchester Institute**	Astex, AstraZeneca, BI, Parsortix, Silicon Biosystems	**G,A,H**	Speaker
Dolcetti	Riccardo	**CRO-IRCCS, Natl. Cancer Institute**	No Relationships		Programme Committee, Speaker
Ekmekcioglu	Suhendan	**UT MD Anderson Cancer Center**	No Relationships		Speaker
Esteller	Manel	**Institut d'Investigació Biomèdica de Bellvitge**	No Relationships		Speaker
Evan	Gerard	**University of Cambridge**	Astra-Zeneca, Genentech Inc., Syros Inc.	**A**	Speaker
Fruci	Doriana	**Ospedale Pediatrico Bambino Gesu**	No Relationships		Speaker
Furnari	Frank	**Ludwig Institute for Cancer Research**	No Relationships		Speaker
Giamas	Georgios	**Imperial College London**	No Relationships		Speaker
Giordano	Silvia	**Fondazione del Piemonte per l'Oncologia**	No Relationships		Programme Committee
Ilic	Nina	**Dana-Farber Cancer Institute**	No Relationships		Speaker
Jänne	Pasi	**Dana-Farber Cancer Institute**	BI, Astra-Zeneca, Pfizer, Genentech, Merrimack Pharmaceuticals, Clovis Oncology, Sanofi, Chugai, Gatekeeper Pharmaceuticals, LabCorp	**C,S,O**	Programme Committee, Speaker

Kenney	Robert	**European Association for Cancer Research**	No Relationships		Programme Committee
Lengauer	Christoph	**Blueprint Medicines**	Blueprint Medicines	**E**	Speaker
Lorenzatti Hiles	Guadalupe	**University of Michigan**	No Relationships		Speaker
Luzzatto	Lucio	**Istituto Toscano Tumori**	No Relationships		Speaker
Maity	Amit	**University of Pennsylvania School of Medicine**	No Relationships		Speaker
Marais	Richard	**Cancer Research UK Manchester Institute**	No Relationships		Programme Committee, Speaker
Mardis	Elaine	**Washington University School of Medicine**	No Relationships		Speaker
Masgras	Ionica	**Universita degli Studi di Padova**	No Relationships		Speaker
Miele	Evelina	**University of Rome La Sapienza; Istituto Italiano di Tecnoligia**	No Relationships		Speaker
Narla	Goutham	**Case Western Reserve University**	Dual Therapeutics	**G,O**	Speaker
Oren	Moshe	**Weizmann Institute of Science**	No Relationships		Programme Committee, Speaker
Ottolini	Barbara	**University of Leicester**	No Relationships		Speaker
Pandolfi	Silvia	**Istituto Toscano Tumori**	No Relationships		Speaker
Peeper	Daniel	**Netherlands Cancer Institute**	No Relationships		Programme Committee, Speaker
Porporato	Paolo	**Catholic University of Louvain School of Medicine**	No Relationships		Speaker
Pouyssegur	Jacques	**Centre Antoine Lacassagne**	No Relationships		Speaker
Ravindran Menon	Dinoop	**Translational Research Institute**	No Relationships		Speaker
Roato	Ilaria	**Ospedale S. Giovanni Battista Molinette**	No Relationships		Speaker
Robert	Caroline	**Gustave Roussy**	Roche, BMS, GSK, Amgen, Novartis, Merck	**C**	Speaker
Rothman	Jeffrey	**Memorial Sloan Kettering Cancer Center**	No Relationships		Speaker
Ruben	Jeff	**American Association for Cancer Research**	Abbott, Dupont, J&J, Merck, Teva, Gilead, Pfizer	**S**	Programme Committee
Schumacher	Ton	**Netherlands Cancer Institute**	No Relationships		Speaker
Shah	Neil	**UCSF School of Medicine**	BMS, Ariad, Pfizer, Plexxikon, Daiichi-Sankyo	**G**	Programme Committee, Speaker
Shaw	Alice	**Massachusetts General Hospital Cancer Center**	Ariad, Chugai, Genentech, Ignyta, Novartis, Pfizer, Roche	**A,C,H**	Programme Committee, Speaker
Sistigu	Antonella	**Regina Elena Cancer Institute**	No Relationships		Speaker
Slack	Frank	**BIDMC Cancer Center/Harvard Medical School**	Mira DX, Mirna Therapeutics	**A**	Speaker
Straussman	Ravid	**Weizmann Institute of Science**	No Relationships		Speaker
Swanton	Charles	**University College London**	Hospitals and Cancer Institute SAB of APOGEN biotechnologies		Speaker
Topalian	Suzanne	**Johns Hopkins Kimmel Comprehensive Cancer Center**	Bristol-Myers Squibb, Five Prime Therapeutics, GlaxoSmithKline, Jounce Therapeutics, MedImmune	**G,C,O**	Speaker
Trono	Paola	**Regina Elena National Cancer Institute**	No Relationships		Speaker
Tuting	Thomas	**University of Bonn**	No Relationships		Speaker
Vander Heiden	Matthew	**MIT Koch Institute for Integrated Cancer Research**	Agios Pharmaceuticals	**A,S**	Speaker
Venkitaraman	Ashok	**Hutchison/MRC Research Centre**	Phroremost Therapeutics, Sentinel Oncology, Astex Therapeutics	**C,A**	Speaker
Vousden	Karen	**Beatson Institute for Cancer Research**	Astex, PMV Pharma	**G,A**	Speaker
Zitvogel	Laurence	**Gustave Roussy Cancer Center**	No Relationships		Speaker

General Information

CONFERENCE SECRETARIAT

c/o ECCO – the European CanCer Organisation
Avenue E. Mounier 83, B-1200 Brussels
EAS2015@ecco-org.eu

CONFERENCE VENUE

Firenze Fiera
(Florence Conference & Exhibition Centre)
Piazza Adua 1
50123 Firenze, Italy
Tel. +39 055 497 21
www.firenzefiera.it/en

APP

All attendees can download the free ECCO App on iPhone, iPad, or Android supported devices. Features include EACR-AACR-SIC related information and news. The App contains the complete list of scientific sessions, session types, speakers, exhibitors, and satellite symposia. Users can save their selected sessions, notes, favourites, as well as exporting sessions to their smartphone calendar. To download the App, search for ECCO cancer on iTunes or Google Play.

Learn more at *www.ecco-org.eu/app* or use the QR code below for direct download.

We gratefully acknowledge Celgene and Roche as sponsors of the App.

BADGES

For security reasons, delegates are requested to wear their badge at all times during the conference. Delegates having lost their badge can obtain a new badge at the registration desk. A replacement fee of 75 EUR per participant will be charged.

CATERING

Coffee Breaks

Coffee breaks, courtesy of the organisers, have been scheduled as follows:

SATURDAY 20 JUNE	15:30–16:00
SUNDAY 21 JUNE	10:15–10:45
	16:15–16:45
MONDAY 22 JUNE	10:15–10:45
	16:15–16:45
TUESDAY 23 JUNE	10:00–10:30

Lunches

Lunches, courtesy of the organisers will be offered to delegates at the following times:

SUNDAY 21 JUNE	12:30 – 14:30
MONDAY 22 JUNE	12:30 – 14:30

All delegates are invited to attend the official EACR-AACR-SIC 2015 **Conference Welcome Reception** to enjoy networking with peers and some light refreshments – this reception will be held on **Saturday 20 June, 18:00 – 19:30.**

All catering will be served in the exhibition area. Free water dispensers are available throughout the Conference Centre.

We gratefully acknowledge Roche as sponsor of the water dispensers.

CERTIFICATE OF ATTENDANCE

Certificates of Attendance will be accessible upon completion of an online Conference Satisfaction Survey. On the last day of the conference you will receive an email link to the questionnaire which also provides the link for you to print your Certificate of Attendance.

We kindly ask you to keep your Congress badge as you will need the unique badge code to print your Certificate of Attendance.

The Conference Secretariat will not mail Certificates of Attendance to participants after the Conference.

For information on CME accreditation see page 7.

CITY INFORMATION

All delegates will receive practical information about Florence, including a city map, in their conference bag. Delegates are also invited to download the free Florence Congress Card which provides special offers and discounted fees for museums, restaurants, car rental, taxis and other services. *www.bit.ly/FCBcard*

CLOAKROOM

A cloakroom is located in room 5 on the ground floor.

Cloakroom Opening Hours

SATURDAY 20 JUNE	10:00 – 20:00
SUNDAY 21 JUNE	06:30 – 20:00
MONDAY 22 JUNE	06:30 – 19:30
TUESDAY 23 JUNE	07:30 – 14:00

CONFERENCE DINNER: MONDAY 22 JUNE 20:00

A seated dinner will take place at Palazzo Borghese. Join us at this unique venue for a warm and friendly networking evening.

The dinner is accessible for all delegates who have a ticket. Price per person: 65 EUR. A limited number of tickets may be for sale at the registration desk at the Conference Centre (not onsite at the dinner venue). Ticket holders will be asked to present their ticket upon arrival at the venue.

EXHIBITION

The exhibition will be held in the Passi Perduti area located around the Auditorium of the Conference Centre on level -1. Entrance is free for registered delegates but limited to researchers, oncology professionals, press and exhibitors.

Exhibition Opening Hours

SATURDAY 20 JUNE	15:30–19:30
SUNDAY 21 JUNE	10:15–17:00
MONDAY 22 JUNE	10:15 - 17:00

For the exhibition floorplan and list of exhibitors, please see the exhibition section (page 14) of this Proceedings Book.

FIRST AID

No first aid room is available in the Conference Centre. In case of medical emergency, please refer to the registration desk at the entrance of the congress centre.

INSURANCE

The organisers do not accept liability for individual medical, travel or personal insurance. Participants are strongly recommended to obtain their own personal insurance policies. The organisers of the EACR-AACR-SIC Special Conference 2015 accept no responsibility for loss due to theft or negligence.

INTERNET WI-FI ACCESS

General Wi-Fi access is available throughout the Conference Centre. For access, activate the Wi-Fi network on your laptop or device, select the network listed as EAS2015, and enter the user name and password EAS2015.

We gratefully acknowledge Roche as sponsor of the Internet WI-FI access.

INTERNET ZONE

The official Conference Internet Zone is available free of charge during the Conference. The terminals provide you with the following services: internet browsing, access to web-based mail, the congress searchable programme and exhibitor information.

We gratefully acknowledge Roche as sponsor of the Internet Zone.

LANGUAGE & TRANSLATION

The official language of the Conference is English. Translation is not provided.

LOST & FOUND

All enquiries should be directed to the registration helpdesk in the entrance hall. The organisers accept no responsibility for loss due to theft or negligence.

POSTER SESSIONS

Each poster will be on display for one day in the dedicated poster areas: on the mezzanine level above the exhibition, and in the Limonaia building (Sunday or Monday, across the various topics, for details please refer to the Scientific Programme).

Poster presenters will be able to mount their poster on the day their poster is to be presented as of 08:30. Posters must be removed by 18:15 on the day the poster was presented. Any posters remaining after this time will be removed by the organisers and cannot be reclaimed.

Registration

The EACR-AACR-SIC Special Conference 2015 is open to all registered participants. Your official name badge is required for admission to the Conference Centre and all conference events. For security reasons, participants are requested to wear their badge at all times.

Registration Opening Hours

SATURDAY 20 JUNE	08:30 - 19:00
SUNDAY 21 JUNE	06:30 - 18:00
MONDAY 22 JUNE	06:30 - 18:00
TUESDAY 23 JUNE	07:30 - 12:00

REGISTRATION PACKAGE

The full conference registration package includes:

- Entry to all scientific sessions and Satellite Symposia;
- Entry to the exhibition (restricted to researchers, oncology professionals and media);
- Proceedings Book;
- Coffee breaks and lunches, as well as the Opening reception on Saturday 20 June;
- Wi-Fi access in the Conference Centre and access to the Internet Zone terminals;
- Conference bag including a city map.

The day registration package includes:

- Access to all scientific sessions and Satellite Symposia on that day;
- Entry to the exhibition (restricted to researchers, oncology professionals and media);
- Proceedings Book (subject to availability);
- Coffee breaks and/or lunches on that day;
- Wi-Fi access in the Conference Centre and access to the Internet Zone terminals;
- Conference bag including a city map (subject to availability).

SATELLITE SYMPOSIA

Two satellite symposia are taking place during the EACR-AACR-SIC Special Conference. For details, please see page 17.

SIC GENERAL ASSEMBLY: SUNDAY 21 JUNE 19:00

The General Assembly of SIC will be held in Sala Verde of the Congress Centre. This event is open to SIC members only.

SOCIAL MEDIA

Twitter is available during the conference – tweet, network and follow updates using hashtag *#EAS2015*. Find links, tutorials and tips: *www.ecco-org.eu/social*

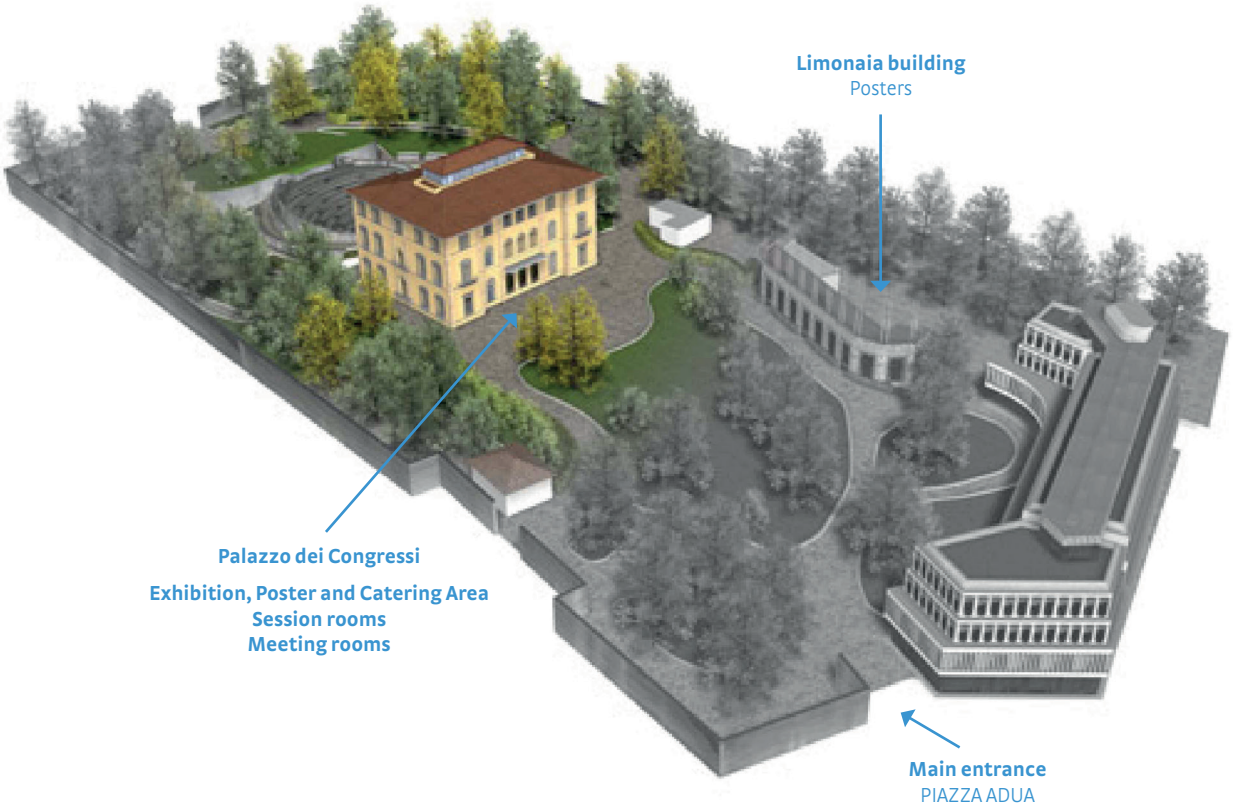
SPEAKER PREVIEW ROOM

The Speaker Preview Room is located in **room 11** (ground floor). Speakers are requested to bring their PowerPoint presentations to the Speaker Preview Room at least 4 hours before their session starts or one day in advance if the session starts early in the morning. Session rooms are not equipped for laptop presentations.

Speaker Preview Room Opening Hours

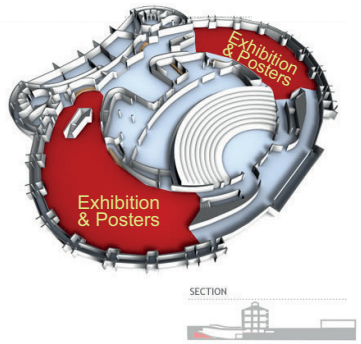
SATURDAY 20 JUNE	10:00 – 17:30
SUNDAY 21 JUNE	06:30 – 19:00
MONDAY 22 JUNE	06:30 – 18:00
TUESDAY 23 JUNE	07:30 – 13:00

Venue Floorplans

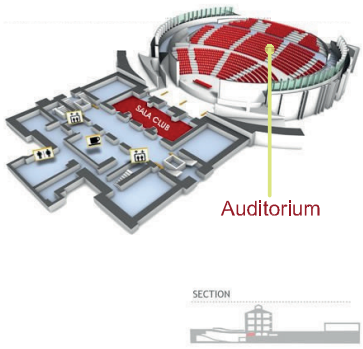


PALAZZO DEI CONGRESSI

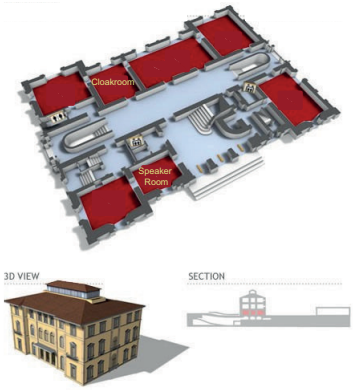
LOWER FLOOR



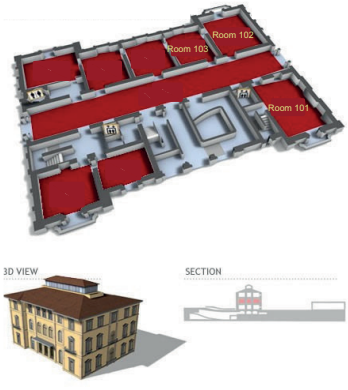
LOWER FLOOR



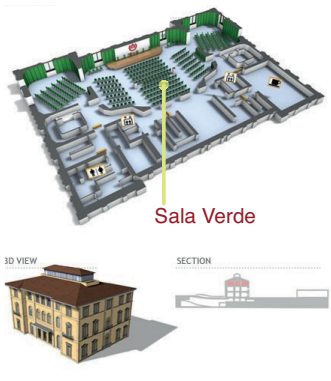
GROUND FLOOR



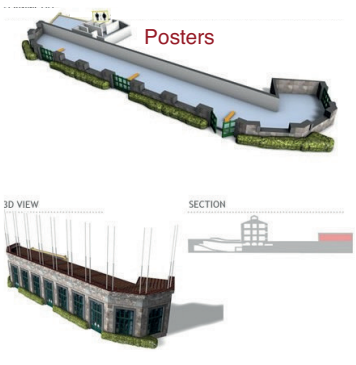
FIRST FLOOR



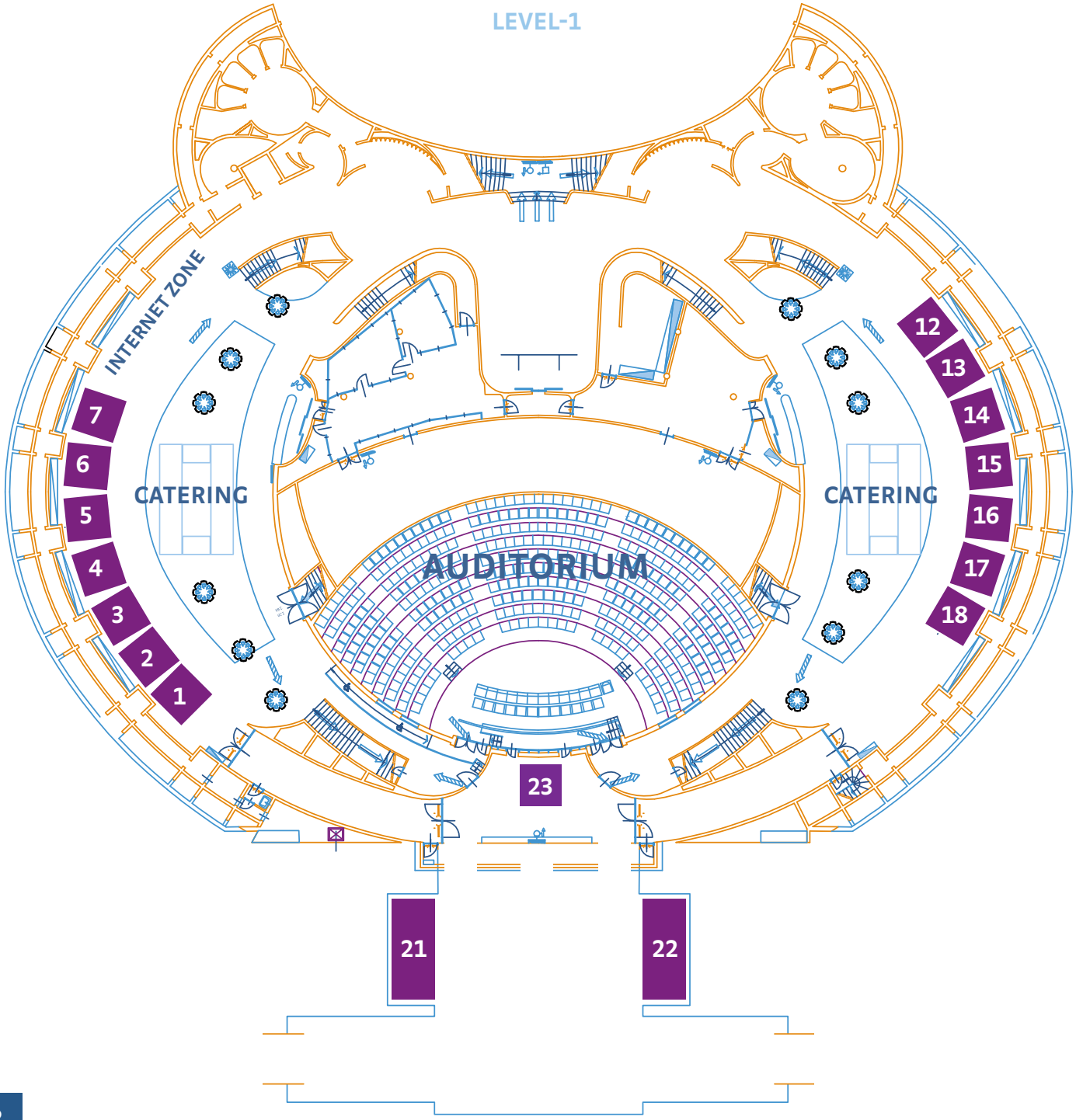
SECOND FLOOR



LIMONAIA



Exhibition Floorplan



Exhibition

LIST OF EXHIBITORS*	Booth number
Agilent Technologies	7
American Association for Cancer Research (AACR)	22
ANGLE plc	18
Crown BioSciences	6
Ephoran Multi Imaging Solutions	3
European Association for Cancer Research (EACR)	21
Fujifilm Visualsonics	4
Illumina	15
LGC Standards	13
Medical and Biological Laboratories	17
Merck Millipore	14
NanoString Technologies	5
Silicon Biosystems	16
Societá Italiana di Cancerologia (SIC)	23
TEMA RICERCA	12

**This list reflects confirmed exhibitors until 22 May 2015*

Exhibitor Profiles

Booth number

Agilent Technologies — 7

www.agilent.com

Agilent Technologies is a leading genomics solutions provider of NGS and microarrays allowing the ability to utilise an integrated workflow from sample preparation to data analysis that enables scientists to study complex biological processes and disease mechanisms, revolutionising cancer research.

American Association for Cancer Research (AACR) — 22

www.aacr.org

The mission of the AACR is to prevent and cure cancer through research, education, communication, and collaboration. Through its programmes and services, the AACR fosters research in cancer and related biomedical science; accelerates the dissemination of new research findings among scientists and others dedicated to the conquest of cancer; promotes science education and training; and advances the understanding of cancer etiology, prevention, diagnosis, and treatment throughout the world.

ANGLE plc — 18

www.angleplc.com

ANGLE is a UK and US based med tech. ANGLE is commercialising its Parsortix technology for the capture and recovery of circulating tumour cells (CTCs) from blood. The resulting liquid biopsy (simple blood test) allows the genetic and protein analysis of the patient's cancer for personalised cancer care.

Crown BioSciences — 6

www.crownbio.com

We provide the comprehensive Translational Platform for Oncology leveraging the world's largest collections of Patient-Derived Xenograft models (HuPrime), in vitro to in vivo human cell line and immuno-oncology models, searchable databases of in vivo models fit for efficacy, and the capacity to perform 100s of in vivo studies in parallel.

Ephoran Multi Imaging Solutions — 3

www.ephoran-mis.com

EPHORAN Multi-Imaging Solutions is a Contract Research Organisation providing imaging expertise and knowledge to study and develop state-of-the-art preclinical imaging for assessing, accelerating and guiding the development of new diagnostic and therapeutic options. Capabilities include access to MRI (1,3,7T), PET/SPECT/CT, High-frequency Ultrasound, Photo-Acoustic, Bioluminescence and Fluorescence imagers and dedicated contrast agents.

European Association for Cancer Research (EACR) — 21

www.eacr.org

The European Association for Cancer Research (EACR) is the largest member society for cancer research in Europe and has a membership of over 9,500. In seeking to advance cancer research, EACR supports its members through a wide range of activities, scientific meetings and other opportunities for communication and interaction.

Booth number

Fujifilm Visualsonics 4

www.visualsonics.com

VisualSonics is the world's leading developer of high-resolution, ultrasound-based, in vivo micro-imaging systems designed specifically for non-invasive preclinical research. The company's enabling technology allows researchers at the world's most prestigious pharmaceutical and biotechnology companies, hospitals and universities to conduct research in cardiovascular, cancer, and developmental biology areas.

Illumina 15

www.illumina.com

At Illumina, our goal is to apply innovative sequencing and array technologies in order to better understand cancer genomics. Our simple, sample-to-data solutions deliver highly accurate, reproducible results for the discovery and validation of cancer variants and epigenetic modifications, which are helping to advance cancer research and make the realisation of personalised medicine possible.

LGC Standards 13

LGC exclusive partnership with ATCC provides efficient access to ATCC's biological resources to scientists throughout Europe. ATCC's unique collections now include panels of cancer cell lines by tissue type or genetic alteration, hTERT-immortalised cell lines and primary cell solutions. Now perform better research with ATCC materials!

Medical and Biological Laboratories 17

www.mblintl.com

Medical and Biological Laboratories Co., Ltd. develops, manufactures, and markets high quality products and solutions for diagnostics and life science research. In this meeting, we more focus on two innovative products, which provide high throughput RAS mutation testing (MEBGEN RASKET KIT) and analyses of antigen-specific T-cell function (MHC tetramers).

Merck Millipore 14

www.merckmillipore.com

Merck Millipore - a division of Merck KGaA- is a leading supplier to the global life science industry, offering a broad range of innovative products and services used in the research, development and production of biotech and pharmaceutical drugs through its three business units.

NanoString Technologies 5

www.nanostring.com

NanoString Technologies provides life science tools for translational research and molecular diagnostic products. The company's nCounter® Analysis System, which has been employed in basic and translational research and cited in 500 peer review publications, has also now been applied to diagnostic use with the nCounter Dx Analysis System and uses the nCounter-based Prosigna™ Breast Cancer Prognostic Gene

Silicon Biosystems 16

wwww.siliconbiosystems.com

Silicon Biosystems has developed an instrument that sorts and collects individual or groups of cells. Using a chip-based microfluidic cartridge and microscopic image analysis the DEPArray™ system recovers also ONE single cell from a suspension of tens of thousands of cells with 100% purity. Applications spans from characterisation of CTC, FFPE, stem cells and many others.

Società Italiana di Cancerologia (SIC) 23

www.cancerologia.it

Società Italiana di Cancerologia (SIC) is the first association of its kind to be established in Italy (1952). The society unites scientists and experts in the areas of experimental and clinical oncology. SIC organises an annual conference and other targeted events in oncology in order to foster knowledge sharing, innovation and scientific collaboration.

TEMA RICERCA 12

TEMA RICERCA is a leading distributor of cutting edge life science research products. Our goal is to identify the most exciting new life science technologies and bring these to our customers, who work in all areas of biological research.

Satellite Symposia*

SUNDAY 21 JUNE

Illumina

12:30 – 13:30, Sala Verde

Title: Revolutionising Cancer Genomics Research

Introduction
Speakers: Nicola Cirenei, Fiona McCartney (Illumina)

Assessing Stromal Contribution to the Neoplastic Transcriptome by RNASeq
Speaker: Enzo Medico (IT)

Applications of next generation sequencing in molecular pathology
Speaker: Silke Lassman (DE)

MONDAY 22 JUNE

NanoString Technologies

12:30 – 13:30, Sala Verde

Title: Simultaneous Multi-Omic Measurement of Nucleic-Acids and Proteins at 800-Plex using Single-Molecule Optical Barcodes: Application to Cancer Immunotherapy

Speaker: Jim White (UK)

**AACR is not providing CME credit for Satellite Symposia.*

Programme at a Glance

SATURDAY, 20 JUNE 2015

Auditorium

10.00 **Annual Meeting of SIC Young Investigators**

10.30 **Pre-Conference Workshop**
"How to create and lead a start-up"
Chairs: Selena Ventura (IT)
Marco Macagno (IT)

14.00 **Opening Address**

14.30 **Opening Lecture**
Non-coding RNA
Frank J. Slack (US)

15.30 Coffee Break

16.00 **Plenary Symposium**
Immunotherapy
James P. Allison (US)
Suzanne Topalian (US)
2 presenters from best abstracts
Ton Schumacher (NL)

18.00 **Welcome Reception**
19.30

Exhibition 15.30 - 19.30

SUNDAY, 21 JUNE 2015

	Auditorium	Sala Verde
07.30	**Meet the Expert** Alice Shaw (US)	**Meet the Expert** Pasi Jänne (US)
08.30	**Keynote Lecture** **Genomically-Driven "Basket" Clinical Trials** José Baselga (US)	
09.15	**Proffered Papers I**	
10.15	Poster Viewing / Coffee Break	
10.45	**Symposium** **Melanoma Targeted Drugs** Richard Marais (UK) Daniel Peeper (NL) 2 presenters from best abstracts Caroline Robert (FR)	
12.30	Lunch	12.30-13.30 **Satellite Symposium: Illumina**
13.15	**Poster Defence**	
14.30	**Symposium** **Tumour Heterogeneity** Charles Swanton (UK) Neil Shah (US) 2 presenters from best abstracts Anton Berns (NL)	
16.15	Poster Viewing / Coffee Break	
16.45	**Symposium** **Circulating Tumour Cells** F. Di Nicolantonio (IT) Caroline Dive (UK) 2 presenters from best abstracts	
18.15	**Keynote Lecture** **Cancer Genomics and Drug Resistance**	
19.00	Elaine Mardis (US)	19.00 - 20.00 SIC General Assembly

Exhibition 10.15 - 17.00

MONDAY, 22 JUNE 2015

	Auditorium	Sala Verde
07.30	**Meet the Expert** Alberto Bardelli (IT)	**Meet the Expert** Silvia Giordano (IT)
08.30	**Keynote Lecture** **Angiogenesis** Peter Carmeliet (BE)	
09.15	**Proffered Papers II**	
10.15	Poster Viewing / Coffee Break	
10.45	**Symposium** **Microenvironment** Erik Sahai (UK) Ravid Strausmann (IL) 2 presenters from best abstracts Mario Colombo (IT)	
12.30	Lunch	12.30-13.30 **Satellite Symposium:** **NanoString Technologies**
13.15	**Poster Defence**	
14.30	**Symposium** **Metabolism** Jacques Pouyssegur (FR) Paola Chiarugi (IT) 2 presenters from best abstracts Karen Vousden (UK)	
16.15	Poster Viewing / Coffee Break	
16.45	**Symposium** **Undruggable Targets** Ashok Venkitaraman (UK) Gerard Evan (UK) 2 presenters from best abstracts	
18.15	**Keynote Lecture** **Animal Models** Mariano Barbacid (ES)	
19.00		
20.00	**Conference Dinner** (access with ticket only)	

Exhibition 10.15 - 17.00

TUESDAY, 23 JUNE 2015

	Auditorium
08.30	**Keynote Lecture** **Precision Medicine in Oncology** Pasi Jänne (US)
09.15	**Keynote Lecture** **Epigenetics** Manel Esteller (ES)
10.00	Coffee Break
10.30	**Giorgio Prodi Lecture** Maurizio D'Incalci (IT)
11.15	**Symposium** **Inflammation** Paola Allavena (IT) Lisa M. Coussens (US) 2 presenters from best abstracts Thomas Tüting (DE)
13.00	**SIC Awards** **Conference Highlights &** **Closing Remarks**
13.30	

EACR-AACR-SIC Special Conference 2015
Scientific Programme

SATURDAY 20 JUNE 2015

		ABSTRACT NR.
14:00 - 14:30	**Opening Address (Auditorium)**	
	Welcome by EACR Chair: R. Marais (United Kingdom)	
	Welcome by AACR Chair: P. Jänne (USA)	
	Welcome by SIC Chair: R. Dolcetti (Italy)	
	Welcome by the Director of Istituto Toscano Tumori Speaker: L. Luzzatto (Italy)	
14:30 - 15:30	**Opening Lecture: Non-coding RNA (Auditorium)**	
	Chair: P. Jänne (USA)	
14:30	MicroRNA-based therapeutics in cancer Speaker: F.J. Slack (USA)	1
16:00 - 18:00	**Plenary Symposium: Immuno-therapy (Auditorium)**	
	Chair: R. Dolcetti (Italy)	
16:00	Immune checkpoint blockade in cancer therapy: New insights, opportunities, and prospects for a cure Speaker: J.P. Allison (USA)	2
16:30	PD-1 pathway blockade: Future clinical directions Speaker: S. Topalian (USA)	3
17:00	Proffered paper: Radiation and dual PD-L1 and CTLA4 checkpoint blockade overcome tumor resistance and distinctly improve immunity A. Maity, C. Twyman-Saint Victor, A.J. Recht, R. Rengan, L.M. Schuchter, D.A. Pryma, S.M. Hahn, E.J. Wherry, R.H. Vonderheide, A.J. Minn (USA)	4
17:10	Proffered paper: Cancer chemotherapy and viral mimicry A. Sistigu, I. Vitale, T. Yamazaki, E. Vacchelli, G. Kroemer, L. Zitvogel (Italy)	5
17:30	What T cells see on human cancer Speaker: T. Schumacher (Netherlands)	6
17:50	Discussion & Roundup	
18:00-19:30	**Welcome Reception**	

SUNDAY 21 JUNE 2015

		ABSTRACT NR.
07:30 - 08:30	**Meet the Expert Session (Auditorium)**	
07:30	Speaker: A.T. Shaw (USA)	7
07:30 - 08:30	**Meet the Expert Session (Sala Verde)**	
07:30	Speaker: P. Jänne (USA)	8
08:30 - 09:15	**Keynote Lecture: Genomically-Driven "Basket" Clinical Trials (Auditorium)**	
	Chair: S. Giordano (Italy)	
08:30	Genomically-driven "basket" clinical trials Speaker: J. Baselga (USA)	9
09:15 - 10:15	**Proffered Papers 1 (Auditorium)**	
	Chair: C. Ambrogio (Spain) Chair: E. Goka (USA)	
09:15	Proffered paper: A kinase-independent role for LMTK3 in chromatin remodeling Y. Xu, H. Zhang, V. Nguyen, J. Nunes, N. Angelopoulos, J. Stebbing, L. Magnani, G. Giamas (United Kingdom)	10
09:25	Proffered paper: Epigenetic silencing of ß-arrestin1 and its intragenic miR-326 controls medulloblastoma growth E. Miele, A. Po, A. Mastronuzzi, S. Valente, A. Carai, I. Screpanti, F. Giangaspero, M. Levrero, F. Locatelli, E. Ferretti (Italy)	11
09:35	Proffered paper: Acquired resistance to ERK inhibitors resulting from a novel P-loop mutation of ERK2 P.A. Clarke, T. Roe, K. Swabey, C. McAndrew, K. Boxall, I. Westwood, R. Van Montfort, B. Al-Lazikani, P. Workman (United Kingdom)	12
09:45	Proffered paper: Mitochondrial metabolism promotes metastatic progression P. Porporato, V.L. Payen, P. Sonveaux (Belgium)	13
09:55	Proffered paper: HEDGEHOG/GLI-E2F1 axis modulates iASPP expression and activation and regulates melanoma cell growth S. Pandolfi, V. Montagnani, B. Stecca (Italy)	14
10:05	Proffered paper: Stress induced phenotypic plasticity drives multi-drug tolerance along with stemness in melanoma D. Ravindran Menon, A. Emran, B. Gabrielli, P. Soyer, N. Haas, R. Somasundaram, M. Herlyn, H. Schaider (Australia)	15
10:45 - 12:30	**Symposium: Melanoma Targeted Drugs (Auditorium)**	
	Chair: A. Berns (Netherlands)	
10:45	Improving patient outcomes to targeted therapies in melanoma Speaker: R. Marais (United Kingdom)	16
11:10	Large-scale genetic in vivo perturbations to reveal novel cancer vulnerabilities Speaker: D. Peeper, (Netherlands)	17
11:35	Proffered paper: Suppression of oncogene transcription - PNA as novel targeted cancer therapy for BRAF-V600E mutant melanoma J. Rothman, O. Surriga, S.D. Vasudeva, G. Ambrosini, G.K. Schwartz (USA)	18
11:45	Proffered paper: Novel therapeutic approaches by targeting CD74 expression in melanoma S. Ekmekcioglu, J. Roszik, S.E. Woodman, E.A. Grimm (USA)	19

ABSTRACT NR.

11:55	Melanoma resistance to targeted therapy: A translation issue? Speaker: C. Robert (France)	20
12:20	Discussion & Roundup	
14:30 - 16:15	**Symposium: Tumour Heterogeneity (Auditorium)**	
	Chair: C. Dive (United Kingdom)	21
14:30	Deciphering drivers of cancer evolution Speaker: C. Swanton (United Kingdom)	
14:55	Heterogeneity of acquired resistance to active targeted inhibitors of BCR-ABL and FLT3 Speaker: N.P. Shah (USA)	22
15:20	Proffered paper: Tumor heterogeneity contributes to anti-EGFR therapy resistance in glioblastoma C. Zanca, W. Cavenee, F. Furnari (USA)	23
15:30	Proffered paper: Prospective identification of RET resistance mutations predicted to emerge from treatment with multi-kinase inhibitors and the development of potent, selective inhibitors that address these mutations E.K. Evans, P. Fleming, J.L. Kim, A.K. Gardino, W. Hu, M. Maynard, K.J. DiPietro, T. Guzi, N.E. Kohl, C. Lengauer (USA)	24
15:40	Mouse models of lung cancer: Tumour heterogeneity and cell-of-origin of thoracic tumors Speaker: A. Berns (Netherlands)	25
16:05	Discussion & Roundup	
16:45 - 18:15	**Symposium: Circulating Tumour Cells (Auditorium)**	
	Chair: A. Bardelli (Italy)	
16:45	Cancer biomarkers in circulating tumor DNA Speaker: F. Di Nicolantonio (Italy)	26
17:15	Versatility of circulating tumour cells in lung cancer: Biology, drug development and biomarkers C. Dive (United Kingdom)	27
17:45	Proffered paper: Long-range regulators of HOTAIR as prognostic biomarkers for the stratification of hormone receptor positive breast cancer M.J. Milevskiy, F. Al-Ejeh, J.M. Saunus, K.S. Northwood, P. Bailey, K.P. Nephew, S.L. Edwards, S.J. Clark, S.R. Lakhani, M.A. Brown (Australia)	28
17:55	Proffered paper: cfDNA profiling of non-small-cell lung cancer using Ion Torrent NGS B. Ottolini, A. Cox, P. Woll, K. Page, D. Guttery, L. Primrose, J.H. Pringle, M. Martins, D. Fennell, J.A. Shaw (United Kingdom)	29
18:05	Discussion & Roundup	
18:15 - 19:00	**Keynote Lecture: Cancer Genomics and Drug Resistance (Auditorium)**	
	Chair: R. Marais (United Kingdom)	
18:15	Cancer genomics and drug resistance Speaker: E. Mardis (USA)	30

ABSTRACT NR.

MONDAY 22 JUNE 2015

07:30 - 08:30	**Meet the Expert Session (Auditorium)**	
07:30	Clonal evolution and drug resistance: From cancer avatars to liquid biopsies Speaker: A. Bardelli (Italy)	31
07:30 - 08:30	**Meet the Expert Session (Sala Verde)**	
07:30	Applications of patient-derived xenograft models to translational cancer research Speaker: S. Giordano (Italy)	32
08:30 - 09:15	**Keynote Lecture: Angiogenesis (Auditorium)**	
	Chair: A.T. Shaw (USA)	
08:30	Angiogenesis revisited: Role and therapeutic potential of targeting endothelial metabolism Speaker: P. Carmeliet (Belgium)	33
09:15 - 10:15	**Proffered Papers 2 (Auditorium)**	
	Chair: S. Ventura (Italy) Chair: M. Macagno (Italy)	
09:15	Proffered paper: p140Cap scaffold protein, a new prognostic marker in ERBB2 breast cancer, limits ErbB2 breast cancer progression in a preclinical animal model S. Grasso, I. Russo, V. Salemme, F. Cavallo, A. Amici, M. Iezzi, P. Di Stefano, E. Turco, P. Defilippi (Italy)	34
09:25	Proffered paper: Overexpression of HER2 and EGFR supports the osseous growth of prostate cancer cells G. Lorenzatti, K.C. Day, S.J. Dawsey, A.R. Paul, S. Daignault-Newton, N. Palanisamy, L. El-Sawy, M.L. Day (USA)	35
09:35	Proffered paper: ATM kinase modulates HER2 tumorigenicity in breast cancer V. Stagni, I. Manni, V. Oropallo, M. Mottolese, A. Di Benedetto, G. Piaggio, R. Falcioni, F. Sperati, M.T. Cencioni, D. Barilà (Italy)	36
09:45	Proffered paper: hMENA11a sustains HER3 activation and contributes to HER3-mediated resistance to PI3K inhibitors P. Trono, F. Di Modugno, R. Circo, S. Spada, R. Melchionna, B. Palermo, S. Matteoni, S. Soddu, R. De Maria, P. Nisticò (Italy)	37
09:55	Proffered paper: Combined inhibition of Ddr1 and Notch signaling is an effective therapeutic strategy to treat K-Ras-driven/p53-null lung adenocarcinomas C. Ambrogio, G. Gómez-López, D. Santamaría, M. Barbacid (Spain)	38
10:05	Proffered paper: Gain of function mutant p53 proteins cooperate with E2F4 to transcriptionally downregulate RAD17 and BRCA1 gene expression F. Valenti, F. Ganci, G. Fontemaggi, A. Sacconi, S. Strano, G. Blandino, S. Di Agostino (Italy)	39
10:45 - 12:30	**Symposium: Microenvironment (Auditorium)**	
	Chair: M. Colombo (Italy)	
10:45	Learning from imaging the failure of targeted therapy Speaker: E. Sahai (United Kingdom)	40
11:10	Tumor microbiome mediated-chemoresistance Speaker: R. Straussman (Israel)	41

ABSTRACT NR.

11:35	Proffered paper: A c-MET inhibitor reduces bone metastases induced by renal cancer stem cells I. Roato, L. Trusolino, L. D'Amico, G. Migliardi, D.C. Belisario, L. Dalle Carbonare, T. Perera, P.M. Comoglio, R. Ferracini (Italy)	42
11:45	Proffered paper: Resistance to MET inhibitors: Which is the role of tumor microenvironment? M. Apicella, A. Bertotti, C. Zanon, S. Giordano, S. Corso (Italy)	43
11:55	Extracellular matrix impinges on immune functions and shapes the tumour microenvironment Speaker: M. Colombo, (Italy)	44
12:20	Discussion & Roundup	
14:30 - 16:15	**Symposium: Metabolism (Auditorium)**	
	Chair: J. Pouyssegur (France)	
14:30	Hypoxic microenvironment and tumor metabolism Speaker: J. Pouyssegur (France)	45
14:55	Reciprocal metabolic deregulation of tumor/stroma interplay: A new druggable synergy Speaker: P. Chiarugi (Italy)	46
15:20	Proffered paper: Metabolic remodeling and dependence on oxoglutarate dehydrogenase induced by oncogenic PIK3CA N. Ilic, A.J. Aguirre, S. Singh, S.E. Moody, N.A. Spardy, B.A. Weir, J.M. Asara, H.R. Widlund, F. Vazquez, W.C. Hahn (USA)	47
15:30	Proffered paper: TRAPping the metabolic adaptations of NF1-associated tumors I. Masgras, G. Guzzo, F. Ciscato, M. Curtarello, S. Indraccolo, F. Calabrese, A.M. Brunati, P. Bernardi, A. Rasola (Italy)	48
15:40	Serine metabolism and cancer therapy – looking for new targets Speaker: K. Vousden (United Kingdom)	49
16:05	Discussion and Roundup	
16:45 - 18:15	**Symposium: Undruggable Targets (Auditorium)**	
	Chair: K. Vousden (United Kingdom)	
16:45	Extending the reach of target discovery and validation to novel classes Speaker: A. Venkitaraman (United Kingdom)	50
17:15	Targeting cancer's engines, not its drivers Speaker: G.I. Evan (United Kingdom)	51
17:45	Proffered paper: An integrated pipeline for pharmaco-genomic studies in patient derived tumour cells A. Bruna, W. Greenwood, M. Callari, A. Sati, O. Rueda, V. Serra, M. Garnett, C. Caldas (United Kingdom)	52
17:55	Proffered paper: Drugging the undruggable: Development of small molecule activators of protein phosphatase 2A for cancer treatment M. Ohlmeyer, M. Galsky, R. Sears, M. Chance, D. Brautigan, W. Xu, Y. Ioannou, A. DiFeo, G. Narla (USA)	53
18:05	Discussion and Roundup	
18:15 - 19:00	**Keynote Lecture: Animal Models (Auditorium)**	
	Chair: M. Oren (Israel)	
18:15	Modeling human cancer in mice: An essential tool for the development of future therapies Speaker: M. Barbacid (Spain)	54

ABSTRACT NR.

TUESDAY 23 JUNE 2015

08:30 - 09:15	**Keynote Lecture: Precision Medicine in Oncology (Auditorium)**	
	Chair: S. Giordano (Italy)	
08:30	Precision medicine in oncology Speaker: P. Jänne (USA)	55
09:15 - 10:00	**Keynote Lecture: Epigenetics (Auditorium)**	
	Chair: D. Peeper (Netherlands)	
09:15	Cancer pharmacoepigenetics: Genes and drugs Speaker: M. Esteller (Spain)	56
10:30 - 11:15	**Award Lecture: "Giorgio Prodi" Lecture (Auditorium)**	
	Chair: S. Giordano (Italy)	
10:30	From seabed to bedside: Lessons learnt from the marine-derived anticancer drug trabectedin Award Lecturer: M. D'Incalci (Italy)	57
11:15 - 13:00	**Symposium: Inflammation (Auditorium)**	
	Chair: L.M. Coussens (USA)	
11:15	Targeting of tumor-associated macrophages and cancer-related inflammation Speaker: P. Allavena (Italy)	58
11:40	Inflammation and cancer: Reprogramming the immune microenvironment as an anti-cancer therapeutic strategy Speaker: L.M. Coussens (USA)	59
12:05	Proffered paper: The T-cell immune landscape predicts clinical outcome in neuroblastoma D. Fruci, M. Mina, R. Boldrini, A. Citti, P. Romania, V. D'Alicandro, M.A. De Ioris, A. Castellano, C. Furlanello, F. Locatelli (Italy)	60
12:15	Proffered paper: Regulation of the immunosuppressive microenvironment of liver metastases by TNFR2 B. Ham, Z. D'Costa, N. Wang, M.C. Fernandez, F. Bourdeau, P. Auguste, P. Brodt (Canada)	61
12:25	Neutrophilic inflammation and melanoma metastasis Speaker: T. Tüting (Germany)	62
12:50	Discussion & Roundup	
13:00 - 13:35	**Closing Session (Auditorium)**	
	Chair: S. Giordano (Italy)	
	SIC Awards	
	"Piero Trivella" Award for the Best Posters Speaker: M.G. Trivella (Italy)	
	"Elena Cappannini" Award for the Best 2014 Publication Speaker: F. Fallarino (Italy)	
	"Pezcoller Foundation" Scholarship Award Speaker: D. Bassi (Italy)	
	Travel Grants	
	Conference Highlights & Farewell Message	

EACR-AACR-SIC Special Conference 2015
Poster Sessions

ABSTRACT NR.

SUNDAY 21 JUNE 2015

Cancer Genomics, Epigenetics and Genome Instability I

Carcinogenesis I

Cell and Tumour Biology I

ABSTRACT NR.

Experimental/Molecular Therapeutics, Pharmacogenesis I

ABSTRACT NR.

Signalling Pathways I

Translational Research I

ABSTRACT NR.

ABSTRACT NR.

Tumour Immunology I

MONDAY 22 JUNE 2015

Cancer Genomics, Epigenetics and Genome Instability II

ABSTRACT NR.

Carcinogenesis II

Cell and Tumour Biology II

ABSTRACT NR.

ABSTRACT NR.

ABSTRACT NR.

Experimental/Molecular Therapeutics, Pharmacogenesis II

ABSTRACT NR.

ABSTRACT NR.

Molecular and Genetic Epidemiology II

Prevention and Early Detection II

Radiobiology/Radiation Oncology II

ABSTRACT NR.

Signalling Pathways II

ABSTRACT NR.

Translational Research II

ABSTRACT NR.

ABSTRACT NR.

ABSTRACT NR.

ABSTRACT NR.

Tumour Immunology II

Abstracts

SATURDAY 20 JUNE 2015

14:30-15:30
Opening Lecture: Non-coding RNA

1 MicroRNA-based therapeutics in cancer

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MicroRNAs are small non-coding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. let-7 and miR-34 are microRNAs implicated in human cancer. Specifically, human let-7 and miR-34 are poorly expressed or deleted in lung cancer, and over-expression of let-7 or miR-34 in lung cancer cells inhibits their growth, demonstrating a role for these miRNAs as tumor suppressors in lung tissue. let-7 and miR-34 regulate the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for their involvement in cancer. We are focused on the role of these genes and other oncomiRs in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis.

Conflict of interest: Ownership: Mirna Therapeutics, Mira Dx. Advisory board: Mirna Therapeutics, Mira Dx

16:00-18:00
Plenary Symposium: Immuno-therapy

2 Immune checkpoint blockade in cancer therapy: New insights, opportunities, and prospects for a cure

No abstract received.

3 PD-1 pathway blockade: Future clinical directions

S. Topalian[1]
1 John Hopkins Cancer Center, Baltimore, USA

The PD-1 pathway, consisting of the immune cell co-receptor Programmed Death 1 (PD-1) and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), mediates immunosuppression within the tumor microenvironment. Several unique monoclonal antibodies designed to "release the brakes" on anti-tumor immunity by blocking PD-1 or PD-L1 have demonstrated significant and durable clinical activity in multiple types of advanced cancers, validating this pathway as a target and ushering in a new age for cancer immunotherapy. Following regulatory approvals in 2014 for pembrolizumab (US) and nivolumab (US and Japan) to treat advanced melanoma in the second- or third-line setting, first- and second-line approvals for additional cancer types are anticipated. The generally manageable safety profile of PD-1/PD-L1 blocking antibodies is consistent with long-term treatment and the development of combinatorial therapies, which are predicted in preclinical models to increase the efficacy of this approach. Studies identifying tumor PD-L1 protein expression as a factor associated with enhanced responsiveness to PD-1 pathway blockade are only scratching the surface of potential biomarkers which might guide patient selection. Such biomarkers hold promise for further enhancing the risk:benefit profile for PD-1/PD-L1 antagonists and for increasing our understanding of the mechanistic underpinnings of this key pathway in tumor biology.

Supported by the National Institutes of Health R01 CA142779, the Melanoma Research Alliance, the Barney Foundation, the Laverna Hahn Charitable Trust, Moving for Melanoma of Delaware, Stand Up 2 Cancer-Cancer Research Institute grant SU2C-AACR-DT1012, and Bristol-Myers Squibb.

Conflict of interest: Ownership: Stock options (spouse): Jounce Therapeutics, Potenza Therapeutics. Advisory board: Consultant for Five Prime Therapeutics, GlaxoSmithKline, Jounce Therapeutics. Board of directors: none. Corporate-sponsored research: Bristol-Myers Squibb. Other substantive relationships: Patent royalties through institution (spouse): Bristol-Myers Squibb, Potenza Therapeutics.

4 Proffered paper: Radiation and dual PD-L1 and CTLA4 checkpoint blockade overcome tumor resistance and distinctly improve immunity

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2 University of Pennsylvania, Medicine, Philadelphia, USA
3 University of Pennsylvania, Abramson Family Cancer Research Institute, Philadelphia, USA
4 University of Washington, Radiation Oncology, Seattle, USA
5 University of Pennsylvania, Radiology, Philadelphia, USA
6 MD Anderson Cancer Center, Radiation Oncology, Houston, USA
7 University of Pennsylvania, Microbiology and Immunology, Philadelphia, USA

Introduction. Optimal results with inhibitors directed against immune checkpoints such as CTLA4 and PD-1 in cancers will likely require combination therapy. Pre-clinical and clinical data indicate that radiation therapy (RT) may augment responses to immune checkpoint inhibition. We therefore evaluated this combination for metastatic melanoma using parallel studies in mice and humans.

Material and method : In a phase I clinical trial, 22 patients with multiple melanoma metastases were given hypofractionated RT (2-3 fractions) to a single index lesion followed by four cycles of the anti-CTLA4 antibody (Ab) ipilimumab. We modeled this in mice using the B16-F10 melanoma cell line. Each flank of C57BL/6 mice was implanted with a tumor to model multiple metastases. Mice received anti-CTLA4 antibody (on days 5, 8, and 11), irradiation of one tumor using an image-guided micro-irradiator (20 Gy x 1 on day 8), or both treatments. Mechanistic studies were performed on material obtained from patients on the clinical trial and from mice.

Results and Discussion. Overall, treatment in the phase I clinical trial was well tolerated, and toxicity was similar to that reported for anti-CTLA4 therapy. Regression of unirradiated tumors was observed in a subset of patients with metastatic melanoma treated with ipilimumab + RT (18% partial response by RECIST criteria). In mice, although combined treatment enhanced the CD8/Treg ratios and improved responses in irradiated and unirradiated tumors, resistance was common. Genome-wide and unbiased analyses revealed that resistant tumors have increased PD-L1, interferon-stimulated genes, and exhausted T cells that depress the CD8/Treg ratio. Patients and mice with high PD-L1-expressing tumors that were treated with RT + anti-CTLA4 Ab poorly reinvigorated exhausted T cells, did not respond, and rapidly progressed. In mice, adding anti-PD-L1/PD-1 Ab to RT + anti-CTLA4 Ab reinvigorated exhausted T cells, leading to complete responses and immunity across multiple cancer types (including breast and pancreatic cancer). The extent of T cell exhaustion/reinvigoration predicts response and can be assessed through peripheral blood.

Conclusion. Resistance to RT + anti-CTLA4 therapy results from depression in the CD8/Treg ratio due to elevated tumor PD-L1 and persistent T cell exhaustion. Our pre-clinical data suggest combining RT with CTLA4 and PD-1 checkpoint blockade is a rational, non-redundant approach to overcoming tumor resistance and improving immunity in multiple cancer types.

No conflict of interest.

5 Proffered paper: Cancer chemotherapy and viral mimicry

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2 Università Tor Vergata, Fisiology, Roma, Italy
3 Gustave Roussy Cancer Campus, U1015, Villejuif, France

Cancer Chemotherapy and Viral Mimicry: Distinct cell death-associated molecular patterns might define cancers proned to respond to a cytotoxic therapy by mounting a protective T cell-based anticancer immunity. Here, we show that immunogenic chemotherapy phenocopies viral infection leading to autocrine IFN$\alpha\beta$/IFNAR1/2 signalling in tumor cells initiated by recognition of self dsRNA by endosomal pattern recognition receptors (PRRs) that usually recognize virus-encoded nucleic acids, namely TLR3/TRIF. In detail, TLR3/TRIF (endosomal dsRNA sensors) and IFNAR1/2 (Type I IFN receptors) must signal within the tumor cells so that chemotherapy can induce downstream CXCL10/CXCR3 axis and elicit therapeutic responsiveness in vivo. Moreover, the IFN fingerprint of human breast cancers allowed to predict tumors proned to benefit from adjuvant anthracyclines.

From an evolutionary viewpoint, while tumors (like viruses) have evolved mechanisms to evade an IFN response, chemotherapy-induced viral mimicry might contribute to bypass such as immunoediting.

No conflict of interest.

6 What T cells see on human cancer

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Human tumors contain large numbers of mutations, of which many hundreds can be present within expressed genes. As the resulting altered protein sequences are foreign to the immune system, immune recognition of such 'neo-antigens' is likely to be of significant importance to the activity of clinically used immunotherapeutics such as anti-CTLA-4 and anti-PD-1 in melanoma. However, the vast majority of the mutations in human cancers are unique to individual patients and, because of this, broadly applicable approaches to link the consequences of DNA damage in human cancer to tumor-specific T cell activity have long been lacking. Using in-house developed technologies for monitoring of T cell activity, we have recently demonstrated the feasibility of cancer exome-driven analysis of both tumor-specific CD8+ T cell reactivity and CD4+ T cell reactivity in human melanoma. The data obtained demonstrate that T cell recognition of the consequences of DNA damage is a common feature in human melanoma. Furthermore, based on the distribution of mutation loads in other major human cancer types, we propose that also in many other human tumors, the repertoire of mutant antigens provided by DNA damage (the 'neo-antigen space') will suffice to allow T cell recognition. Collectively, these data indicate that mutational load may form a biomarker in cancer immunotherapy, and that the development of 'personalized immunotherapies' that exploit cancer genome information to target patient-specific mutant antigens should be explored.

No conflict of interest.

SUNDAY 21 JUNE 2015

07:30-08:30
Meet the Expert Session

7 No abstract received.

07:30-08:30
Meet the Expert Session

8 No abstract received.

08:30-09:15
Keynote Lecture: Genomically-Driven "Basket" Clinical Trials

9 No abstract received

09:15-10:15
Proffered Papers 1

10 Proffered paper: A kinase-independent role for LMTK3 in chromatin remodeling

Y. Xu[1], H. Zhang[1], V. Nguyen[1], J. Nunes[1], N. Angelopoulos[1], J. Stebbing[1], L. Magnani[1], G. Giamas[1]
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Introduction. Lemur Tyrosine Kinase 3 (LMTK3) is an oncogenic receptor tyrosine kinase (RTK) implicated in various types of cancer including breast, lung, gastric and colorectal. It is localized in different cellular compartments but its nuclear function has not been investigated thus far.

Material and method. We performed:

- ChIP-seq to map LMTK3-chromatin interaction events;
- rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) to investigate LMTK3 binding partners on the associated chromatin;
- immunoprecipitation and GST pull-down to validate LMTK3 binding partners;
- fluorescence in situ hybridization (FISH) and formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) to study the function of LMTK3 on chromatin remodeling and repositioning.

Results. We mapped genome-wide LMTK3 bindings and found that LMTK3 binding events are correlated with repressive chromatin markers.

We further identified KRAB associated protein-1 (KAP1) as a novel binding partner of LMTK3.

KAP1/LMTK3 interaction suppresses genes transcription at the promoter regions, while it inhibits genes expression at enhancer regions by tethering the chromatin to the nuclear periphery.

Mechanistically, KAP1 phosphorylation is suppressed by the LMTK3/PP1α complex specifically at LMTK3-associated chromatin regions, inducing chromatin condensation and resulting in transcriptional repression of LMTK3-bound tumor suppressors.

Conclusions. We propose a new model where a non-kinase function of nuclear LMTK3 promotes cancer progression through chromatin remodeling and direct transcriptional regulation, suggesting that its therapeutic targeting may require approaches other than the inhibition of its kinase activity.

No conflict of interest.

11 Proffered paper: Epigenetic silencing of ß-arrestin1 and its intragenic miR-326 controls medulloblastoma growth

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7 University of Rome "Sapienza", Internal Medicine DMISM, Roma, Italy
8 University of Rome "Sapienza", Experimental Medicine, Roma, Italy

Introduction. Medulloblastoma (MB) is the most common malignant brain tumors in childhood and leading cause of cancer morbidity and mortality in this age group. High-throughput studies have classified MBs into four subgroups characterized by distinct mutations and deregulation of specific signaling pathways as well as different clinical outcomes. A series of pathways and epigenetic mechanisms are deregulated across multiple subtypes, thus representing a challenge for the success of tailored therapies in different MBs subgroups, but also a window of opportunity for the identification of common targets. A feature common to all MBs is the presence of stem-like cells (SLCs) that represent a fraction of the tumor bulk, which retain the ability to sustain tumor growth and may represent the progenitors that give rise to MB. Recent studies have highlighted the crucial role of microRNAs tumor signaling pathway deregulation. We have previously identified microRNAs deregulated medulloblastoma. Among them we showed that miR-326 is strongly downregulated and represses Hedgehog/Gli signalling.

Material and Method. Human and murine SLCs were derived and cultured as oncospheres. We evaluated the expression levels of miR-326 and its host gene β-arrestin1 in medulloblastoma specimens as well as in

SLC. We investigated the role of the two molecules in MB context. We studied the regulation of miR-326/ β-arrestin1 transcriptional unit in SLC. A pharmacological approach was utilized to modulate the expression of miR-326/ β-arrestin1 in MB both in vitro and in vivo.

Results and Discussion. We found that miR-326 cooperates with its host gene β-arrestin1 as tumor suppressors, lost in medulloblastoma and in SLC. Such unit suppresses Hedgehog signaling at multiple levels: β-arrestin1 inhibits Hedgehog through the modulation of Gli1 K518 acetylation while miR-326 controls Gli2 and Smo, activatory molecules of the pathway. Then, we analyzed the potential mechanisms involved in the downregulation of this transcriptional unit, finding that β-arrestin1/ miR-326 are silenced through epigenetic mechanisms at histone levels. Indeed, epigenetic drugs are able to reactivate the miR-326/β- arrestin1 expression and suppress MB and SLC growth in vitro and in vivo.

Conclusion. Our study reveals a new microRNA/host gene network in MB and proposes miR-326/β-arrestin1 as tumor suppressors for medulloblastoma patients, susceptible to be re-expressed by epigenetic treatments.

No conflict of interest.

12 Proffered paper: Acquired resistance to ERK inhibitors resulting from a novel P-loop mutation of ERK2

P.A. Clarke[1], T. Roe[1], K. Swabey[1], C. McAndrew[1], K. Boxall[1], I. Westwood[1], R. Van Montfort[1], B. Al-Lazikani[1], P. Workman[1]
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Introduction. Genomic aberrations frequently activate the MAPK and PI3K pathways in cancers and combined inhibition of these pathways is being explored widely in the clinic.

Results and Discussion. In a panel of 45 colorectal cancer cells we found combination of MEK/PI3K inhibition overcame resistance to monotherapy with these agents and exhibited potent synergy, particularly in the hard to treat KRAS mutant cells. Resistance to individual targeted agents is challenge in the clinic and can be overcome by combining treatments. However, as with the monotherapy we found prolonged exposure to the PI3K/MEK inhibitor combination regime also resulted in resistance. This was due to acquisition of a mutation of the allosteric drug-binding site that abrogated binding of the MEK inhibitor to MEK2. The clinical development of small-molecule ERK inhibitors is of considerable interest as ERK signaling represents a key downstream effector of RAS mutations, and ERK inhibitors are predicted to retain activity in cells resistant to RAF and MEK inhibitors. Consistent with the latter, the combination-resistant line retained sensitivity to an ERK inhibitor (VTX-11e), which also rescued the synergistic interaction when combined with PI3K inhibition. However, prolonged exposure of this MEK/PI3K combination resistant line to the combination of ERK/PI3K inhibitors resulted in the acquisition of additional resistance to the ERK inhibitor component of the combination. Resistance to VTX-11e was a result of an ERK2 mutation, novel to protein kinases, in a conserved P-loop residue of the kinase domain. Recombinant wild-type and P-loop mutant ERK2 had similar Vmax and ATP Km values, but exhibited an increased IC50 for VTX-11e. Development of an additional VTX-11e resistant line, in this case by exposure to prolonged monotherapy, resulted in the acquisition of the same ERK2 mutation. The repeated development of resistance to the MEK/ERK inhibitor arm of the combination is intriguing as the parent cells were not particularly sensitive to treatment with MEK or ERK inhibitors alone, with a GI50 30-fold higher than BRAF mutant cell lines that are sensitive to these inhibitors.

Conclusion. Our data show that dual combination of agents, at least for PI3K and MEK pathway inhibition, is insufficient to block the acquisition of resistance. Finally we recommend sequencing of ERK1/2 in clinical studies of ERK inhibitors to determine whether active site mutations will occur in patients following treatment with these inhibitors.

Conflict of interest: Other substantive relationships: The authors are employees of The Institute of Cancer Research, which has a commercial interest in the development of PI3K inhibitors, including pictilisib (GDC-0941), and operates a rewards-to-inventors scheme.

13 Proffered paper: Mitochondrial metabolism promotes metastatic progression

P. Porporato[1], V.L. Payen[1], P. Sonveaux[1]
1 UCL Institute of Experimental and Clinical Research (IREC), FATH, Brussels, Belgium

Introduction. It is broadly accepted that tumor cells rewire metabolic fluxes in order to promote cell proliferation and resistance to apoptosis. However, the specific impact of metabolism on metastatic progression is still poorly characterized. Since most metastases are abnormally avid for glucose (which is the rationale for their clinical detection using FDG-PET), it is often assumed that mitochondrial metabolism is detrimental to metastatic progression. Still, mechanistic evidence is missing. In this study, we assessed the specific contribution of mitochondrial metabolism on metastasis development.

Methods. Several clones with increased invasive and metastatic potential were generated following in vitro (starting from human cervix adenocarcinoma SiHa cells) and in vivo (starting from B16F10 murine melanoma cells) selection. A complete

metabolic on these clones characterization was performed for measuring oxidative and glycolytic metabolism, as well as redox status and mitochondrial mass. The specific contribution of cancer cell metabolism to metastasis was assessed by the use of specific metabolic inhibitors or modulators.

Results and discussion. We identified that a mitochondrial switch, corresponding to an overload of the TCA cycle with preserved mitochondrial functions but increased mitochondrial superoxide production, promotes metastasis. This switch provided a metastatic advantage that was reproduced by moderate OXPHOS inhibition associated with a moderate increase of mitochondrial superoxide levels. Both conditions involved protein tyrosine kinase PTK2B/Pyk2 increased expression and Src activation as downstream effectors. Coherently, the complete blockade of mitochondrial respiration, as well as the specific scavenging of mitochondrial superoxide prevented cancer cell invasion. Finally, we report that antioxidants specifically targeting mitochondria inhibit metastatic dissemination from primary murine and human tumors in mice.

Conclusion. Two different events (i.e., OXPHOS overload and moderate OXPHOS inhibition) promote superoxide-dependent tumor cell metastasis. Overall, this work demonstrates the central role of mitochondrial superoxide generation in the pathogenesis of metastasis and the potential impact of its scavenging for metastasis prevention.

No conflict of interest.

14 Proffered paper: HEDGEHOG/GLI-E2F1 axis modulates iASPP expression and activation and regulates melanoma cell growth

S. Pandolfi[1], V. Montagnani[1], B. Stecca[1]
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Introduction. Melanoma is the most aggressive skin cancer. Although p53 is rarely mutated in melanoma, its function can be impaired by multiple mechanisms, including the binding to inhibitor of apoptosis-stimulating protein of p53 (iASPP). HEDGEHOG (HH) signaling is a key regulator of tissue development and it is aberrantly activated in several cancer types, including melanoma, where it sustains cell growth and survival. Here we show that E2F1 is a crucial target of HH pathway in melanoma and that HH/GLI-E2F1 axis controls iASPP expression and activation.

Material and Method. HH signaling was activated by silencing the negative regulator PTCH1 alone or in combination with silencing of E2F1 in melanoma cells. Cellular and molecular biology techniques, and orthotopic xenografts of human melanoma cells in athymic nude mice were used to investigate the relevance of HH/GLI-E2F1 axisin vitro and in vivo.

Results and discussion. We showed that GLI1 and GLI2, the last mediators of the HH signaling, directly regulate the expression of E2F1 in melanoma cells, by binding to a functional non-canonical GLI consensus sequence in E2F1 promoter. Interestingly, E2F1 expression correlates with that of PTCH1, GLI1 and GLI2 in human metastatic melanomas. Functionally, we show that E2F1 is a crucial mediator of HH signaling, which is required for melanoma cell proliferation and xenograft growth induced by active HH pathway. We show that the HH/GLI-E2F1 axis positively modulates iASPP at multiple levels. Activation of HH signaling induces iASPP expression through E2F1, which directly binds to iASPP promoter. HH pathway also contributes to iASPP phosphorylation and activation, by induction of CyclinB1 and by E2F1-dependent regulation of CDK1. Our data show that E2F1 dictates the outcome of the activation of HH signaling, resulting in enhanced proliferation in presence of E2F1, but leading to apoptosis in its absence. Indeed, melanoma cells with activated HH signaling resulted more sensitive to treatment with the CDK1 inhibitor JNJ-7706621, which reproduced the effect of E2F1 silencing on iASPP activation.

Conclusion. Our findings identify a novel HH/GLI-E2F1-iASPP axis that regulates melanoma growth and survival, providing a novel mechanism through which HH signaling contributes to restrain p53 function in cancer cells.

No conflict of interest.

15 Proffered paper: Stress induced phenotypic plasticity drives multi-drug tolerance along with stemness in melanoma

D. Ravindran Menon[1], A. Emran[2], B. Gabrielli[3], P. Soyer[4], N. Haas[3], R. Somasundaram[5], M. Herlyn[5], H. Schaider[1]
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5 The Wistar Institute, Melanoma Research Center, Philadelphia, USA

Introduction. Drug tolerant cancer stem cell like subpopulations constitute a major challenge for effective cancer therapies resulting in relapse of the disease. However, their mode of formation and the dynamics leading to permanent resistance are poorly understood but are important to design better treatment strategies. Thus we set to answer this question in melanoma cells by characterizing this population, identifying their mode of origin and the temporal dynamics of their transition into a permanent acquired resistant state.

Materials and Methods. Melanoma cells were exposed to sublethal stressful conditions involving drug exposure, hypoxia or low glucose treatment and changes in their phenotype and epigenetic characteristics were determined. In parallel, we monitored their cell cycling characteristics in the course of acquired drug resistance against BRAF inhibitors using fluorescence ubiquitous cell cycle indicator by live cell imaging techniques.

Results and Discussion. We show that cancer cells in general exhibit a universal early innate response as a primary survival reaction towards unfavorable environmental conditions or drug exposure, inducing a transition into a slow cycling, multi- drug tolerant stem like state expressing markers like CD271, ABCB5 and high ALDH activity, termed induced drug tolerant cells (IDTC). This response is led by global chromatin remodeling, through the modulation of histone methylation patterns in H3K4, H3K9 and H3K27, in turn leading to activation of multiple signaling cascades and gain of a high tumorigenic potency. IDTCs exhibit persistent rewiring capabilities of signaling cascades making them a demanding target. Upon prolonged drug exposure IDTCs undergo drug specific secondary transitions resulting in permanent resistance characterized by a reversal into proliferative state. **Conclusion.**Our results imply that there is a requirement for alternative treatment strategies including drug holidays to prevent or delay the emergence of IDTCs and acquired drug resistance.

No conflict of interest.

10:45-12:30
Symposium: Melanoma Targeted Drugs

16 Improving patient outcomes to targeted therapies in melanoma

R. Marais[1]
1 Cancer Research UK Manchester Institute, Manchester, United Kingdom

The RAS/RAF/MEK/ERK pathway plays an important role in melanomagenesis. The NRAS gene is mutated in 20-30% of melanomas and the BRAF gene is mutated in a further 45-50% of cases. Drugs that inhibit this pathway are effective in melanoma patients bearing BRAF or NRAS genes, but most patients only achieve a transient response and develop resistance after a relatively short period of disease control. We and others have investigated the mechanisms of resistance to BRAF and MEK inhibitors and find that in most cases resistance is due to pathway reactivation, which is often mediated by upregulation of receptor tyrosine kinases (RTK) that signal through SRC, or by acquisition of mutations in NRAS that signal through CRAF, a close relative of BRAF. We have developed second-generation panRAF inhibitors that also inhibit SRC. These agents are effective in treatment-naïve BRAF mutant melanomas because they inhibit mutant BRAF directly. They are effective in BRAF mutant melanomas when resistance is mediated by upregulation of RTK signalling because they inhibit SRC, and in tumours where resistance is mediated by NRAS, because they inhibit CRAF. Compounds from this series are being advanced to the clinic for testing in resistant patients and to ensure the most efficient use of these and other drugs, we are implementing a precision medicine approaches in melanoma patients to ensure the best outcomes for our patients.

No conflict of interest.

17 Large-scale genetic in vivo perturbations to reveal novel cancer vulnerabilities

D. Peeper[1]
1 Netherlands Cancer Institute, Amsterdam, Netherlands

Melanoma is the most aggressive type of skin cancer and its incidence is steadily increasing. It tends to spread rapidly, which is associated with a grim prognosis. Until recently, most advanced stage melanomas were refractory to the available therapeutic options, but recent developments have been offering better perspectives. In particular, the discovery of the activating BRAFV600E mutation in roughly half of the melanomas has spurred the development of targeted therapies, which are associated with unprecedented clinical benefits. The small molecule inhibitor vemurafenib, specifically targeting the mutant BRAFV600Ekinase, was the first standard of personalized care for patients diagnosed with mutant BRAF metastatic melanoma. Although this compound initially reduces tumor burden dramatically, eventually melanomas become resistant and progress while on treatment. This occurs by acquisition of additional mutations or other alterations that affect the mitogen-activated protein kinase (MAPK) pathway, often leading to reactivation of extracellular signal-regulated kinase (ERK), thereby restoring signaling of the oncogenic BRAF/MEK/ERK pathway.

Therefore, in spite of these new perspectives, there is a dire need to identify additional targets amenable to therapeutic intervention, to be used in combination with vemurafenib or other specific inhibitors to overcome or prevent drug resistance and achieve more durable responses. To achieve this, we set out to identify melanoma factors that are required for proliferation and survival specifically in an in vivo setting. Thus, we performed negative selection RNAi screens parallel in vitro and in vivo and focused on the hits that were preferentially depleted in tumors relative to the corresponding cells in culture. The results from these screens will be discussed.

No conflict of interest.

18 Proffered paper: Suppression of oncogene transcription - PNA as novel targeted cancer therapy for BRAF-V600E mutant melanoma

J. Rothman[1], O. Surriga[2], S.D. Vasudeva[2], G. Ambrosini[2], G.K. Schwartz[2]
1 Memorial Sloan-Kettering Cancer Center, New York City, USA
2 Columbia University Medical Center, Herbert Irving Cancer Center, New York City, USA

Introduction. Our aim is to target tumor cells specifically by directly suppressing their oncogenes with peptide nucleic acid (PNA) oligonucleotide analogues. PNA oligonucleotides bind to DNA over 1000-fold more avidly than its native complement,

are completely resistant to intra and extra cellular enzymatic degradation, show no nonspecific toxicity at therapeutic levels, and when conjugated to delivery peptides can be made nuclear and cell membrane permeable. We have employed these PNA oligomers to target BRAFV600E which is prevalent in cutaneous melanoma in a sequence-specific complementary manner in order to disrupt transcription by strand invasion.

Materials and Methods. For these studies, we have employed a novel delivery peptide conjugated to PNA modified to increase both cellular delivery and PNA stability towards its target. We have assessed its ability to obstruct BRAFV600E transcription specifically in variety of cell lines by monitoring suppression of cell proliferation, BRAFV600E protein expression, and mRNA transcription. Tumor reduction was assessed through Xenograft mouse models.

Results and Discussion. Our results indicate that exposure of the melanoma cell lines to a modified PNA-peptide conjugate selective for BRAFV600E results in a concentration-dependent inhibition of cell growth that is specific for the BRAFV600E mutant melanoma cell lines with an IC50 range of 250 to 500 nM. Moreover, there is no inhibition of BRAFWT cell growth at these concentrations. This is associated with suppression of BRAFV600E protein over time with no effect on BRAFWT protein levels. Furthermore, BRAFV600E protein expression was suppressed for up to 6 days following initial exposure proving the durability of this type of inhibition. Exposure to this modified PNA-peptide down-regulates BRAFV600E mRNA transcription exclusively in the mutant cell lines. Live cell imaging of BRAFV600E mutant cells indicates localization of fluorescein-labeled PNA-delivery peptide specific to BRAFV600E to the nucleus within 3 hours of treatment. Xenograft mouse studies show reversal of tumor burden after four doses continuing for days following the last dose with a maximum tolerated dose to at least 50mg/kg.

Conclusion. Our results indicate that these PNA-peptide derivatives could represent a novel and promising new therapy for patients with BRAFV600E mutant melanoma, and this technology could be applied to a multitude of other cancers either with specific translocations or mutations differing from wild-type cells even by only a single base pair.

No conflict of interest.

19 Proffered paper: Novel therapeutic approaches by targeting CD74 expression in melanoma

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Features of chronic inflammation are accepted as contributing to progression of many cancers, including melanoma. We have recently showed that CD74 expression significantly associates with decreased risk in death and disease recurrence on univariate and multivariable analyses of stage III melanoma patients. CD74 percentage and intensity in tumor were significant predictors of a lower risk of death (HR 0.71, P = .004 for percentage; HR 0.59 P= .007 for intensity). CD74 in tumor infiltrating lymphocytes (TIL) was also highly significantly associated with overall survival (OS) with HRs of 0.54 (P < .0001) and 0.61 (P = .004). Additionally, higher CD74 in tumor cells was significantly associated with a lower risk of disease recurrence or death (HR, 0.73; P < .001 and HR, 0.6; P = .002). CD74 in TIL was also highly significantly associated with recurrence-free survival (RFS) with HRs of 0.65 (P = .002) and 0.68 (P = .001). We were also able to validate our findings in discovery set for CD74 in tumor (OS: HR, 0.27; P < .001 and RFS: HR, 0.45; P = .001). Therefore, we have identified and validated CD74 expression that is strongly predictive of survival in patients with stage III melanoma. More importantly, expression of CD74 adds substantial predictive information beyond stage and mutational status of stage III melanoma. Now, our focus is regulating CD74 expression and thus, the purpose of our current study is to identify novel drugs by screening a panel of sensitive compounds. We have found that potent and also very selective gamma secretase inhibitor, L-685458, strongly associates with CD74 upregulation. It also regulates CXCR4 and VEGFR2 expression, most likely through inhibition of Notch signaling. Our current effort is defining signaling pathways and biologic functions of this drug to inhibit melanoma growth, in vitro, by regulating CD74 expression in melanoma cells.

No conflict of interest.

20 Melanoma resistance to targeted therapy: A translation issue?

No abstract received.

14:30-16:15
Symposium: Tumour Heterogeneity

21 No abstract received.

22 Heterogeneity of acquired resistance to active targeted inhibitors of BCR-ABL and FLT3

No abstract received.

23 Proffered paper: Tumor heterogeneity contributes to anti-EGFR therapy resistance in glioblastoma

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Introduction. Glioblastoma is the most aggressive tumor type affecting the adult brain, with an overall patient survival from the time of diagnosis of about 15 months. Tumor promoting proteins such as EGFR and its mutant form, EGFRvIII, are amplified in a subgroup of patients and have attracted attention as potential therapeutic targets. Unfortunately, kinase inhibition of EGFR or EGFRvIII has been proven to be ineffective due to resistance mechanisms that prevent the onset of cell death. Here we hypothesize that the presence of cytokines in the tumor microenvironment, in addition to their previously shown promotion of glioblastoma growth, also promote resistance to EGFR-directed therapies. To address this, we investigated core pathways known to protect cancer cells from induced cell death and tested novel treatment strategies of EGFR blockade in combination with inhibitors of cytokine-induced signaling effectors, activated by inter-clonal communication between heterogeneous tumor cell populations expressing EGFRvIII and EGFR.

Material and method. For these studies we used human and mouse glioma cell lines engineered to express wild type (wt) EGFR, EGFRvIII, or an analog-sensitive (as)-allele of EGFRvIII, as well as primary human glioma neurospheres expressing amplified EGFRvIII. Expression of cell death-related molecules was determined by RT-qPCR to identify potential survival factors regulated by conditioned medium (CM) produced from EGFRvIII-expressing glioma cells. Findings were validated in vivo by orthotopic engraftment of heterogeneous tumors and measurement of tumor burden by fluorescence molecular tomography technology during single and combined drug therapy regimens.

Results and discussion. Previous results demonstrated that CM from EGFRvIII-expressing glioma cells enhances in vitro and in vivo growth of wtEGFR cells through an EGFRvIII-prompted IL-6/LIF paracrine mechanism. Here, our data indicate that EGFRvIII-CM additionally promotes resistance to EGFR inhibition in cells and tumors expressing wtEGFR. We show that IL-6/gp130-mediated signaling specifically promotes this resistance through mTORC2/NF-kB upregulation of the anti-apoptotic protein, Survivin (BIRC5). Blocking Survivin expression or pharmacologically inhibiting mTORC2 restores sensitivity to EGFR inhibitors.

Conclusion. The mTORC2/NF-kB/Survivin axis promotes resistance to EGFR inhibitors in glioblastoma and nominates targeting this pathway as a means to enhance efficacy of EGFR blockade.

No conflict of interest.

24 Proffered paper: Prospective identification of RET resistance mutations predicted to emerge from treatment with multi-kinase inhibitors and the development of potent, selective inhibitors that address these mutations

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Introduction. Treatment of numerous cancer types with targeted therapies, including ALK- and ROS-fusion positive lung cancers, has generated on-target resistance mutations driving disease progression. Oncogenic fusions of the RET receptor tyrosine kinase have more recently been identified in 1-2% of non-small cell lung adenocarcinomas. Consequently, a number of multi-kinase inhibitors such as cabozantinib, vandetanib, and ponatinib have moved into phase 2 trials in the RET fusion lung cancer patient population. We sought to prospectively identify RET resistance mutations that may arise after treatment with these multi-kinase inhibitors.

Materials and Methods. The structure of RET bound to several inhibitors was analyzed to predict amino acid substitutions that would disrupt the protein-inhibitor interaction, and in vitro resistance screens with cabozantinib, ponatinib, regorafenib and vandetanib in a Ba/F3-KIF5b-RET cell line confirmed these predictions. The proprietary Blueprint Medicines kinase inhibitor library was screened to identify inhibitors of wild type and resistance mutant RET which were used as medicinal chemistry starting points for lead optimization.

Results and Discussion. Both structural analysis and in vitro resistance screening revealed that only a handful of positions within the RET kinase domain provide resistance mutations to the multi-kinase inhibitors. This suggests a narrow mutational spectrum to this group of inhibitors and defines the profile of a next generation RET inhibitor encompassing both wild type RET and resistance mutant activity. Using our library of de novo designed kinase inhibitors, we have identified potent, orally bioavailable inhibitors of RET that maintain activity against both the wild type and resistance mutants in both KIF5b-RET-driven cell lines and in vivo xenograft models while sparing the majority of the human kinome. Furthermore, we have established a PK-PD-efficacy relationship in these RET fusion models which

demonstrates that over 80% target suppression is required for maximal tumor growth inhibition.

Conclusion. This work reveals the potential to create selective RET inhibitors addressing oncogenic RET fusions while overcoming the on-target resistance mutations likely to emerge from treatment with currently available therapies and provide therapeutic benefit to patients with RET-driven disease.

Conflict of interest: Ownership: Blueprint Medicines

25 Mouse models of lung cancer: Tumour heterogeneity and cell-of-origin of thoracic tumors

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Lung cancer and mesotheliomas belong to the most lethal human malignancies with poor prognosis. The majority of these tumors is associated with carcinogen exposure (smoking and asbestos). Small cell lung cancer (SCLC) and mesothelioma patients show very poor survival statistics due to their late detection, invasive and high metastatic potential, and chemo-resistance. Using the Rbf/f;p53f/f mouse model for SCLC, we found that the tumors are often composed of phenotypically different cells, characterized by mesenchymal and neuroendocrine markers. These cells often share a common origin. Crosstalk between these cells can endow the neuroendocrine component with metastatic capacity, illustrating the potential relevance of tumor cell heterogeneity in dictating functional tumor properties. Also specific genetic lesions appear to be associated with metastatic potential. We have studied the nature of this crosstalk and identified the components responsible for paracrine signaling and the downstream effector pathway critical for promoting metastatic spread.

We have also evaluated the relevance of additional lesions that were frequently acquired in the mouse SCLC, such as amplification of Myc and Nfib. Therefore, we have derived ES cells from Rbf/f;p53f/f, equipped these cells with an exchange cassette in the Col1A1 locus, and shuttled a conditional L-Myc and Nfib under a strong promoter into this locus. This accelerated tumorigenesis and resulted also in a shift in the metastatic phenotype.

To investigate the cell-of-origin of thoracic tumors, we have inactivated a number of tumor suppressor/oncogene combinations (Trp53, Rb1, Nf2, Cdkn2ab-p19Arf, mutant Kras) in distinct cell types by targeting Cre-recombinase expression specifically to Clara cells, to neuroendocrine cells, alveolar type II cells and cells of the mesothelial lining (origin of malignant mesothelioma) using adenoviral or lentiviral vectors with Cre recombinase driven from specific promoters. Dependent on the induced lesions and the cell-type specific targeting, SCLC, NSCLC, or mesothelioma could be induced. We show that multiple cell types can give rise to these tumors but that the cell-of-origin is an important factor in determining tumor phenotype.

Our data indicate that both cell type specific features and the nature of the oncogenic lesion(s) are critical factors in determining the tumor initiating capacity of lung (progenitor) cells. Furthermore, the cell-of-origin appears to influence the malignant properties of the resulting tumors.

Conflict of interest: Corporate-sponsored research: Janssen Pharmaceutica N.V. Specific drug target evaluation in a SCLC mouse model

16:45-18:15
Symposium: Circulating Tumour Cells

26 Cancer biomarkers in circulating tumor DNA

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The usefulness of tissue biopsies is hampered by intratumor molecular heterogeneity as well as by cancer genomic evolution occurring over time and upon therapy. Recent works have proposed to use minimally invasive 'liquid biopsies' as an alternative to tissue biopsies. Liquid biopsies include circulating tumor cells and fragments of circulating tumor DNA (ctDNA) that are shed by dying neoplastic cells or otherwise actively secreted in the bloodstream. I will summarize here potential clinical applications of ctDNA.

First, ctDNA has been found to recapitulate genetic and epigenetic alterations found in the tumor and therefore can be used to characterize cancer molecular profiles in lieu of tissue. However, since ctDNA can be diluted with nucleic acids deriving from non-cancerous cells, highly sensitive and sophisticated methods are needed for the analysis. Despite these technical limitations, in some instances ctDNA might be preferable to core biopsies as it could better reflect and capture the molecular heterogeneity seen in metastatic malignancies.

Second, prominent works have shown that ctDNA often provides the earliest measure of treatment response in solid cancers. This approach has some caveats, since the use of ctDNA for monitoring tumor volume may be negatively impacted by several variables, including tumor heterogeneity, copy number alterations, rate of release, tumor histology, vascularity, lymphatic drainage, renal function and clearance. Nevertheless, ctDNA has shown superior sensitivity to that of other circulating biomarkers and has a greater dynamic range that correlates with changes in tumor burden.

Third, levels of ctDNA usually increase with tumor stage and are higher in metastatic patients. Early-stage cancer patients have less ctDNA – yet the ability to detect ctDNA in these patients may be of greater utility. Indeed, analysis of cancer specific genetic or epigenetic alterations, such as point mutations or methylation changes, could prove valuable for diagnostic purposes (early detection) as well as for monitoring minimal residual disease after resection of solid tumors.

Lastly, ctDNA can be used to study tumor evolution with therapy. Acquired resistance to target therapies is often caused by the emergence of clones carrying different molecular lesions. To investigate tumor changes upon therapy, tissue biopsies can be obtained with inherently risky procedures and could induce sampling errors. On the other hand, ctDNA analyses are minimally invasive and could better capture tumor complexity, making them ideal for repeated genotyping over multiple timepoints. Indeed, ctDNA has been key to discover novel mechanisms of secondary drug resistance in solid tumors. In the future, the analysis of ctDNA may also aid in testing novel therapeutic strategies to delay or overcome acquired drug resistance.

No conflict of interest.

27 Versatility of circulating tumour cells in lung cancer: Biology, drug development and biomarkers

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The ability to obtain serial biopsies from the majority cancer patients remains a significant challenge and for some patients this is not without risk. Moreover, it is becoming clear that a small biopsy from a primary tumour of a metastatic lesion fails to capture intratumoural heterogeneity sufficiently well and selecting and obtaining adequate biopsy material from oligometastatic patients presents further challenge. With this in mind, the development of circulating biomarkers to monitor tumour evolution and to inform the delivery of precision medicine is paramount. Our laboratory has focussed on the detection, enumeration and molecular characterisation of circulating tumour cells (CTCs) in lung cancer, and specifically in small cell lung cancer (SCLC). In SCLC, CTCs detected by the EpCAM dependent CellSearch platform are prevalent (up to 1000s of CTCs in 7.5ml blood), the CTC number thus detected is prognostic ('cut off' 50 CTCs/7.5ml blood), the dynamic range is sufficient for a pharmacodynamic biomarker and predictive CTC based biomarkers are feasible (examples will be presented). New technology platforms can now isolate individual CTCs and we are beginning to interrogate CTC heterogeneity and its relevance to patient outcomes. Most SCLC patients respond well to platinum based chemotherapy intially but relapse wihtin 3-18 months with progressive disease, whilst ~20% SCLC patients are initially chemorefractory. What CTCs offer at multiple timepoints is a full genome and transcriptome analysis at the single CTC level. We are currently comparing the whole exome sequences of single CTCs from chemosenstive and chemorefractory patients, and conducting longitudinal analysis of patients CTCs sampled both at baseline and again at relapse following initial treatment response to identify genetic changes associated with therapy resistance. Most recently we expanded the utility of CTCs by developing CTC derived patient explant models (CDX), Hodgkinson et al Nature Medicine, 2014)). We developed CDX where CTCs were enriched at baseline from chemosensitive or chemorefractory SCLC patients and for some patients a second model was derived at patient progression. CDX recapitulate the patients' responses to chemotherapy and are now being exploited to search for new drug targets (via genomic, transcriptomic and proteomic analyses), to test treatment combinations and explore treatment resistance mechanisms. Whilst there have been significant breakthroughs in recent years, one remaining limitaiton for CTC research and its more routine application to clincal trials remains the lack of a fully validated marker independent technology platform for CTC enrichment that enriches for both epithelial and mesenchymal CTCs. However, an increasing number of promising novel CTC technologies are under evaluation and will be discussed.

No conflict of interest.

28 Proffered paper: Long-range regulators of HOTAIR as prognostic biomarkers for the stratification of hormone receptor positive breast cancer

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Introduction. Prediction of breast cancer progression and response to therapy is essential for effective clinical management. Whilst a number of histological and molecular biomarkers are available, their predictive power is limited. Overexpression of the non-coding RNA HOTAIR is known to promote metastasis and be predictive

of poor prognosis in breast cancer. The study uncovers the molecular mechanisms of HOTAIR transcriptional regulation in breast cancer in order to identify clinical biomarkers of disease progression and endocrine therapy resistance.

Materials and Methods. HOTAIR control elements were identified using a combination of bioinformatic analysis of ENCODE data and experimental validation using cell-based reporter assays. Analysis of chromatin interactions was done using quantitative chromosome conformation capture. A range of cell-based assays were used to explore the role of estrogen and transcription factors in the regulation of HOTAIR. Hierarchical clustering analysis used TCGA data, while stratification of breast cancers was performed using clinically informative METABRIC and UNC datasets. The clinical significance of the identified HOTFOX module was established by comparing expression with standard clinico-pathological indicators of the METABRIC cohort using univariate and multivariate survival analysis.

Results and discussion. A long-range transcriptional enhancer of the HOTAIR gene that binds several hormone receptors and associated transcription factors, interacts with the HOTAIR promoter and augments HOTAIR transcription is described. HOTAIR is co-expressed with a network of genes implicated in regulating the cell cycle and chromosome instability. Expression of this gene network (named HOTFOX) is strongly associated with chromosome instability in estrogen receptor positive tumours and stratifies luminal breast cancer on the basis of survival and response to endocrine and chemotherapy. Importantly, a minimal set of three HOTFOX genes displays greater predictive power than the clinically used Oncotype Dx and Mammprint gene panels.

Conclusion. Our studies elucidate the transcriptional regulation of HOTAIR, implicate HOTAIR in the regulation of cell cycle and chromosome instability, and identify a new network of genes with prognostic potential for breast cancer.

No conflict of interest.

29 Proffered paper: cfDNA profiling of non-small-cell lung cancer using Ion Torrent NGS

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1 University of Leicester, Cancer Studies, Leicester, United Kingdom
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Background. Lung cancer is a leading cause of cancer-related death worldwide. Tumour heterogeneity and late diagnosis are major challenges for successful treatment. As cancer patients have variable amounts of circulating free DNA (cfDNA) in the plasma, accurate identification of tumour specific mutations in the tumour derived fraction of cfDNA, termed circulating tumour DNA (CtDNA), could help inform therapy. With the same venepuncture it is also possible to isolate lymphocyte DNA as a normal control. The first phase of this study aimed to evaluate SNPs calling concordance between matched cfDNA and lymphocyte DNA in a cohort of 20 healthy controls, using Ion AmpliSeq™ Cancer Panel v2 and AmpliSeq customised Colon and Lung Cancer Panel. The second phase saw the use of AmpliSeq customised Colon and Lung Cancer Panel for detection of mutations in cfDNA of 20 patients with advanced non-small-cell lung cancer (NSCLC). The third ongoing phase is focussed on the development and testing of a new customised AmpliSeq panel for NSCLC.

Methods. cfDNA was extracted using the Qiagen circulating nucleic acids kit. 1ml of plasma was used from 40 patients with advanced NSCLC (ReSoLuCENT study). 3 ml of plasma was used for all the healthy controls. cfDNA yield was determined by real-time qPCR. All samples with cfDNA yield between 3ng and 10ng were sequenced and variants were identified using the recommended Variant Caller plugin and stringency settings. The NSCLC AmpliSeq panel was designed using the Ion AmpliSeq Designer Software.

Results. No differences were observed between cfDNA and genomic DNA SNPs profiles in the 20 healthy controls, for all the panels in analysis. With the customised Colon and Lung Cancer Panel, it was possible to identify somatic variants in 25% of patients with advanced NSCLC. In particular we observed 4 distinct TP53 missense mutations, an intronic low frequency SNP present in two different patients in ERBB4 and a coding silent substitution in EGFR. None were present in matching genomic DNA from lymphocytes.

Conclusions. The first two phases of this study confirm the potential of Ion Torrent NGS for the detection of somatic mutations in cfDNA, with the caveat that samples with lower cfDNA concentration tend to produce less confident calls with lower coverage. The Colon and Lung Cancer AmpliSeq Panel detected somatic variants in cfDNA of a subgroup of the patients with NSCLC. The development of a more focussed NSCLC-specific panel with shorter amplicons and with better coverage of fewer key genes driving lung cancer progression could help to increase the diagnostic power of this sequencing approach. Moreover, through larger cohort studies it will be possible to identify common mutations suitable for qPCR or droplet digital PCR screening at higher sensitivity for ctDNA.

No conflict of interest.

18:15-19:00
Keynote Lecture: Cancer Genomics and Drug Resistance

30 Cancer genomics and drug resistance

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Genomic studies of cancers in the targeted therapy setting are providing novel insights to the nature of drug resistance. This trend is further enabling monitoring of tumor response and progression from liquid biopsy and other direct monitoring procedures that further utilize the genomic information to inform oncology care. Our work has focused on developing this paradigm along three different lines of research with an aim toward clinical translation. AML studies are exploring the use of massively parallel sequencing-based monitoring to detect the persistence or loss of mutations during the timeframe of induction chemotherapy to indicate patients who are treatment refractory or likely to quickly relapse. Our breast cancer studies are focused on determining a primary tumor signature that is indicative of late relapse disease development in the ER+ setting. A new application of genomics will study how standard of care therapies such as chemo- and radiotherapy are perhaps creating a mutational landscape predictive of response to immunotherapeutic modalities.

MONDAY 22 JUNE 2015

07:30-08:30
Meet the Expert Session

31 Clonal evolution and drug resistance: From cancer avatars to liquid biopsies

A. Bardelli[1]
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It is now evident that colorectal cancers (CRC) indistinguishable by light microscopy are molecularly distinct diseases requiring unique therapeutic approaches. Tissue and liquid biopsies can be used to define CRC molecular subtypes and to monitor clonal evolution during therapy. Using these approaches, CRC patients were found to respond selectively to targeted agents interfering with oncogenic nodes of the EGFR signaling pathway. Notably, the patient-specific responses can be recapitulated and paralleled in cellular and mouse clinical proxies (CRC-avatars). The inevitable development of acquired resistance to inhibitors of the EGFR signaling pathway presently limits further clinical advances. Strategies to prevent or overcome resistance are therefore essential to design the next generation of molecularly-driven clinical trials for CRC patients.

No conflict of interest.

07:30-08:30
Meet the Expert Session

32 Applications of patient-derived xenograft models to translational cancer research

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In the last few years there has been a growing interest in the development of patient-derived tumor xenograft (PDX) models for cancer research. These models, in fact, offer several advantages compared to in vitro and in vivo models derived from conventional stable cell lines.

Usually PDX models recapitulate the histologic and genetic characteristics of the tumors from which they derive and do not change across further in vivo passages.

Several works have shown that PDXs are endowed with predictive clinical value and that they are of incredible importance in the field of precision medicine as they can be used for preclinical trials testing drug efficacy and for biomarker identification.

We will present current techniques to generate PDX models, discuss the pro and cons of using these models and their current applications, and summarize the most important results obtained with PDX models in the field of cancer therapy

No conflict of interest.

08:30-09:15
Keynote Lecture: Angiogenesis

33 Angiogenesis revisited: Role and therapeutic potential of targeting endothelial metabolism

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Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to starve cancer cells from nutrients. Clinically approved anti-angiogenic drugs prolong the survival of cancer patients, but their success is limited by intrinsic refractoriness and acquired resistance. New strategies are thus needed to block tumor angiogenesis via alternative mechanisms. We recently reported that PFKFB3-driven glycolysis regulates the endothelial tip cell function during vessel sprouting, even capable of overruling the potent pro-stalk activity of Notch, and that its loss in endothelial cells causes vascular hypobranching defects. Moreover, partial and transient reduction of glycolysis by blocking PFKFB3 reduced pathological angiogenesis in several disease models. Ongoing studies explore the role of lipid and amino acid metabolism in vessel sprouting, and assess the therapeutic potential of targeting these metabolic pathways for anti-angiogenic therapy.

No conflict of interest.

09:15-10:15
Proffered Papers 2

34 Proffered paper: p140Cap scaffold protein, a new prognostic marker in ERBB2 breast cancer, limits ErbB2 breast cancer progression in a preclinical animal model

S. Grasso[1], I. Russo[1], V. Salemme[1], F. Cavallo[1], A. Amici[2], M. Iezzi[3], P. Di Stefano[1], E. Turco[1], P. Defilippi[1]
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2University of Camerino, Bioscience and Veterinary Medicine, CAMERINO, Italy
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Introduction. p140Cap is a multisite docking protein that behaves as a negative regulator of breast tumor growth and metastasis formation. We have previously shown that the tuning of p140Cap expression, via gain and loss of function approaches in breast cancer cells, is sufficient to dramatically change cell migratory and invasive properties as well as in vivo tumor growth. Based on our data showing that p140Cap expression correlates with a lower risk of developing distant metastases and improved patient survival of ERBB2 patients, we aimed to investigate whether the p140Cap protein may be causally linked in the negative control of ERBB2 tumorigenesis.

Materials and method. The NeuT mice, a preclinical model of spontaneous ERBB2 breast tumors, were crossed with mice over-expressing p140Cap in the mammary gland. Primary cancer cells were analyzed in 2D and 3D conditions and in vivo with biochemical and functional assays.

Results and discussion. p140Cap expression in the NeuT mice delayed in vivo tumor onset and caused decreased tumor growth, leading to the development of more differentiated, lower grade mammary carcinomas. To further assess p140Cap molecular mechanisms, primary cancer cells were used. p140Cap strongly limited the in vivogrowth and invasive features of primary NeuT tumor cells. In 3D Matrigel, while NeuT cells formed large multi acinar structures of irregular shape without lumen, p140Cap expressing cells formed acina-like small sphere structures that often present a lumen, indicating that p140Cap restores mammary cell polarity disrupted by NeuT. Rac1 small GTPase has been reported as a major effector in HER2-mediated breast cancer progression to metastasis. p140Cap negatively modulated the activity of Rac1 and of its main GEF, TIAM1. Furthermore, p140Cap bound to TIAM1, suggesting that this association leads to its inhibition. Indeed, treatment of NeuT cells with a Rac1 inhibitor has restored the acina phenotype as p140Cap does. Taken together, these data show that, in the context of the NeuT oncogene, p140Cap effectively controls the activation of Rac1.

Conclusion. Together, our preclinical model and 3D analysis reveals that p140Cap is causative in limiting NeuT tumor growth exerting a suppressive function on signaling molecules involved in tumor progression, like the Rac1 GTPase pathway. Inhibition of these pathways allows restoring mammary acina morphogenesis in breast tumors.

No conflict of interest.

35 Proffered paper: Overexpression of HER2 and EGFR supports the osseous growth of prostate cancer cells

G. Lorenzatti[1], K.C. Day[1], S.J. Dawsey[1], A.R. Paul[1], S. Daignault-Newton[2], N. Palanisamy[3], L. El-Sawy[1], M.L. Day[1]
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3University of Michigan Medical School, Pathology, Ann Arbor MI, USA

Introduction. Epidermal Growth Factor Receptor 1 and 2 (Erb1/EGFR, ErbB-2/HER2) overexpression in prostate cancer revealed their association with disease progression, tumor behavior and patient outcome. The most prominent site of prostate cancer metastasis is the bone. The intent of the current study was to evaluate functional significance of HER2 and EGFR over-expression in the osseous growth of human prostate cancer cells.

Materials and Methods. Analysis of prostate tumor microarrays (TMA) (including primary cancer, lung and liver metastasis as well as bone metastasis) were performed using two distinct HER2 antibodies and a EGFR monoclonal antibody. HER2 gene copy number was tested by FISH analysis. C42B cells were used as a model of prostate bone cancer growth. Cells were tested for tumor initiating ability (sphere formation assay) and tumor growth in the bone (tibia injection model in SCID mice). Inhibitors of EGFR and HER2 were utilized as treatment in these animals.

Results and Discussion. Analysis of patient's prostate tumors revealed elevation of HER2 and EGFR protein expression in metastatic bone lesions compared to primary tumors. Increased HER2 expression in bone metastases was independent to gene amplification. To analyze the tumor initiating cell potential C42B cells were grown under detachment conditions. We found high EGFR expressing cells demonstrated a higher sphere forming potential than high HER2 positive prostate cancer cells. In addition, we found a reduced number of prostate spheres, correlating with low EGFR expression. HER2 knockdown slightly reduced bone associated tumor growth that promoted reduced Ki67 expression in xenografted tibiae. However, EGFR levels remained high; where a suggestive compensatory role for EGFR may support survival mechanisms of tumor cells in the absence of HER2. This was confirmed by simultaneous targeting of both receptors with a combination of therapeutic antibodies or a dual tyrosine kinase inhibitor that ablated the viable tumors.

Conclusion. We found that both HER2 and EGFR are highly expressed in bone metastasis. These two receptors are key players in metastatic prostate cancer and collaborate to the progression of the osseous lesions.

No conflict of interest.

36 Proffered paper: ATM kinase modulates HER2 tumorigenicity in breast cancer

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Background. ATM kinase is a major guardian of genome stability and it is mainly considered a tumor suppressor gene. However, its identification as a component of several signalling pathways, activated in cancer cells, suggests a dualism for ATM in cancer. HER2/ERBB2 is a Receptor Tyrosine Kinase (RTK) aberrantly activated in a subset of aggressive breast cancers. The identification of ATM kinase activation downstream RTKs, among which HER2, along with the observation that ATM sustains tumorigenic signals such as AKT activation, suggest that HER2 tumors may provide a model system suitable for testing whether ATM may differently modulate tumor progression depending on the specific signalling pathways that sustain tumorigenicity.

Material and methods.RNA interference approaches to genetically downregulate ATM expression; KU-55933 inhibitor to pharmacologically downregulate ATM kinase activity; immunoprecipitation and immunoblotting analysis; in vitro and in vivo tumorigenicity assays; immunohistochemistry (IHC).

Results and discussion. We demonstrated that ATM targeting by small RNA interference significantly impairs HER2-dependent tumorigenicity in vitro and in vivo. Moreover, the pharmacological inhibition of ATM activity significantly decreases tumor multiplicity and growth in transgenic MMTV-NeuT mice, which represent a well-established model for HER2-dependent breast cancer development. We identified ATM as a novel modulator, in vitro and in vivo, of HER2 protein stability. ATM promotes HER2 interaction with HSP90 chaperone, therefore preventing its ubiquitination and degradation. Importantly, ATM activity modulates the response to therapeutic approaches, supporting the idea that ATM expression sustains HER2 function. Consistently, patients (not treated with trastuzumab) bearing ATM-phosphorylation-positive/HER2-positive tumors have a shorter disease free survival compared to ATM-phosphorylation- negative/HER2-positive tumors, suggesting that the identification of ATM phosphorylation may have a prognostic and therapeutic significance.

Conclusions. Our findings provide evidence for a novel role of ATM as promoter of HER2 tumorigenicity suggesting a more complex function of ATM in cancer, in addition to the canonical role of ATM as pure tumor suppressor.

No conflict of interest.

37 Proffered paper: hMENA11a sustains HER3 activation and contributes to HER3-mediated resistance to PI3K inhibitors

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Introduction. Human Mena (hMENA), an actin regulatory protein of the ENA/VASP family, is overexpressed in high-risk preneoplastic lesions and in primary breast tumors where it correlates with HER2 overexpression and an activated status of AKT and MAPK. The concomitant overexpression of hMENA and HER2 identifies breast cancer patients with a worse prognosis.

The aim of this study is to investigate the role of hMENA epithelial isoform, namely hMENA11a, in HER3 activation and HER3-mediated mechanisms of resistance to PI3K inhibition in HER2+ breast cancer cells.

Material and Methods. The phospho-proteomic profiles of MDA-MB-361 cells, before and after hMENA11a-specific silencing (si-hMENA11a), untreated or NRG-1 treated, were assessed by the Reverse Phase Protein Array (RPPA). MDA-MB-361 and MCF7-HER2 cells, control and si-hMENA11a, untreated or treated with PI3K/mTOR inhibitor BEZ235, were analyzed for: HER3/AKT axis activation by Western Blotting (WB), HER3 mRNA levels by qRT-PCR, FOXO3a sub-cellular localization by immunofluorescence and cell cycle distribution by flow cytometry. hMENA11a phosphorylation status was evaluated by 2D WB. Cell resistance to BEZ235 was analyzed in 2D and 3D cells.

Results and discussion. By RPPA we identified a novel role for the epithelial associated hMENA11a isoform in sustaining HER3 activation and pro-survival pathways in HER2 overexpressing breast luminal cancer cells. The specific hMENA11a depletion counteracted the up-regulation and activation of HER3-mediated by BEZ235 and impaired the nuclear accumulation of FOXO3a transcription factor induced by PI3K inhibitors. PI3K inhibitor treatment increased hMENA11a phosphorylation and affected its sub-cellular localization. At functional level, we found that hMENA11a sustains cell proliferation and survival, whereas hMENA11a silencing increases molecules involved in cancer cell apoptosis and sensitizes cells to PI3K inhibition, as seen in 2D and in 3D cultures.

Conclusions. hMENA11a sustains HER3/AKT axis activation and contributes to HER3-mediated resistance mechanisms to PI3K inhibitors in HER2+ breast cancer cells. hMENA11a expression can be proposed as a marker of HER3 activation and of

resistance to PI3K inhibition therapies, to select patients who can benefit from these combined targeted treatments. hMENA11a activity may represent a new target for anti-proliferative therapies in breast cancer.

No conflict of interest.

38 Proffered paper: Combined inhibition of Ddr1 and Notch signaling is an effective therapeutic strategy to treat K-Ras-driven/p53-null lung adenocarcinomas

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Introduction. K-RAS is the driver oncogene in 30% of lung adenocarcinomas. Unlike patients bearing other driver mutations such as those in the EGF or ALK receptors, there are no selective therapies available for these tumors. In an attempt to identify targets whose activity might be imperative for all stages of tumor progression, we focused on the identification of genes involved in the earliest stages of tumor development.

Materials and Methods. We used a GEM model of lung adenocarcinoma driven by a resident K-RasG12V oncogene. In this GEM, the K-RasG12V allele also expresses a color marker that allows identification of K-RasG12V expressing cells at the single cell level in tissue sections. Thanks to this strategy we isolated early K-RasG12V-driven hyperplastic lesions (about 500 cells) and analyzed their gene expression profiling by Affimetrix GeneChip hybridization.

Results and discussion. We found that K-RasG12V-driven hyperplastic lesions display two independent transcriptional profiles. Whereas one of them is related to that of normal lung cells, the other resembles the profile of advanced human tumors. This aggressive signature is characterized by increased expression of the protein tyrosine kinase receptor Ddr1. Genetic analysis using mice devoid of Ddr1 revealed that it plays a key role in tumor initiation and progression in a p53-proficient background. Pharmacological inhibition of Ddr1 mimicked these genetic results. Moreover, concomitant inhibition of Ddr1 and Notch signaling, a downstream mediator of Ddr1 activity, induced significant anti-tumor effects even in aggressive K-RasG12V/p53-null tumors. Indeed, the therapeutic activity of the targeted therapy combining Ddr1+Notch inhibitors was improved compared to that observed with standard chemotherapy. This targeted treatment had lower toxic side effects. HES1 and DDR1 display a strong tendency to co-expression in human K-RAS driven adenocarcinomas. Furthermore, preliminary data indicate that activating mutations in DDR1 mediate chemoresistance suggesting that its pharmacological inhibition may be therapeutically relevant.

Conclusions. The aggressive profile of K-RasG12V lung adenocarcinoma is determined early during tumor initiation.

- The Ddr1 tyrosine protein kinase receptor is required for tumor development.
- Combined pharmacological inhibition of Ddr1 and Notch signaling hampers the growth of K-RasG12V/p53-null lung adenocarcinomas and is more effective than standard chemotherapy.

No conflict of interest.

39 Proffered paper: Gain of function mutant p53 proteins cooperate with E2F4 to transcriptionally downregulate RAD17 and BRCA1 gene expression

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Introduction. Genomic instability (IN) is a common feature of many human cancers. The TP53 tumour suppressor gene is mutated in approximately half of human cancers. In vitro and in vivo evidences pointed out that GOF (Gain of Function) mutant p53 proteins promote invasion, metastasis and structural chromosomal changes resulting in high levels of genomic instability in tumors of different tissue origin. Failure of DNA repair results in apoptosis to prevent accumulation of unrepaired DNA and propagation of mutated DNA. HNSCC samples present high IN and TP53 is frequently mutated (78%). This is consistent with the fact that proteins involved in DNA repair, such as Rad17, ATM and MRE11 are often down-regulated in HNSCC. Surprisingly, despite the prevalence of mutp53 in human cancers, the understanding of how mutp53 imposes its GOF and how it is regulated are poor. Materials: CAL27 (head and neck cancer cell line; mutant p53), SKBr3 (breast cancer cell line; mutant p53), H1299 (lung carcinoma cell line; p53 null); expression vector mutantp53R175H; siRNAs for p53 protein; Antobodies to detect p53, E2F4, RAD17, BRCA1, GAPDH. Oligonucleotides for RT-qPCR analysis.

Methods. RT-qPCR analysis for gene expression; western blotting and immunoprecipitation experiments; Chromatin immunoprecipitation (ChIP); Comet assays; NHEJ DNA repair in vitro assay

Results and Discussion. Here we show that RAD17 and BRCA1 genes, whose derived proteins play a pivotal role in DNA damage repair, are transcriptional targets of gain-of-function p53 mutant proteins. Indeed, high levels of mutant p53 protein facilitate DNA damage accumulation and severely impair RAD17 and BRCA1 expression in proliferating cancer cells. By chromatin immunoprecipitation assay (ChIP) we show that the recruitment of mutp53/E2F4 complex onto specific regions of RAD17 and BRCA1 promoters leads to the inhibition of their expression.

Furthermore, BRCA1 and RAD17 protein expression is reduced in Nead and Neck carcinoma patients (HNSCC) carrying TP53 mutations when compared to those bearing wt-p53 gene.

The analysis of gene expression databases for breast cancer patients reveals that low expression of DNA repair genes correlates significantly with reduced relapse free survival of patients carrying TP53 gene mutations. Collectively, these findings highlight the direct involvement of transcriptionally active gain of function mutant p53 proteins in genomic instability through an aberrant impairment of DNA repair mechanisms. **Conclusions.** Since inhibitors of the aberrant kinase activity of DNA damage components are already used in cancer therapy, our data might contribute: a) to better define the molecular events underlying inefficient DNA repair in mutp53 tumour cells and consequently to tailor more accurately target specificity; b) to design therapeutic protocols that might combine kinase inhibitors with compounds interfering with mutp53 oncogenic activities.

No conflict of interest.

10:45-12:30
Symposium: Microenvironment

40 Learning from imaging the failure of targeted therapy

No abstract received.

41 Tumor microbiome mediated-chemoresistance

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Resistance to chemotherapy in advanced cancer patients is a pressing problem. Despite a sharp surge in novel anti-cancer drugs, complete clinical response to chemotherapy is very rare and the onset of resistance is almost always the rule. Previously, we have studied the effects of the tumor microenvironment on the innate, up-front resistance to chemotherapy, demonstrating that normal (non-cancer) cells inside tumors can render cancer cells resistant to chemotherapy. In the current work we extended our work to study the role that bacteria in the tumor microenvironment might have on chemoresistance – focusing on the sensitivity of pancreatic ductal adenocarcinoma to cytotoxic therapies like gemcitabine and oxaliplatin. After careful characterization of the bacteria that reside in human pancreatic tumors we used in-vitro and in-vivo models to test for the effects of these bacteria on chemoresistance and dissected the molecular mechanisms that underlie these effects. In the talk I will also present our recent efforts to characterize the tumor microbiome across many other tumor types and the development of high throughput screens to allow a more systematic study of the effects of the tumor microbiome on cancer progression and chemoresistance.

No conflict of interest.

42 Proffered paper: A c-MET inhibitor reduces bone metastases induced by renal cancer stem cells

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Introduction. Renal cancer patients develop a high rate of destructive bone metastases. In solid tumors, cancer stem cells (CSCs) are directly involved in the bone metastatic process due to their interaction with bone microenvironment, thus therapeutic strategies to block it are currently under investigation. Since activating mutations of c-MET are associated to some renal tumors and C-MET mediates the interaction between cancer cells and mesenchymal cells of the bone microenvironment, we hypothesized that targeting c-MET will lead to bone metastases inhibition. Materials and Method We utilised NOD/SCID mice, previously implanted with a small fragment of human bone to study the ability of renal CD105+ CSCs, isolated from human cancer patients, to metastasise bone and how a c-MET inhibitor (JNJ) interfere with this process. After the injection of CSCs, mice were daily treated or not with JNJ for 90 days, then sacrificed and implanted bone, lungs and blood were retrieved for analysis.

Results and Discussion. Renal CSCs colonized human implanted bone but not mice bone, leading to a specie-specificity of those cells to metastasize human bone. We then found that the JNJ treatment delayed the tumor growth and inhibited the metastases at bone implant site. To study the effect of JNJ on bone turnover and on the activity of osteoclasts (OCs) and osteoblasts (OBs), we performed histomorphometry. This analysis shows that CSCs induced an activation of OCs corresponding to an increase erosion surface, whereas the OB activity diminished with a reduction of the osteoid thickness. The treatment with JNJ restored the normal activity of OCs and OBs, comparable to the control mice. In particular,

JNJ induces a reduction of bone turnover consistent with the inhibition of bone metastases. To investigate the effect of JNJ on OCs and OBs, without the presence of bone microenvironment, we perform in vitro cultures of human OCs and OBs, stimulated or not with JNJ, showing that JNJ inhibited osteoclastogenesis and did not significantly affect the number of BAP+ OBs. Furthermore, we analysed mice sera by a multi-analyte detection system, showing that CCL20, a chemokine involved in the tumor progression process, was reduced in mice treated with JNJ, corroborating the inhibitor role of JNJ both in tumor progression and in bone metastasis formation.

Conclusion. Our results highlight the ability of this c-MET inhibitor to abrogate the bone metastastis formation induced by renal CSCs.

No conflict of interest.

43 Proffered paper: Resistance to MET inhibitors: Which is the role of tumor microenvironment?

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Introduction. Resistance to targeted therapy represents a major challenge to the treatment of cancer patients. Many data support the role of tumor stroma in modulating tumor progression, but little is known about its potential role in drug resistance since until now resistance to targeted therapies was mainly studied in vitro. This sustains the need of in vivo models that better mimic human tumors. Since MET, the tyrosine kinase receptor for Hepatocyte Growth Factor, is frequently implicated in resistance to kinase-targeted therapies and since MET Tyrosine kinase inhibitors (TKIs) are currently in advanced phases of clinical trials, our aim was to investigate a possible role of non-cell-autonomous mechanisms in sustaining resistance to MET-TKIs.

Materials and method. We generated in vivo models of MET-addicted tumors resistant to anti-MET TKIs, subcutaneously injecting the non–small cell lung carcinoma cell line-EBC-1 in NOD-SCID mice and treating them with MET inhibitors (JNJ-38877605® or crizotinib) until resistance onset. In parallel, tumors grown in untreated animals were used as control. We analyzed both the tumors and the cells that we were able to isolate and put in culture from wild type (WT) and resistant (RES) tumors (tumor cells and murine fibroblasts).

Result and discussion. Through cell viability assays we demonstrated that six out of seven xeno-derived tumor cell lines were not resistant in vitro to MET-TKIs, suggesting a possible role of microenvironment in sustaining resistance. To support this idea, murine-gene array on WT and RES tumors and on the corresponding fibroblasts in vitro showed a strongly different gene expression profile between WT vs RES murine-stroma in tumors, and this was also maintained in the corresponding fibroblasts in vitro. To understand whether the stroma may confer resistance to MET TKIs, we performed in vitro and in vivo co-culture. We demonstrated that stroma of resistant tumors can protect sensitive cells from the effect of MET-TKIs in in vivo experiments. We also showed that fibroblasts derived from RES tumors can render tumor cells resistant to the MET inhibitors in vitro and that the resistance is mediated by soluble factor(s).

Conclusion. We demonstrated that the microenvironment of RES tumors induces resistance to MET TKIs both in vitro and in vivo. Now, we would identify which molecule(s) are responsible of resistance in order to discover new possible therapeutic targets in tumor microenvironment.

No conflict of interest.

44 Extracellular matrix impinges on immune functions and shapes the tumour microenvironment

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Among the relevant modifications occurring in stromal compartment of bone marrow (BM) and secondary lymphoid organs (SLO) we have uncovered the importance of the matricellular protein SPARC in the homeostatic and pathogenic modifications of hematopoietic and immunological settings. Specifically, the high expression of SPARC in the stroma favors aberrant osteoblastic niche expansion towards myelofibrosis when in presence of a myeloproliferative spur. On the contrary, stroma cells unable to secrete SPARC induce alterations of the hematopoietic niche that favor myeloid precursor expansion in the BM. In SLO, SPARC deficiency alters the mesenchymal follicular dendritic cells (FDC) networking and impairs humoral immunity while promotes lymphomagenesis in the case of persistent perturbation of lymphoid tissue homeostasis. In this latter case, the absence of SPARC results in defective collagen assembly and lack of inhibitory signals through the collagen receptor LAIR-1 on myeloid cells, particularly neutrophils. Hyper-activated neutrophils acquire an IFN-related signature and eventually die through NETosis that promotes CD5+ B cell transformation via NF-kB. The emerging scenario indicates that stromal SPARC expression has effect on lymphomagenesis controlling the behavior of bystander immune cells rather than influencing CD5+ B cells directly. These data found correlation with human B-CLL, which shows reduced microenvironmental SPARC expression and ECM deposition as well as signs of NETotic neutrophil among the activated infiltrating myeloid cells.

Nonetheless, also solid tumors are deeply influenced by SPARC produced either by tumor cells or infiltrating leukocytes toward local inflammation, epithelial to mesenchymal transition, metastasis and drug resistance.

No conflict of interest.

14:30-16:15
Symposium: Metabolism

45 Hypoxic microenvironment and tumor metabolism

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In metazoans, sensing the availability of oxygen and key nutrients such as glucose and amino acids is integrated with growth factor and hormone signaling. This multiple nutrient checkpoint, which converges on the activation of mTORC1 protein kinase, is critical for cells to engage and progress through the cell cycle. Therefore, rapidly growing cells have developed sophisticated regulatory systems to rapidly respond to fluctuations in oxygen and nutrients in the microenvironment.

Early on in evolution, oxygen sensing emerged as a central control mechanism of energy metabolism and vasculogenesis. At the heart of this regulatory system are the Hypoxia-Inducible Factors, HIFs, which control the expression of numerous gene products including VEGF-A, Angiopoïetin-2 and Notch-ligand, three key angiogenic factors in vertebrates. This finding has placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF-1 and HIF-2 can also induce a vast array of gene products controlling import of nutrients (GLUT1, LAT1), glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so are recognized as strong promoters of tumor growth. It is therefore not surprising that HIF-1 also promotes access to another source of nutrients by inducing via BNIP3 macro-autophagy.

In this presentation we will highlight two anticancer targets induced by HIFs and highly expressed in rapidly growing tumors: the plasma membrane transporters LAT1 (SLC7A5) importing essential amino acids and MCT4 (SLC16A4), a lactate/H+ symporter regulating pHi by efficient lactic acid extrusion. The second target, MCT4, is particularly interesting in the context of this symposium. Lactic acid generated from tumors is emerging as a major metabolite that impacts on the tumor microenvironment not only as a signaling molecule, but also as an acid stressor compromising immune surveillance. Thus an approach that blocks lactic acid export could have a dual effect as an anticancer strategy.

No conflict of interest.

46 Reciprocal metabolic deregulation of tumor/stroma interplay: A new druggable synergy

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The acquisition of malignant traits during tumorigenesis is strongly influenced by the surrounding microenvironment and cancer associated fibroblasts (CAFs), key players of cancer progression, have also been correlated with resistance to therapy. CAFs induce epithelial mesenchymal transition (EMT), metabolic reprogramming towards oxidative phosphorylation (OXPHOS) and activation of a lactate shuttle, promoting tumor growth and metastatic dissemination. The effect of stromal cells on EMT undergoing cancer cells is mainly mediated by the metabolic regulator pyruvate kinase M2 (PKM2). EMT-driven oxidative signaling leads to PKM2 oxidation and Src-mediated phosphorylation, nuclear migration, association with hypoxia inducible factor-1, down-regulation of miR205 and activation of OXPHOS through SIRT1-PGC1-α regulation. PKM2 and OXPHOS targeting in vivo confirms the relevance of the pathway for stromal reprogramming of tumor cells.

We also observed a clear reprogramming towards OXPHOS in relapsing colon cancers, resistant to 5-fluorouracil (FU) therapy, with an inversion of the PKM1/PKM2 ratio, activation of penthose phosphate pathway and a respiratory phenotype. OXPHOS inhibitors are effective on 5-FU resistant cells stemness, mesenchymal phenotype and survival to therapy.

Collectively, the metabolic profile of aggressive and relapsing cancers suggests that they take advantage from shifting towards OXPHOS, which is thus a leitmotif of malignant cells and an attractive therapeutic target, involving unusual functions of PKM2 only in lactate addicted cells exploiting stromal trophism.

No conflict of interest.

47 Proffered paper: Metabolic remodeling and dependence on oxoglutarate dehydrogenase induced by oncogenic PIK3CA

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Introduction. Oncogenic activation of PIK3CA is one of the most frequent mutational lesions across multiple human cancers. However, to date PIK3CA inhibitor development has faced numerous challenges, including poor isoform- and oncogene-specificity with modest results in early clinical studies.

Materials and Methods. Project Achilles is a genome-wide shRNA screen conducted in a large cohort of cancer cell lines, which measures gene suppression effects on cell proliferation. Utilizing this dataset, our goal was to identify genes that when suppressed specifically inhibit proliferation of the PIK3CA mutant cancers, while having a negligible effect on their wild-type counterparts, thus uncovering unique dependencies within the mutant class. As proof-of-concept, this approach identified 'anticipated' dependencies in the PI3K-pathway, including multiple components of the mTOR-signaling cascade and downstream translation factors.

Results and discussion. Not having been previously linked to the oncogenic PI3K-pathway, a tight dependency was found with all components of the oxoglutarate dehydrogenase complex (OGDH-C), an integral mitochondrial enzyme catalyzing a rate-limiting step in the Krebs cycle. To that end, in vitro and in vivo experiments validate OGDH as a specific vulnerability in PIK3CA mutant cancer cells. Furthermore, metabolomic profiling confirms a specific block of the TCA cycle upon OGDH suppression and reveals distinct metabolic profiles of PIK3CA mutant cell lines and xenograft tumors. Moreover, PIK3CA-mutant lines specifically display suppression of mTOR signaling upon OGDH suppression, driven by an increase in the levels of its substrate, a-ketoglutarate. These results suggest that PIK3CA-mutant cancers depend on an altered metabolism to maintain their proliferative capacity, and specifically identify and characterize the OGDH-complex as a novel therapeutic candidate in these tumors.

Conclusions. We described a hypothesis-generating methodology aimed at identifying cancer cell vulnerabilities, here specifically associated with PIK3CA mutant tumors. In effect, we detail a collective means to identify specific cellular oncogene-dependencies in human cancers and define OGDH function as a unique cancer cell sensitivity in PIK3CA mutant tumors.

No conflict of interest.

48 Proffered paper: TRAPping the metabolic adaptations of NF1-associated tumors

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Introduction. NF1 is a genetic disease characterized by an elevated propensity to develop a variety of tumors, mainly complex neoplasms called neurofibromas composed by transformed Schwann cells, mast cells and fibroblasts. The disease is caused by loss of function of neurofibromin, a negative regulator of Ras encoded by the NF1 gene. The Ras/ERK transduction axis, dysregulated in NF1, is emerging as an important player in metabolism rewiring, which is crucial in many tumors; despite this, nothing is known about metabolic adaptations occurring in NF1 tumorigenesis.

Materials and Methods. We investigated the metabolic and the mitochondrial bioenergetic properties in several cell models with or without neurofibromin by measuring the Oxygen Consumption Rate (OCR) and the amount and activity of the respiratory chain complexes I (NADH dehydrogenase) and II (SDH, succinate dehydrogenase) using blue native gel electrophoresis and spectrophotometric assays, respectively. Moreover, we analyzed cell tumorigenic properties by in vitro and in vivo assays.

Results and discussion. The absence of neurofibromin confers tumorigenic properties that can be abrogated by MEK/ERK pathway inhibition. Nf1-/- cells show a decreased mitochondrial respiration with lower Complex II and Complex I activities, the latter being paralleled by a decrease in the expression level of Complex I subunits. ERK inhibition increases the expression of Complex I subunits resulting in its augmented assembly and activity. Complex II inhibition correlates with an interaction between SDH and TRAP1, a mitochondrial chaperone that prompts neoplastic transformation by increasing intracellular levels of succinate, thus stabilizing HIF1α. Remarkably, TRAP1 silencing abolishes the tumorigenic potential of neurofibromin deficient cells both in vitro and in xenograft models, where we also observe stabilization of HIF1α.

Taken together, these observations demonstrate that aberrant activation of Ras/ERK signaling impacts on metabolism, providing a selective advantage to NF1 tumor cells. Remarkably, TRAP1-mediated metabolism rewiring plays a key role in the tumorigenic potential of neurofibromin deficient cells, thus opening a new perspective for anti-neoplastic drug design toward NF1-associated tumors.

Conclusion. We propose that neurofibromin loss, and the ensuing induction of Ras/ERK signaling, is upstream to the regulation of mitochondrial bioenergetics. Tackling the problem of neurofibroma development by the original perspective of mitochondrial metabolism will pave the way for the design of novel, highly specific anti-neoplastic drugs for NF1-associated tumors against which there are no current treatments.

No conflict of interest.

49 Serine metabolism and cancer therapy – looking for new targets

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Cancer cells show changes in metabolism that help support enhanced proliferation and tumour growth in abnormal environments and under conditions of fluctuating nutrient availability. However, these metabolic alterations can also impose vulnerabilities that may be exploited for therapy. Recent studies have shown that many cancer cells have a high dependency on serine, which can be synthesized de novo or taken up from the medium. The serine synthesis pathway (SSP) enzymes are overexpressed in several tumor types, but those cancer cells that do not show up-regulation of this pathway are highly dependent on the uptake of exogenous serine. Indeed, dietary depletion of serine in vivo reduces cancer growth without impacting general health.

We have been examining approaches to improve the therapeutic response to serine depletion. Our previous studies showed that adaptation to serine starvation requires an increase in OXPHOS and antioxidant defenses to deal with the accompanying increased ROS. We have therefore tested the effect of serine depletion and inhibitors of OXPHOS, and shown a cooperative effect on limiting tumor progression in vivo. Importantly, not all cancer types are affected by this therapy, possibly reflecting differential dependencies of cancers on the uptake of serine from the circulation.

An alternative approach has been to examine the effects of serine starvation under conditions of depleted antioxidant response. To this end, we have used mice lacking the gene for TIGAR, an enzyme that regulates the level of fructose-2,6-bisphosphate (F-2,6-BP) and redirects glycolysis to increase NADPH and GSH levels. Our previous studies demonstrated that the antioxidant function of TIGAR helps to support survival in regenerating epithelial tissue and developing cancers. Using in vivo models we have shown that TIGAR expression is enhanced during early stages of malignant progression and that depletion of TIGAR can inhibit tumor development. The effect of serine starvation on the development of tumors in the absence of TIGAR will be discussed.

Conflict of interest: Advisory board: Raze Therapeutics; PMV Pharma. Corporate-sponsored research: Astex Pharmaceuticals.

16:45-18:15
Symposium: Undruggable Targets

50 Extending the reach of target discovery and validation to novel classes

No abstract received.

51 Targeting cancer's engines, not its drivers

No abstract received.

52 Proffered paper: An integrated pipeline for pharmaco-genomic studies in patient derived tumour cells

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Introduction. The high attrition rate of new oncological molecules in drug development occurs at phase II/III, mainly due to a lack of efficacy. This is largely because currently used preclinical models fail to recapitulate the heterogeneous and dynamic nature of the cancer genomes. Cancer cell lines have proven to be a useful tool as a biomarker drug discovery tool (Garnet et al.). However, established cell lines have recognized limitations, specially the limited clinical predictive power. To circumvent these problems and improve our understanding on the molecular mechanisms underlying breast cancer, we have been implanting human breast cancer samples in immune-compromise mice and generated a bio-bank of life human breast cancer tissue or patient derived tumour xenografts (PDTXs). We have generated a panel of PDTX models that represent breast cancer intra- and inter-tumour heterogeneity and used them in high-throughput molecular and functional assays.

Materials and Methods. Cambridge patients were recruited and samples collected as part of the Adult Breast Stem Cell Study at Addenbrooke's Hospital, Cambridge, UK, approved by the local research ethics committee (REC reference no. 08/H0308/178). PDTX models are derived by subcutaneous implantation of surgical breast cancer specimens in immune-compromised mice. This includes matched primary/metastasis samples from the same patient. We currently have generated 75 PDTXs, which are able to generate 2nd and subsequent generations of xenografts upon serial transplantation. We have extensively profiled 25 models at several passages (whole exome sequencing, Illumina arrays, reduce representation bisulphite sequencing (RRBS) and immunohistochemistry (IHC)) and most have been compared with the primary tumour material from which they are derived to evaluate clonal heterogeneity and evolution in a complex heterogeneous tumour (Bruna et al. in preparation, Eirew et al. Nature 2015). PDTX were dissociated to single cells following protocol described in Bruna et al. (Nat. Comms., 2012), and plated in triplicates into 384 well plates for high-throughput drug activity studies following the pipeline described in Garnett et al. (Nature, 2012). 108 different compounds in different stages of clinical development were used in all our models in at least 2-3 different passages for most.

Results and discussion. We have performed DNA sequencing, expression arrays, RRBS and IHC in 25 established breast cancer PDTX models, at different passages for most. We have dedicated a lot of effort to develop a pipeline for the analysis of combined mouse/human samples derived from our PDTX DNA to accurately call for

somatic/germline mutations and cancer related CpG methylation changes. These data shows breast cancer PDTXs represent the functional complexity and heterogeneity of breast cancer genomes and can also be used to uncover mechanisms of clonal selection and evolution. We hypothesized the difficulty on discriminating sample specific cancer drivers solely on genomic data could benefit from the incorporation of functional information. We thus aimed to use these state-of-the art breast cancer pre-clinical models in high-throughput drug efficacy assays in a feasible and cost-effective manner. We combined in vivo maintenance of human derived samples (as low passaged PDTXs) with ex vivo short-term cultures (PDTX cells or PDTCs). We have comprehensively characterized this PDTX_PDTC integrated platform and shown can successfully recapitulate patient-specific treatment and rationally predicted targeted treatment responses. We have further validated in vivo, ex vivo drug responses that recapitulate clinically relevant targeted treatment strategies (such as hormone therapies in ER positive tumours), and rationally predicted targeted treatment responses (such as the effective response of PI3K inhibitors in ER+ samples). In combination with the deep molecular characterization data, we identified known mechanisms of drug resistance and sensitivity and, importantly, de novo biomarkers and novel mechanisms of resistance to targeted treatments. We further used clinically operating imaging approaches and have identified potential new biomarkers of response to PI3K inhibitors.

Conclusion. Our ambition is to accelerate personalized medicine by integrating multiparametric genomic data with preclinical drug-drug vulnerabilities to develop signatures of targeted therapy responses with clinical applicability.

No conflict of interest.

53 Proffered paper: Drugging the undruggable: Development of small molecule activators of protein phosphatase 2A for cancer treatment

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While oncogenic kinases have proven to be successful targets for cancer treatment, the therapeutic targeting of tumor suppressor phosphatases, the key negative regulators of these same pathways, has remained largely unexplored. Through reverse engineering of tricyclic neuroleptic drugs, we developed a first-in-class series of small molecule activators of PP2A activators (SMAPs) with favorable pharmaceutic properties that directly bind and activate the serine/threonine phosphatase 2A (PP2A). A critical role for PP2A as a tumor suppressor has previously been established, and PP2A inactivation is common feature in many human cancers and its dominant and best-defined targets are major oncogenic signaling pathways including ERK, AKT, MYC and AR. Here we report results from extensive mechanistic investigations using a combination of radiolabelled equilibrium dialysis, photoaffinity labeling, and hydroxyl radical drug footprinting studies demonstrating that SMAPs directly bind to the PP2A A-scaffold subunit and induce conformational changes in the C-terminus of the C-catalytic subunit. This conformational change results in a loss of inhibitory post-translational modifications in the C-terminus (e.g. phosphorylation of Y307) thereby reactivating PP2A. Global phosphoproteomic studies of SMAP treated demonstrate marked dephoshorylation of a number of oncogenic PP2A substrates in drug treated cell lines including ERK, AKT, MYC and AR. Target specificity was demonstrated using genetic loss of function (shRNAs to PP2A subunits), drug binding and naturally occurring tumor associated PP2A A-alpha mutation expressing cell lines and through pharmacological PP2A inhibition with okadaic acid. Advanced SMAP candidates have been extensively profiled in xenografts, GEMM models, and PDX (patient-derived xenograft) models and have shown excellent activity, comparable or superior to standard of care agents, in numerous tumor types including non-small cell lung cancer (NSCLC), castrate-resistant prostate cancer (CRPC), endometrial cancer, and Burkitt's Lymphoma. In studies currently underway, SMAPs are showing dramatic activity in models representing significant unmet medical need including triple-negative breast cancer and pancreatic cancer. Additionally, the compounds demonstrate favorable pharmacokinetics and show no overt toxicity. We are currently expanding both the breadth and depth of in vivo profiling and are working to identify patient-selection and target engagement biomarkers to facilitate the clinical evaluation of SMAPs. While research and clinical effort has largely focused on development of inhibitors of oncogenic kinases, the identification of small molecule activators of tumor suppressor proteins has remained elusive. Activation of such proteins could offer the opportunity to identify novel synergistic strategies for the treatment of a number of cancer types. Nevertheless, translation of a PP2A activation strategy into clinical medicine has required pharmaceutically tractable agents for development. Our studies represent a first step into that new territory and highlight the potential for the development of small molecule activators of other protein phosphatases and tumor suppressor proteins.

Conflict of interest: Corporate-sponsored research: Dual Therapeutics
Other substantive relationships: Co Founder Dual Therapeutics

18:15-19:00
Keynote Lecture: Animal Models

54 Modeling human cancer in mice: An essential tool for the development of future therapies

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K-RAS oncogenes have been implicated in about one fifth of all human cancers including lung adenocarcinoma and pancreatic ductal adenocarcinoma, two tumor types with some of the worse prognosis. In order to identify effective therapeutic strategies to treat these tumors, we have developed genetically engineered mouse (GEM) models that closely recapitulate their natural history. We are using these mice to validate targets of potential therapeutic value with the ultimate goal to translate these findings to the clinic. In previous studies, we have crossed these GEM models with mice that carried either germ line or lox-Cre conditional knock out loci encoding each of the downstream kinases of the Raf/Mek/Erk pathway as well as the cell cycle interphase Cdks to interrogate their role in the development of K-Ras driven lung adenocarcinomas. These studies have led us to validate the c-Raf and Cdk4 kinases as essential targets for these tumors (Puyol et al., Cancer Cell 2010; Blasco et al., Cancer Cell 2011). Following a similar strategy, we have demonstrated that the EGF Receptor and c-Raf were also absolutely essential for the development of pancreatic ductal adenocarcinomas (Navas et al., Cancer Cell 2012). Importantly, systemic ablation of either c-Raf or Cdk4 has no significant effect on normal homeostasis. Now, we have decided to interrogate the role of these targets in advanced tumors. To this end, we have generated new GEM models in which expression of the resident K-Ras oncogene, as well as ablation of the p53 tumor suppressor is mediated by the frt-FLp(o) targeting system, a strategy that allows temporal separation of tumor development from target ablation. In addition, we have generated lox-Cre based conditional knock-in strains that upon recombination, direct the expression of kinase dead isoforms instead of inducing protein ablation. We feel that these strains should serve as better models to mimic subsequent drug intervention in the clinic. Finally, we are now combining the inactivation (or ablation) of these and other previously validated therapeutic targets in order to identify combinations of targets that may eventually eradicate advanced tumors, with the ultimate goal to guide the design of future clinical trails based on the used of combined targeted therapies.

Conflict of interest: Ownership: Pfizer, corporate sponsored research
Advisory board: Eli Lilly, corporate sponsored research

TUESDAY 23 JUNE 2015

08:30-09:15
Keynote Lecture: Precision Medicine in Oncology

55 Precision medicine in oncology

No abstract received.

09:15-10:00
Keynote Lecture: Epigenetics

56 Cancer pharmacoepigenetics: Genes and drugs

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For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs.

No conflict of interest.

10:30-11:15
Award Lecture: "Giorgio Prodi" Lecture

57 From seabed to bedside: Lessons learnt from the marine-derived anticancer drug trabectedin

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1 Department of Oncology, IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

The lecture will focus on the importance of translational research for the successful development of modern anticancer agents exemplified by trabectedin as prototype of a new class of drugs which exert their activity on both cancer cells and the tumour microenvironment. The lecture will illustrate how a robust international collaboration of scientists and clinicians working in academia and the pharmaceutical industry can be scientifically productive and profitable teasing out intricate multiple mechanisms ultimately leading to the rapid application of laboratory findings to the clinic. Trabectedin, a tetrahydroisoquinoline alkaloid initially isolated from the Caribbean marine tunicate Ecteinascidia Turbinate and currently prepared synthetically, is registered in Europe and several other countries for the second line therapy of sarcoma and ovarian cancer. It binds with some degree of sequence specificity in the DNA minor groove to exocyclic N2 amino groups of guanines. DNA repair and transcription mechanisms are involved in the drug's ability to block the cell cycle and induce differentiation and cell death. The drug is unique in that it is more effective against cells in Go/G1 than those in the S phase of the cell cycle. It is much more effective against cells harbouring homologous recombination defects than those without this defect, but less active against cells deficient in Nucleotide Excision Repair. The unusual and distinctive ability of the drug to displace certain transcription factors from DNA appears to be a major mechanism of action of the drug against sarcomas characterized by certain gene translocations. For example, myxoid liposarcomas are exquisitely sensitive to trabectedin, and experimental evidence obtained in patient-derived xenografts is consistent with the clinical finding that the drug modulates with high selectivity the expression of genes involved in adipocytic differentiation, angiogenesis and inflammation. The striking antitumor activity of trabectedin observed in preclinical experimental models, including immune deprived mice bearing human-derived ovarian cancer or sarcoma xenografts, as well as its unique mode of action prompted its clinical development. The preclinical toxicological evidence of a transient drug hepatotoxicity represented a potential drawback, although this toxicity was not cumulative and could be reduced significantly by pretreatment with anti-inflammatory steroids, a finding obtained in preclinical systems and then successfully applied in the clinic. Recent studies indicate that trabectedin significantly reduces the number of tumor associated macrophages (TAM) thus modifying tumor microenvironment and angiogenesis. Finally the lecture will focus on perspectives pertinent to the design of "intelligent combinations" which exploit the distinctive ability of trabectedin to modulate transcription and tumor microenvironment. Some of these combinations have already been tested at the preclinical level and are currently under clinical investigation.

No conflict of interest.

11:15-13:00
Symposium: Inflammation

58 Targeting of tumor-associated macrophages and cancer-related inflammation

P. Allavena[1], C. Belgiovine[1], R. Frapolli[2], M. Liguori[1], A. Anselmo[1], E. Bello[2], A. Mantovani[1], M. D'Incalci[2]
1 IRCCS Istituto Clinico Humanitas, Immunology and Inflammation, Rozzano (Milan), Italy
2 IRCCS Istituto Mario Negri, Oncology, Milan, Italy

Tumor-induced immune dysfunction is a hallmark of cancer and a major obstacle to successful anti-tumor therapies. In the evasion from immune surveillance and promotion of cancer-related inflammation, myeloid cells populating tumor tissues, and especially the most abundant macrophages, play a crucial role. Macrophages are plastic cells responding to local cues with different functional programmes. In most tumors, macrophages are trained by cancer cells to differentiate into non-cytotoxic, trophic and pro-angiogenic effectors, which support neoplastic cell survival. High density of Tumor-Associated Macrophages (TAM) in experimental and human tumors has been associated with rapid disease progression and resistance to treatment. Therapeutic targeting of TAM, either by direct depletion, inhibition of their recruitment or by re-stimulation of their cytotoxic function, is now considered a promising approach.

We have reported that the registered anti-tumor agent trabectedin, currently used in soft tissue sarcoma and ovarian cancer, and under clinical investigations in several other human malignancies, has interesting peculiar effects on the tumor micro-environment. It induces a rapid apoptosis selectively on cells of the monocyte-macrophage lineage, including TAM, in vitro and in vivo; this selective pro-apoptotic mechanism was dependent on the differential expression of specific death receptors in distinct leukocyte subsets, and was an important determinant of its anti-tumour efficacy. Furthermore, trabectedin inhibits the production of several bioactive mediators relevant in the tumor context (e.g. CCL2, IL-6, VEGF), overall fading cancer-related inflammation and angiogenesis in the tumor micro-environment. These results shed light on the unexpected modes of action of a clinically useful and available anti-cancer agent, and open interesting perspectives for the rational exploitation of its peculiar properties in therapeutic settings. Furthermore these data provide the basis for testing trabectedin in combination with other immunological therapies.

Conflict of interest: Advisory board: P.Allavena and M.D'Incalci participated at the Advisory Board meeting of the organization PharmaMar

59 Inflammation and cancer: Reprogramming the immune microenvironment as an anti-cancer therapeutic strategy

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The concept that leukocytes are critical components of solid tumors is now generally accepted, however, their role(s) in regulating aspects of neoplastic progression, and/or response to cytotoxic therapy is only beginning to be understood. Utilizing de novo mouse models of organ-specific cancer development, we now appreciate that adaptive leukocytes differentially regulate myeloid cell recruitment, activation and behavior, and in turn, engaged tumor-infiltrating myeloid cells activate tissue-based programs to foster malignancy, as well as repress anti-tumor immunity by a diversity of mechanisms. Treatment of tumor-bearing mice with therapeutic agents that disrupt lymphocyte-myeloid cell interaction, myeloid cell activation, or myeloid cell functionality invariably results in slowing of primary tumor growth, and also improved responses to cytotoxic therapies, and significantly diminished presence of metastatic disease. To be presented will be our recent insights into organ and tissue-specific regulation of cancer development by adaptive and innate immune cells, and new studies evaluating how attenuating protumor properties of select lymphoid and myeloid cells can be exploited to enhance therapeutic responses to cytotoxic therapy.

LMC acknowledges generous support from the NIH / NCI, the Department of Defense Era of Hope Scholar Expansion Award, Susan G. Komen Foundation, the Breast Cancer Research Foundation, and a SU2C award supported by the AACR and Lustgarten Foundation.

No conflict of interest.

60 Proffered paper: The T-cell immune landscape predicts clinical outcome in neuroblastoma

D. Fruci[1], M. Mina[2], R. Boldrini[3], A. Citti[3], P. Romania[1], V. D'Alicandro[1], M.A. De Ioris[1], A. Castellano[1], C. Furlanello[2], F. Locatelli[1]
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2 Fondazione Bruno Kessler, FBK, Trento, Italy
3 Ospedale Pediatrico Bambino Gesù, Pathology, Rome, Italy

Neuroblastoma grows within an intricate network of different cell types including epithelial, stromal and immune cells. The presence of tumor-infiltrating T cells is considered an important prognostic indicator in many cancers, but the role of these cells in neuroblastoma remains to be elucidated. Herein, we examined the relationship between the type, density and organization of infiltrating T cells and clinical outcome within a large collection of neuroblastoma samples by quantitative analysis of immunohistochemical staining. We found that infiltrating T cells have a prognostic value greater than, and independent of, the criteria currently used to stage neuroblastoma. A different in-situ structural organization and variable concurrent infiltration of T-cell subsets were detected in tumors with different outcome. Low-risk neuroblastomas were characterized by a higher number of proliferating T cells and by a more structured T-cell organization, which was gradually lost in tumors with poor prognosis. We defined an immunoscore based on the presence of CD3+, CD4+ and CD8+ infiltrating T cells that associates with favorable clinical outcome in MYCN-amplified tumors, improving the prediction of patient survival when combined with the MYCN status.

These findings support the hypothesis that infiltrating T cells influence the behaviour of neuroblastoma and might be of clinical importance for the treatment of patients.

No conflict of interest.

61 Proffered paper: Regulation of the immunosuppressive microenvironment of liver metastases by TNFR2

B. Ham[1], Z. D'Costa[1], N. Wang[2], M.C. Fernandez[3], F. Bourdeau[3], P. Auguste[4], P. Brodt[5]
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4 Université de Bordeaux, INSERM LAMC, Talence, France
5 McGill University Health Centre, Montreal, Canada

Introduction. Cancer metastasis to vital organs such as the liver is the major cause of cancer-related death. We have previously shown that the entry of metastatic colorectal or lung carcinoma cells into the liver triggers a rapid inflammatory response mediated by Kupffer cell-derived TNF-α.

Methods. Here we analyzed the role of host innate immunity, TNF-α signaling in particular, in liver metastasis of colon and lung carcinoma cells using mice with tnfr gene deficiencies. We investigated parameters of the host response and the outcome of metastasis in these mice, using the highly metastatic mouse colon carcinoma MC-38 and lung carcinoma H-59 cells and a combination of flow cytometry, immunohistochemistry and confocal microscopy

Results. We found a marked reduction in the number of experimental liver metastases in TNFR2-/- mice. Analysis of immune cell recruitment to sites of metastases in these mice revealed a significant depletion (75% reduction) in a subset of CD11b+GR-1+ myeloid-derived cells enriched in arginase activity, identifying them as myeloid derived suppressor cells (MDSC). This coincided with a reduction in the recruitment of CD4+CD25+FoxP3+ Treg cells to the liver. Reconstitution of TNFR2-/- mice with normal bone marrow restored MDSC recruitment and increased liver metastasis and this was also observed in TNFR2-/-mice into which TNFR2+, but

not TNFR2-null MDSC were adoptively transferred. TNFR1 deficiency did not alter MDSC recruitment or liver metastasis, although it reduced vascular endothelial cell adhesion receptor expression and chemokine production in response to TNF-α. Finally, treatment with TNFR2 antisense oligodeoxynucleotides significantly reduced liver metastasis.

Conclusions. The data implicate TNFR2 in the regulation of the immune microenvironment associated with liver metastases and identify TNFR2 as a targetable molecule for liver metastasis prevention.

No conflict of interest.

62 Neutrophilic inflammation and melanoma metastasis

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Genetically engineered mouse melanoma models enable new insights into pathogenesis and treatment of melanoma. We established the genetically engineered HGF-CDK4(R24C) mouse strain in our laboratory, where invasively growing primary melanomas fail to spontaneously activate the type I IFN system in the tumor microenvironment and consequently do not effectively stimulate a tumor-specific T cell response. HGF-CDK4(R24C) mouse melanomas morphologically imitate the subgroup of lymphocyte-poor human melanomas that tend to ulcerate and are associated with a poor prognosis. We found that repetitive UV exposure of primary HGF-CDK4(R24C) mouse melanomas selectively promoted systemic metastatic disease progression independent of its tumor-initiating effects. Specifically, UV irradiation enhanced the expansion of tumor cells along blood vessel surfaces in a pericyte-like manner and increased the number of lung metastases. This effect depended on TLR4-driven recruitment and activation of neutrophil leukocytes which stimulated angiogenesis and promoted the ability of mouse melanoma cells to migrate towards endothelial cells. Furthermore, endothelial cells possess selective cues to promote melanoma cell motility on their surfaces. Our results reveal how UV radiation is sensed by epidermal keratinocytes and show that the resulting neutrophilic inflammatory response catalyses reciprocal melanoma-endothelial interactions and drives perivascular melanoma cell invasion, a phenomenon originally described as angiotropic growth by histopathologists in human melanomas. Angiotropism represents a hitherto underappreciated mechanism of metastasis that also increases the likelihood of intravasation and dissemination via the blood. Consistent with our findings, neutrophil infiltration and reactive angiogenesis in ulcerated primary melanomas is associated with angiotropism and metastatic progression in this patient subgroup. A better understanding of inflammation-induced interactions between tumour and endothelial cells will reveal new treatment approaches to prevent the metastatic progression of high-risk primary melanomas.

No conflict of interest.

Poster Sessions

SUNDAY 21 JUNE AND MONDAY 22 JUNE 2015

Poster Session: Cancer Genomics, Epigenetics and Genome Instability I

100 Identification of non-coding RNAs involved in cell cycle control

F. Corrà[1], M. Galasso[1], C. Zerbinati[1], L. Minotti[1], M. Previati[1], C.M. Croce[2], S. Volinia[1]
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Introduction. Non-coding RNAs (ncRNAs) are functional RNA molecules that are not translated into protein. They include functionally important RNAs, such as transfer RNA and ribosomal RNA, as well as small interfering RNAs, microRNAs (miRNAs), PIWI-associated RNAs, small nucleolar RNAs, promoter-associated RNAs and the burgeoning class of long non-coding RNAs (lncRNAs) (Mercer et al,2009). Recent data suggest that some ncRNAs, including transcribed ultraconserved regions (T-UCRs), contribute to molecular alterations in cancer (Calin et al, 2007). We performed a large-scale study to identify T-UCR activity in human cancer and used information theory and statistics to identify associations between some key miRNAs and T-UCRs.

Materials and Methods. We performed a genome-wide study of correlation of T-UCRs expression with miRNAs. We took advantage of our database of over 6000 expression profiles in human cancer and normal samples. We used Maximal Information Coefficient (MIC) and Pearson correlation to identify significant associations between miRNAs and T-UCRs. Transfection in cell lines, cell cycle assay, RT-PCR, q-PCR were perform to investigate the T-UCR/miRNA expression and activity.

Results. miRNA-221, a miRNA involved in cell cycle (Pineau et al, 2010; Di Leva et al, 2010) would serve as a bait to detect positively associated T-UCRs, also involved in cell cycle. Analogous rationale would be valid for a negative correlation. We split the T-UCR/miRNA expression database randomly in two groups of 3000 samples each, a test and a validation cohort. We identified the 23 most important associations of miR-221 with the other ncRNAs in the test cohort. The large sample size guaranteed that the detected associations were highly significant. We then generated a Bayesian networks for these 23 miRNA/UCR pairs using the additional 3000 expression profiles in the validation cohort. We propose that this miR-221 based miRNA/T-UCR network indicates ncRNA with candidate function in cell cycle. Lastly, we transfected breast cancer cell lines with siRNA for silencing the T-UCRs. We performed RT and q-PCR to assess the extension of UCR and miRNA silencing. Cell cycle was analyzed using standard techniques upon T-UCR silencing.

Conclusion. Our result identified candidate and novel non-coding RNAs that exert their role in cell cycle control.

No conflict of interest.

101 Characterization of circulating miRNAs in lung cancer patients: Origin and release

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Introduction. We previously reported the identification of diagnostic miRNA signatures in plasma samples of lung cancer patients detected by low dose computed tomography (LDCT) screening. Circulating miRNAs are released into the bloodstream by different mechanisms such as passive leakage from broken cells or active secretion through microvesicles or protein complexes.

Material and methods. To evaluate the origin and the release of the 24 miRNAs of the diagnostic signature we analyzed their expression in different cell types including fibroblasts, hematopoietic, endothelial, bronchial epithelial (HBECs) and tumor cells and in the respective culture medium (CM) as well as in plasma samples. miRNAs expression was evaluated using custom microfluidic cards.

Results and discussion. miRNAs were expressed differently in all cell types, with an increased value in specific cellular components (mir-16-17-19b-106-145 in fibroblasts, mir-126 in endothelial cells, mir-451 and mir-142-3p in hematopoietic cells). The analysis of miRNAs released in CM showed that miRNAs were secreted differently and independently from their cellular levels and that tumor cells were the less involved in this release. Furthermore, we observed an higher release of miRNAs (mir-28-3p, mir-30b, mir-30c, mir-92a mir-197, mir-320) from HBECs than tumor cells. Interestingly, we identified miRNAs that were expressed and released from blood cells only such as mir-451, mir-133 and mir-142-3p. To better characterize the origin of plasma miRNAs, we isolated exosomes from CM of different cell types. We detected the presence of all 24 miRNAs in exosomes and identified exosomal miRNAs that were secreted only by specific cellular components (mir-101 from blood cells, mir-145 from fibroblasts and mir-126 from endothelial cells). Furthermore, analysis of plasma samples from 14 lung cancer patients confirmed the presence of the 24 miRNAs inside exosomes and their levels correlated with those observed in total plasma (correlation=0.75, p<0.001). Similar analysis will be done for miRNA associated with proteins such as Ago2. Future experiments will be performed using digital PCR for miRNAs quantification and flow cytometry for exosomes characterization.

Conclusion. Our preliminary findings on the origin of miRNAs support the conclusion that plasma miRNAs are heterogeneous and contributed by different cellular components, and not specifically by tumor cells. Furthermore, data on exosomes isolated from CM and plasma indicate that our 24-miRNA were actively secreted by lung microenvironment.

No conflict of interest.

102 The potential role of miR-143 targeted FHIT gene in breast cancer cells

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Introduction. MicroRNAs (miRNAs) are small non-protein-coding RNAs that play important roles in cell proliferation, differentiation, apoptosis, and development and so it is involved in cancer development. Recent studies focus on identification of miRNA target genes and detection of altered its mRNA levels. miR-143 is associated with cancer tumorigenesis in multiple types of cancer because it shows tumor-suppressive activity in some human cancers. Besides, miR-143 was frequently downregulated in 80 % of breast carcinoma tissues. However, the function and mechanism of miR-143 in breast cancer cells remains unknown.

The FHIT (Fragile histidine triad) gene is located in 3p14 region, represents the most active chromosome fragile site in the human genome. Large deletions and/or point mutations in the FHIT locus have been identified in many cancer-derived cell lines, primary human tumors including breast, lung, head and neck, colon and esophagus. Genetic alterations with reduced expression of this gene are found in about 30% of breast cancers. The aim of this study was to determine the role of the expression levels of FHIT gene in miR-143 transfected breast cancer cells and to investigate whether to use the potential effect of miR-143 as miRNA-based treatment for breast cancer.

Material and Methods. The expression levels of FHIT gene was determined by TaqMan real-time PCR based expression assays before and after transfection in breast cancer cells. Additionally, we evaluated the apoptotic effects of miR-143 transfection on breast cancer cells by Annexin-PI and cell cycle analysis.

Results. Although the mRNA expression of FHIT gene in the untreated MCF-7 cells was very low; this level was increased (1.69 fold) when transfected with mir-143 for 72 h. At 72 h after transfection, total apoptosis (early and late apoptosis) rate was found 36.75±2.4 % and arrest of G0/G1 phase was increased from 49.1±0.7 to 61.8%±1.2 compared to control group.

Conclusion. In conclusion, miR-143 transfection resulted in increasing Go/G1 arrest and inducing apoptosis. These findings could potentially use in the development of miRNA-based treatment for breast cancer.

Keywords: miR-143, FHIT, MCF-7 cells, apoptosis.

No conflict of interest.

103 MicroRNAs expression analysis in high-fat diet induced NAFLD-NASH-HCC progression: Study on C57BL/6J mice

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Introduction. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of the liver. Non alcoholic fatty liver disease (NAFLD) is a frequent chronic liver disorder in developed countries. It can progress through the more severe non alcoholic steatohepatitis (NASH), cirrhosis and, lastly, HCC. Genetic and epigenetic alterations of coding genes and deregulation of microRNAs (miRNAs) activity play a role in HCC development. MiRNAs are small non-coding RNAs acting as regulators of gene expression at the post-transcriptional level. In this study, a mouse model predisposed to diet-induced obesity and NAFLD was used to investigate the effects of a high-fat (HF) diet during the transition from steatosis to hepatitis and then HCC development. We analyzed miRNAs' expression to identify molecules involved in the progression of the hepatic damage up to HCC onset.

Material and method. C57BL/6J male mice were fed a high fat (HF) or low fat (LF) diet for 3, 6 and 12 months. After sacrifice, blood and hepatic tissues were collected. Clinical chemistry assays and histological analysis were performed using standard procedures. RNA was extracted from liver tissues and miRNAs' profiles were analyzed by a TaqMan-based real time PCR method.

Results and discussion. Significant increase of cholesterol, HDL, LDL, triglycerides and alanine aminotranferase was detected in HF mice. Gross anatomical examination revealed hepatomegaly and paler color in livers from HF animals. Depending on the treatment's length, histological analysis highlighted different degrees and levels of steatosis, inflammatory infiltrate and fibrosis, in LF vs HF groups, demonstrating the progression from NAFLD through NASH. Macroscopic nodules, showing typical neoplastic features at the microscopic examination, were observed in 20% of HF mice. MiRNAs analysis showed several miRNAs differentially modulated through the progression of the liver disease, by comparing HF vs LF hepatic tissues and HF vs tumor tissues (i.e. miR-155, miR-193b, miR-20a, miR-125a-5p, miR-182, miR-200a, miR-200c, miR-27a, miR-99b).

Conclusions. MiRNAs expression was evaluated in a mouse model fed a HF diet. Several microRNAs were found differentially expressed in the transition through steatosis, hepatitis, and HCC development, indicating their potential role in the progression of the hepatic damage and initiation of HCC. Further experiments are ongoing to clarify in which way deregulation of these miRNAs impact on progression from NAFLD to NASH and HCC.

No conflict of interest.

105 Stromal contribution to the colorectal cancer transcriptome

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Introduction. Recent studies have identified a poor prognosis stem/serrated/mesenchymal (SSM) transcriptional subtype of colorectal cancer (CRC). We noted that genes upregulated in this subtype are also prominently expressed by stromal cells, which led to the hypothesis that SSM transcripts could indeed derive from stromal rather than epithelial cancer cells.

Materials and Methods. To test this hypothesis, we analyzed CRC expression data from patient-derived xenografts (PDXs) on both microarray and RNAseq platforms. In this scenario human cancer cells are supported by murine stroma; we therefore exploited human mouse orthologous diversity to discriminate human from murine transcripts and quantify the contribution of epithelial and stromal transcripts to the global gene expression profile.

Result and discussion. Species-specific expression analysis revealed that mRNA levels of SSM genes are mostly due to stromal rather than epithelial expression in CRC. Immunohistochemistry of representative genes (ZEB1, MAP1B and TAGLN) confirmed the specific stromal expression of SSM markers. Three gene signatures specifically reporting the abundance of cancer-associated fibroblasts (CAFs), leucocytes or endothelial cells, were strongly associated with the SSM subtype. A high CAF signature was associated with poor prognosis in untreated CRC patients (log rank $p < 0.005$), while in rectal cancer high stromal signatures jointly predicted radio resistance.

Conclusion. These data show that the distinctive transcriptional and clinical features of the SSM subtype can be ascribed to its particularly abundant stroma.

No conflict of interest.

106 An integrated multi level '-omics' approach identifies Dlk1-Dio3 locus associated with miR-506 expression in epithelial ovarian cancer. A new player in disease recurrence?

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Background. A major challenge in Epithelial Ovarian Cancer (EOC) treatment is prediction of chemoresistant relapse. We previously identified an 8 miRNAs cluster, located in the ChrXq27.3 region and belonging to the miR-506 family, independently associated with early relapse in advanced stage EOC. Here we propose a multi-level, integrated molecular approach driven by miRNAs expression to decipher molecular signatures underlying chemoresistance and disease recurrence in EOC patients.

Materials and Methods. RNA from fresh-frozen primary tumor samples of 72 consecutive advanced-stage, high grade EOC patients homogeneous for optimal debulking and standard treatment (platinum + taxane) was profiled for: miRNA expression, methylome profiling (Illumina); mRNA/lncRNA expression (Agilent). After a single-level analysis, we applied a miRNA-driven step by step integration approach to identify the key molecules/pathways.

Results. miRNA expression profile identified two groups of patients associated to a differential expression (DE) of miR506-cluster. Their class comparison identified 147 DE miRNAs at FDR<0.15 and among the miRNA concurrently down-regulated with miR506-cluster, known epigenetically regulated and tumor suppressor miRNAs (miR-34, miR-30 family and miRNAs of the Dlk1-Dio3 locus -Chr14q32.2 region) were detected. Poor prognosis of patients with low miR506-cluster expression was confirmed ($p=0.014$).

Applying the miR506-cluster expression level as molecular classifier, we identified 526 significant DE genes (FDR<0.15) in high (H) vs low (L) miR506-cluster expressing patients. Gene Set Enrichment Analysis combined with functional pathway (IPA) analysis identified enrichment in signalling pathway related to EMT, cell cycle, DNA replication and repair in L group as well as in the platinum resistant subgroup, thus suggesting the complementarity of clinical and molecular data. lncRNAs analysis, driven by the same criteria, identified 141 DE lncRNAs (pval<0.05) in H vs L patients and one of the lncRNAs down-modulated in L patients, MEG3, is located in the previously identified Dlk1-Dio3 locus. Preliminary data on methylome profiling indicate an epigenomic regulation of this region. Interestingly, a role in chromatin remodeling and EMT events has been described for these molecules and functional assays are currently ongoing to validate these observations in in-vitro EOC models.

Conclusions. Combining miRNA, lncRNA and mRNA profiles we identified a multilayer molecular portrait associated with EOC chemoresistance and early relapse whose related functions were cell plasticity, DNA repair and cell cycle.

The correlated expression of Dlk1-Dio3 locus and miR-506 cluster suggests a potential mechanism of cross regulation which deserves to be further investigated to elucidate the mechanisms underlying EOC biology (Partially supported by AIRC IG10302 to SC, CARIPLO to DM).

No conflict of interest.

107 Magnetic beads-based sensor for cisplatin-modified DNA

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Introduction. Cisplatin is a drug used for the cancer treatment. The mode of cisplatin action has been linked to its ability to crosslink purine bases in the DNA, interfere with DNA repair mechanisms and induce DNA damage and then cancer cells apoptosis.

In this work, the sandwich ELISA-like method was exploited for the selective analysis of DNA modified with cisplatin. Sandwich based on magnetic particles (MPs) with anti-dsDNA antibody and secondary anti-cisplatin-modified DNA antibody labelled with quantum dots (QDs) was proposed.

Material and method. In the sandwich immunoassay, the MPs - anti-dsDNA antibodies complex interacted with cisplatinated DNA (41 nM lambda DNA was interacted with 0, 8 and 17 μM cisplatin) and the anti-cisplatin modified DNA antibodies (2 mg/mL) were added and incubated for 60 min. Subsequently 10 μL of CdTe QDs modified by HWRGWVC peptide were added and incubated for 60 min. The fluorescence of coupled QDs was measured by multifunctional microplate reader Tecan Infinite 200 PRO 132 (TECAN, Switzerland). Excitation wavelength was 360 nm and emission wavelength ranged from 390 to 850 nm per 2 nm steps.

Results and discussion. In this work, a bead-based sensor for cisplatinated DNA was proposed. The sensor consisted of bead – anti-dsDNA antibody – cisplatinated DNA – quantum dot-labelled anti-cisplatin-modified DNA antibody. Compared to DNA without cisplatin, at all applied cisplatin concentrations the 5- or 7- fold increase in fluorescence was observed.

The concentration of Pt in eluted DNA was determined by LA-ICP-MS. When compared the applied and eluted cisplatin, in the eluted DNA solution 32% of applied cisplatin was present for 8 μM cisplatin and 42% for 17 μM cisplatin. This confirms that the recorded fluorescent signal is caused by presence of cisplatin-modified DNA.

Conclusion. In this work a bead-based sensor for cisplatin-modified DNA detection was constructed.

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No conflict of interest.

108 Genetic characterization of pure tumor cell sub-populations from a formalin-fixed paraffin-embedded sample

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Introduction. The complex heterogeneity of the tumor tissue, composed by intermixed tumor cells and stromal cells, reduces the genetic analysis accuracy and hides less represented genetic modifications: here we present a method for isolation and genetic characterization of pure cell populations from a FFPE tissue.

Materials & Methods. Archival FFPE tumor tissues (11 lung, 9 colon, 3 pancreatic, 1 breast) were disaggregated down to single cells suspensions and stained with Keratin, Vimentin and DAPI. The analysis with DEPArray™ technology, an image-based cell sorting platform with single cell resolution, allowed the identification of multiple cells sub-sets, considering both cells staining and ploidy. Interestingly, also in the VIM+/KER+/DAPI+ cells, hyperdiploid cells were identified, leading to the identification of an epithelial to mesenchymal transitions (EMT) cell sub-population.

Pools of pure sorted cells (mean= 112, range= 5-600), along with an aliquot of the corresponding unsorted cells and the corresponding DNA extracted from FFPE tissue, were used as input for the AmpliSeq CHP v2 for the libraries production, sequenced with Ion Torrent Platform (mean depth>2,000x) and analyzed using the related software.

Results & Discussion. On several loci, we detected somatic mutations with 100% variant frequency, only observable as heterozygous in the unsorted samples and as wild-type in stromal cells of same patient, confirming 100% purity of sorted cells. Moreover, in the EMT-phenotype subpopulations we identified clear somatic mutations, different from tumor cells majority and undetectable in unsorted samples. Frequently, for loci harboring germ-line heterozygous SNPs with variant frequency around 50% for pure stromal cells, we readily detected loss-of-heterozygosis in tumor cells subpopulations as binary (0%/100%) variants. Quantitative traits such as copy number gains and losses were also reproducibly identified in tumor cell replicates as deviations from the 50% variant frequency of germline SNPs of pure stromal cells.

Conclusions. This innovative technology solves the heterogeneity of the tumor tissues, by isolation and genetic analysis of pure tumor cells from tiny clinical samples and allows reliable, accurate and more sensitive genetic analysis of a FFPE tumor tissues, highlighting a deeper genetic characterization undetectable in unsorted samples.

No conflict of interest.

110 A new method for discovering disease-specific miRNA-target regulatory networks

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Introduction. Genes and their expression regulation are among the key factors in the comprehension of the genesis and development of complex diseases. In this context, microRNAs (miRNAs) are post-transcriptional regulators that play an important rolein gene expression since they are frequently deregulated in pathologies like cardiovascular disease and cancer. In vitro validation of miRNA - targets regulation is often too expensive and time consuming to be carried out for every possible alternative. As a result, a tool able to provide some criteria to prioritize trials is becoming a pressing need. Moreover, before planning in vitro experiments, the scientist needs to evaluate the miRNA-target genes interaction network.

Material and method. In this study we describe the miRable method whose purpose is to identify new potentially relevant genes and their interaction networks associate to a specific pathology. To achieve this goal miRable follows a system biology approach integrating together general-purpose medical knowledge (literature, Protein-Protein Interaction networks, prediction tools) and pathology specific data (gene expression data).

Results and discussion. A case study on Prostate Cancer (PCa) has shown that miRable is able to: 1) find new potential miRNA-targets pairs, 2) highlight novel genes potentially involved in a disease but never or little studied before, 3) reconstruct all possible regulatory subnetworks starting from the literature to expand the knowledge on the regulation of miRNA regulatory mechanisms. In our top ranking list of miRNAs we found miR-494 and miR-548c-3p and the Estrogen Receptor 1 (ESR1) as protein coding gene.

Conclusion. In this study we proposed miRable, a new method aimed at exploring the complex world of miRNA regulation in the context of a disease. Starting from the literature information our method applies different constraints and filters to build an extended regulatory network, and exploits it to find all the regulatory subnetworks involved in a disease. By using this approach our method has been able to find new candidate genes and miRNAs related to PCa. Some of these genes have already been associated with PCa, but the majority of them are novel candidate genes.

No conflict of interest.

112 Reversing DNA methylation of androgen responsive genes by curcumin analogue

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Introduction. Prostate cancer is the most frequently diagnosed cancer among men and the second leading cause of male cancer deaths. DNA hypermethylation and loss of androgen receptor (AR) function are known to contribute to the progression of prostate cancer from a hormone sensitive to a hormone refractory state. Dibenzoylmethane (DBM), a curcumin analogue, exhibits antineoplastic effects in rodent models of skin and mammary cancers and human prostate cancer cells. To date, few mechanisms explaining the growth inhibitory effects of DBM on prostate cancer cells have been proposed. We have used microarray technology to investigate global gene expression changes in hormone refractory prostate cancer observed after treatment with DBM.

Material and Method. The HU133 Plus 2.0 Affymetrix 3'expression microarray chip was used to examine the mRNA profile of the LNCaP prostate cancer cell line, treated 72 hours with DBM. Methylation sensitive genes uncovered from the microarray data were further validated using a DNA methylation PCR system.

Results and Discussion. Our results revealed that out of 47,000 + transcripts, 524 differentially expressed genes (274 up-regulated; 294 down-regulated) were found to have a greater than or equal to log2 change (treated vs. control). Of the 524 named genes, 7 KEGG pathways were resolved including cell cycle, DNA replication, p53 signaling and metabolism of xenobiotics by cytochrome P450. Ten percent of the differentially regulated genes were also found to be sensitive to methylation.

Conclusion. Our data suggests that DBM exerts a strong epigenetic effect on the AR and its' associated co-activators. DBM's ability to reverse DNA methylation patterns has potential as targeted therapy for hormone refractory prostate cancer.

No conflict of interest.

114 Targeting MPS1 to kill tumor cells

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Introduction. Monopolar spindle 1 (MPS1, o.n. TTK) is a mitotic kinase up-regulated in multiple human tumors, including breast and lung cancers. MPS1 contributes to mitosis progression by regulating the alignment of chromosomes to the metaphase plate and the spindle assembly checkpoint (SAC). The aim of this study was to evaluate MPS1 as a potential target for anticancer therapy.

Material and method. Apoptotic parameters, including the quantification of plasma membrane integrity and inner mitochondrial membrane potential, and cell cycle distribution, including the percentage of DNA-replicating and mitotic cells, were assessed by means of cytofluorometric studies. Immunofluorescence microscopy and videomicroscopy analyses were conducted to evaluate the organization of the mitotic spindle and the execution of mitosis. The anti-neoplastic effect of the inhibition of MPS1 was also evaluated in vivo, in xenograft mouse models.

Results and discussion. We first observed that the depletion of MPS1 by means of cell transfection with specific siRNAs directed against this kinase or its pharmacological inhibition abrogates SAC function, both in vitro and in vivo. This leads to aberrant chromosome segregation, the generation of aneuploid/polyploid cells and the activation of mitotic catastrophe executed by the mitochondrial pathway of apoptosis. We then gave evidence that MPS1 inhibition/depletion kills tetraploid cancer cells more efficiently than their parental diploid counterpart. Of note, tetraploidy constitutes a genomically metastable state observed in the early stages of multiple neoplasms, and associated with aneuploidization and genomic instability. The anti-tetraploid effect of MPS1 inhibition/depletion seems to be due on the one hand to the intrinsic propensity of tetraploid cells to incur in mitotic aberrations (and thus activate mitotic catastrophe) and on the other hand to their dependency on a very robust mitotic machinery for a correct chromosome segregation.

Conclusion. Taken together, these results suggest that MPS1 inhibitors may exert robust anticancer/antitetraploid activity and support the further clinical development of such agents.

No conflict of interest.

116 A novel intragenic regulatory region controls RUNX2 expression in cancer cells

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Introduction. RUNX2 is a transcription factor necessary for skeletal morphogenesis. Factors crucial during embryogenesis are often hijacked during cancer progression, and RUNX2 is not an exception. RUNX2 is increasingly recognized in cancer biology for its oncogenic properties. Numerous papers link RUNX2 expression with progression and metastasization of different tumors. The mechanisms regulating the re-

activation of this factor in cancer are still unknown. In this work we dissect some of the molecular mechanisms controlling RUNX2 expression in cancer.

Materials and Methods. We used the annotation data of the ENCODE project to identify DNA regions within the RUNX2 locus with features of regulatory elements (conservation, DNAse I hypersensitivity, specific histone modifications). Three potential enhancers (ENH1-3) were identified and tested for their ability to activate the RUNX2 P2 promoter by luciferase assay. Sequential deletions and site directed mutagenesis were employed to assess ENH3 functional elements. To characterize the transcriptional complex controlling ENH3 we used EMSA, ChIP, DNA-pulldown assay and mass-spectrometry. To functionally validate our observations we used siRNA mediated silencing and pharmacological approaches.

Results and discussion. Using the ENCODE data, we identified a new enhancer region within the RUNX2 locus named ENH3. We showed that ENH3 presents features of active chromatin and that it is necessary to drive activation of the P2 promoter in several cancer cell lines. We mapped the ENH3 core in a 30bp bipartite region and we showed that a multi-protein complex binds to the ENH3 core and is responsible for its activation. We identified the c-JUN transcription factor as crucial component of this complex and as positive regulator of RUNX2 expression. By DNA-pull down assay and proteomic approaches we isolated the multiprotein complex bound to ENH3 and identified additional components. Finally, we showed that HDACi profoundly inhibits RUNX2 expression in cancer cells by disassembling the transcriptional complex on ENH3.

Conclusion. This work demonstrates that the major transcriptional activity driving the expression of RUNX2 in cancer resides in a newly identified enhancer, located in an intronic region of the gene, which is able to activate the P2 promoter and to respond to external regulatory stimuli. Furthermore, these data envisage a possible targeting strategy to counteract the oncongenic function of RUNX2 in cancer cells, and provide evidence that the cytotoxic activity of HDACi in cancer is not only dependent on the reactivation of silenced oncosuppressors but also on the repression of pro-oncogenic factors.

No conflict of interest.

117 STARD3: A potential therapeutic target in colorectal cancer

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Introduction. Every year, more than one million of people worldwide are diagnosed with colorectal cancer (CRC). Even though preventive screenings have been able to reduce incidence and mortality, nearly half of the patients die following the diagnosis and the treatment. To improve the therapeutic success and options, repositioned or new drugs and novel therapeutic targets should be taken in consideration.

In the last years, comprehensive cancer genomic analyses have shed new light on previously unknown genetic and biological determinants of the disease.

Starting from a collection of amplified genes selected by The Cancer Genome Atlas (TCGA) in CRC samples, the aim is to identify new oncogenes for targeted therapy utilising a RNAi-based screening approach.

Materials and Methods. In this study were used 3 colon cancer cell lines, corresponding to 3 CRC subtypes. The cell lines were transduced with a lentiviral based vector where shRNAs where cloned. The stable cell lines were cultured and their cell viability evaluated by a luciferase-based assay. The RNA of cell lines was extracted and quantitative PCR was used to evaluate silencing activity

Results and discussion. Using a set 3 colon cancer cell lines, (HCT116, HT29 and DLD1) corresponding to different CRC subtypes, we set up a functional screening, by RNAi, to address the impact of the putative oncogene on cell viability. Preliminary results have shown that out of 6 genes tested, in HCT116 and HT29, but not in DLD1, the STARD3 gene reduced cell growth, after 96 hours of culture, by 50% compared to the control. These results may indicate that STARD3, a cholesterol binding and shuttling protein, could reduce cell viability in a subtype-dependent context. More investigation are needed to elucidate its role on CRC and identify old or new drugs to regulate its activity.

Conclusions. Among the potential oncogenes only STARD3 has been found to decrease cell viability in 2 CRC cell line. This data suggest a subtype-specific mechanism of action that might further explain the differences in diverse CRCs and, if identified, a novel therapeutic chance to treat these types of cancers.

No conflict of interest.

118 Open resource CRISPR/Cas9 genome-wide sgRNA library screening platform

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Recent studies demonstrate that CRISPR/Cas9 gene knock-out technology can be used as a powerful tool for large-scale functional genomic analysis in mammalian cells. With the goal to establish a cost-effective functional genomics platform for the discovery of therapeutic targets, we developed a public resource lentiviral sgRNA screening platform, enabling scientists to perform pooled format genome-wide CRISPR/Cas9 genetic screens. Three 55K sgRNA libraries were designed to cover the whole protein-encoding human genome with a redundancy of 8 sgRNAs per gene. As supporting tools, we developed protocols, reagents, and software tools for hit validation and target prioritization. The performance of CRISPR/Cas9 and RNAi screening platforms was compared in genetic screens aimed at delineating the processes underlying tumorigenesis in a panel of PDX-derived cancer cell lines. These newly developed sgRNA screening, validation, and software tools are freely available for academic researchers through the open resource www.decipherproject.net website.

Conflict of interest: Other substantive relationships: Employee of Cellecta, Inc.

Poster Session: Cancer Genomics, Epigenetics and Genome Instability II

119 Frameshift mutation of a histone methylation-related gene SETD1B and its regional heterogeneity in gastric and colorectal cancers with high microsatellite instability

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Introduction. Histone methyltransferase (HMT), which catalyzes a histone methylation, is frequently altered in cancers at mutation and expression levels.

Material and Method: The aims of this study were to explore whether SETD1B, SETDB2, and SETD2, SET domain–containing HMT genes, are mutated and expressionally altered in gastric (GC) and colorectal cancers (CRC). In a public database, we found that SETD1B, SETDB2, and SETD2 had mononucleotide repeats in coding sequences that might be mutation targets in cancers with microsatellite instability (MSI).

Results and Discussion. We analyzed the mutations in 76 GCs and 93 CRCs and found SETD1B (38.7% of GC and 35.6% of CRC with high MSI [MSI-H]), SETDB2 (11.1% of CRC with MSI-H), and SETD2 frameshift mutations (6.7% of CRC with MSI-H).

These mutations were not found in stable MSI/low MSI.

In addition, we analyzed intratumoral heterogeneity (ITH) of SETD1B mutation in 6 CRCs and found that 2 CRCs harbored regional ITH of SETD1B. We also analyzed SETD1B expression in GC and CRC by immunohistochemistry. Loss of SETD1B expression was identified in 15% to 55% of the GC and CRC with respect to the MSI status. Of note, the loss of expression was more common in those with SETD1B mutations than those with wild-type SETD1B. We identified alterations of SET domain–containing HMT at various levels (frameshift mutations, genetic ITH, and expression loss), which together might play a role in tumorigenesis of GC and CRC with MSI-H.

Conclusion. Our data suggest that mutation analysis in multiple regions is needed for a better evaluation of mutation status in CRC with MSI-H.

No conflict of interest.

120 CpG island methylation profiling in bortezomib-treated multidrug-resistant cancer cell line

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Introduction. Multiple drug resistance (MDR) is a major obstacle to attenuating the effectiveness of chemotherapy to many human malignancies. Proteasome inhibition induces apoptosis in a variety of cancer cells and is recognized as a novel anticancer therapy approach. Despite its success, some multiple myeloma patients are resistant or become refractory to ongoing treatment by bortezomib. We recent reported that the existence and participation of ABCB1 and the Wnt pathway in an MDR cell line that attenuated bortezomib-induced apoptosis. To study the chemoresistant cancer cells may have developed a novel mechanism directed against the bortezomib-induced cell death, we used human CpG island microarray to identify epigenetic change and genes expression profile in bortezomib-treated multidrug-resistant cancer cells MES-SA/Dx5 cells.

Material and method. The human sarcoma cell line MES-SA (ATCC CRL-1976) and the multiple drug-resistant cell line MES-SA/Dx5 (ATCC CRL-1977) were used in this study. An Oligo CpG islands microarray was developed by Dr. CM Chen laboratory and the 240,000 spots on these chip was used to screen the difference of genome-wide methylation pattern by using the Genechip differential methylation hybridization (DMH) protocol.

Results and discussion. Our data has shown that bortezomib inhibits DNA methyltransferase gene expression; the global genes were hypomethylated in both bortezomib-treated MES-SA and MES-SA/Dx5 cell line. Furthermore, the Wnt signaling pathway and PKCδ signaling pathway related genes have significant altered in DNA methylation pattern of bortezomib-treated MES-SA/Dx5 cells.

Conclusion. These data may help us to discover the novel mechanism directed against the bortezomib-induced cell death, and to develop a new strategy for treatment of multiple drug resistance cancer cells.

No conflict of interest.

122 A combination of expression biomarkers PSA, PCA3 and AMACR in urine outperforms the PSA test alone in diagnostics of prostate cancer

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Introduction. Prostate cancer (PC) is the second most commonly diagnosed male cancer worldwide. Currently used prostate specific antigen (PSA) biomarker and digital rectal examinations (DRE) have low specificity for detecting PC and they poorly predict the presence of aggressive disease. New generation of noninvasive biomarkers for screening and prognosis is emerging that may supplement PSA testing, or replace it. Nevertheless, larger prospective studies comparing these biomarkers are necessary to evaluate definitely their value in the management of early PC.

Material and Method. We analyzed PCA3 (DD3), PSA (KLK3), AMACR and GOLM1 (GOLPH2) in urine samples collected after DRE of 54 PC patients and 13 BPH controls using quantitative real-time polymerase chain reaction (qPCR), TaqMan technology. For normalization B2M was used as reference control. Data were analyzed by SPSS. To evaluate diagnostic accuracy we performed ROC curve analysis. Correlation analysis was also made.

Results and Discussion. In the ROC curve analysis the best diagnostic accuracy was observed for PCA3 (AUC=0.778, 95% CI: 0.654-0.901, p=0.003) with sensitivity of 85.2% and specificity of 58.3%. PCA3 outperformed serum PSA levels (AUC=0.767) and urine PSA levels (AUC=0.694) as well as urine expression levels of GOLPH2 (AUC=0.678) and AMACR (AUC=0.728). Even better discriminating performance was reached when combinations of the studied biomarkers were used. Sensitivity of 77.8% and specificity of 75% were achieved when PCA3 and AMACR were combined (AUC=0.832, 95% CI: 0.700-0.954, p<0.001). The three urine biomarker combination PSA, PCA3 and AMACR delimitate PC from BPH with 75.9% sensitivity and 91% specificity (AUC=0.823, 95% CI: 0.696-0.950, p=0.001). Expression levels of the studied biomarkers in urine did not show correlations with clinicopathological characteristics probably due to the small sample size.

Conclusion. Combinations of the studied urine biomarkers have the potential to be used as noninvasive diagnostic biomarkers for PC screening outperforming the serum PSA testing alone. To validate the results a study with larger sample size is needed.

No conflict of interest.

123 The histone methyltransferase EHMT2/G9a epigenetically activates BECN1 to control of autophagy

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Purpose. High expression of EHMT2/G9a (euchromatic histone-lysine N-methyltransferase 2; EHMT2), which contributes to transcriptional repression of tumor suppressors, has been implicated in promoting tumorigenesis, metastasis and poor prognosis. Although the cytoplasmic machinery that orchestrates autophagy induction has been widely studied, the key epigenetic events that initiate and maintain the autophagy process remain unknown. In this study, we provide experimental evidence supporting the role of the EHMT2 in the transcriptional regulation of BECN-1.

Materials and Methods. We investigated the specific targets of EHMT2 were identified by using PCR array (RT2 profiler PCR array) and ingenuity pathway analysis (IPA). To test whether EHMT2 transcriptionally regulates the BECN1 expression, we performed chromatin immunoprecipitaion (ChIP) assays and to understand the underlying mechanism in the epigenetic regulation mediated by G9a inhibition, we examined several signal transduction pathways by nuclear fraction immunoblot, fluorescent microscopy analysis. Lastly, synergistic transcriptional activation of BECN-1 were confirmed with methylation specific PCR (MS-PCR) in the combination treatment of 5-aza-2'-deoxycytidine(5-Aza-dC), a DNA methyltransferase inhibitor..

Results. Inhibition of EHMT2 by BIX and EHMT2 siRNA induced expression of autophagy-related genes (ATGs), especially a tumor suppressor gene, BECN1. BIX and EHMT2 siRNA decreased dimethylation of lysine 9 of histone H3 (H3K9me2) and recruited RNA polymerase II on promoter of BECN-1. Increase in ROS by BIX activated NF-κB and translocated it onto the promoter of BECN1, leading to transcriptional activation. Moreover, treatment of BIX in the presence of 5-aza-2'-deoxycytidine(5-Aza-dC), a DNA methyltransferase inhibitor had synergistic effect on transcriptional activation of BECN-1.

Conclusions. These findings suggest that EHMT2 directly represses ATGs and tumor suppressor genes and the inhibition of EHMT2 may be an important mechanism of cancer prevention by activating autophagy.

No conflict of interest.

124 Bioinformatic approaches for somatic mutation calling, annotation and selection in in matched sensitive-resistant ovarian tumor pairs

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Introduction. Epithelial ovarian cancer (EOC), is generally sensitive to first line platinum based therapy, however more than 80% of patients experience relapse within 18 months and become resistant to subsequent cycles, until the disease becomes incurable. Despite recent advances, the mechanisms underlying drug resistance in EOC have yet to be clearly identified. Earlier results from our laboratory, identified the EMT pathway as a key player (Marchini et al., 2013). To go deeper in detail on the genomic defects driving tumor resistance at relapse, we performed targeted DNA re-sequencing over 65 genes on a cohort of matched sensitive-resistant samples from 33 EOC pairs. Here we describe the development of a bioinformatic approach to call, annotate and select potential somatic mutations in these matched sample pairs.

Materials and Methods. We built our approach on existing open-source solutions. For somatic mutation calling, we improved an already existing, community developed, pipeline: bcbio-nextgen (http://github.com/chapmanb/bcbio-nextgen), adding support for existing somatic variant callers after evaluation of existing programs (MuTect, VarScan2, and FreeBayes), and adopting the pipeline to support them We developed post-processing methods leveraging on annotation software (snpEff and VEP) and categorization and filtering programs (GEMINI) to select mutations of interest among all paired samples.. The pipeline was then tested on a high performance cluster computing platform (Cloud4CARE project). Tumor comparisons were performed using blood from the same patient as normal reference.

Results. Initial tests were made on reduced data sets from the Cancer Genome Atlas to assess the proper generation of the data and their correctness. Once the proper functionality was assessed, we ran the pipeline on the complete data set of EOC samples. The pipeline correctly discriminated germline mutations from somatic ones, selecting potential mutations of interest then confirmed through external validation.

Conclusions. Our results suggest that our bioinformatic approach is sensitive, robust, reproducible and viable for analysis of matched EOC samples.

No conflict of interest.

125 High MALAT 1 expression induce metastasis process in early stage colorectal cancer patients

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Introduction. Metastasis-associated lung adenocarcinoma transcript1 (MALAT1), one of the first defined cancer-associated long noncoding RNAs (lncRNAs), has a significant role in the development and progression of many types of tumors. According to recent studies, MALAT1 might also suppress the tumor growth and metastasis via PI3K/AKT signaling pathway in osteosarcoma. However, the exact molecular mechanism of MALAT1 in metastasis process of colorectal carcinoma remains largely unknown. Furthermore, the down-regulation of nm23H1 is responsible for the up-regulation of growth and invasion by MAPK/Erk and Akt-integrins and other signaling pathways. The aim of this study was to identify the altered expression of MALAT1 depending on the nm23H1 expression status of early stage CRC and determine prognostic biomarker that could provide novel therapeutic molecular target.

Materials and Methods. MALAT1 expression levels were investigated in tumors and surgical margin tissue samples from 60 early onset sporadic CRC patients in whom nm23H1 gene expression profiles were analyzed previously. The relationship between the MALAT1 expression profiles depending on nm23H1 expression status and the characteristics of tumors and patients was evaluated using SABioscience Data Analysis Software.

Results. The expression level of MALAT 1 was significantly up-regulated (P=0.01) in early stage tumor tissues compared with the normal tissues. High expression level of MALAT1 was significantly associated with distant metastasis in 5 years follow-up period (4.07-fold; P<0.05). MALAT1 up regulation is also correlated with down regulated expression of nm23H1 which involves in PI3K/AKT signaling pathway in tumor tissues (P=0.004).

Conclusion. High expression levels of MALAT1 in early stage tumors correlated with formation of metastasis and poor prognosis in CRC patients. Further studies and validations are required; MALAT1 is a promising therapeutic target related with AKT signaling pathway for anti-tumor and anti-metastatic intervention as a new remarkable biomarker for early stage of CRC patients.

Keywords. MALAT1; lncRNAs; Colorectal cancer; Early stages; Metastasis

No conflict of interest.

126 Genetic variations in miRNA-binding sites of KRAS related with invasion in Turkish Pancreatic Cancer patients

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Introduction. Oncogenic KRAS mutation is the signature genetic event in the progression and growth of pancreatic cancer (PC), an almost universally fatal disease. Although it has been appreciated for some time that nearly 95% of PC harbor mutationally activated KRAS, to date no effective treatments that target this mutant protein have reached the clinic. MicroRNAs (miRNAs) are small non-coding RNAs that inhibit mRNA stability and/ or translation by binding to the 3' untranslated region (UTR) of target mRNAs. miRNAs regulate genes involved in functions ranging from development, differentiation and proliferation to stress processes. Some studies shown that KRAS 3'UTR polymorphisms disrupting miRNA binding can be functional and can act as genetic markers of cancer risk. The aim of this study was to analyze the 3'UTR regions of the KRAS in PC patients and to identify the possible miRNA-binding sites of the specific 3'UTR variations in the Turkish population.

Materials and Methods. 43 patients with PC was selected in from Uludag University, Medical Faculty, Department of General Surgery. 3'UTR of KRAS gene were amplified for sequencing using PCR and DNA Sequencing System. The results of the sequencing analysis were compared with wild- type samples and the wild-type sequences of 3'UTR regions the KRAS. The relationships between the defined alterations and miRNA binding-sites were verified using web-based programes. Chi-squared tests (SBSS) was used to compare the determined results of mutation analysis and clinical features of patients.

Results. Two variations (rs34719539 and rs57698689) were identified in 23% and 7% of patients. These variations are important for miRNA binding-side such as let-7c, miR-155 and miR-216b in the 3'UTR region of the KRAS. In terms of variations in the 3'UTR region of the KRAS gene and the invasion status of the patients there was a statistically significant relationship (p =0.047).

Conclusion.miRNas may act as a tumor suppressor gene and suppressed cellular migration and invasion by targeting KRAS in PC. The ability of miRNAs to bind to the 3'UTR of KRAS is critical for regulating KRAS levels and protein expression, and this binding can be affected by variations. In this study reveals new information of the approximate 3'UTR region of KRAS variations and frequencies in the Turkish population. Functionally further experiments are needed to reveal the therapeutic targets controlling these processes.

Keywords: 3'UTR ; miRNA; Pancreatic Cancer; Invasion

No conflict of interest.

127 Down–expression of miR-152 and miR-212 in leiomyosarcoma and undifferentiated pleomorphic sarcoma

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Introduction. Soft tissue sarcoma (STS) are a heterogeneous group of rare mesenchymal tumours, representing 1% of adult and 15% of childhood cancers. While benign STS are relatively common, the rarity of malignant STS causes problems with respect to diagnosis, grading, or optimal therapeutic approach. The cytogenetic abnormalities associated with STS include a wide spectrum from gains to losses of chromosomal material such as trisomies, deletions, translocations. Clinical outcome of patients with advanced disease remains strongly unfavourable for the lack of effective therapies.

microRNAs, a class of post-transcriptional regulators, act either as tumour suppressor genes or oncogenes and control cellular signalling events leading to cell growth and survival. miRNA expression pattern may serve as a new tool in establishing tumour clinical-biological peculiarities.

Our aim was to analyze microRNA expression profile in STS clinical specimens in order to identify specific histotype-related signatures predictive of clinical course of disease .

Methods. miRNA array was performed on 20 selected STS samples including 10 high-grade LMS (Leiomyosarcoma) and 10 UPS (Undifferentiated Pleomorphic Sarcoma).

Statistical analysis using two-group tests was performed to obtain differentially expressed miRNAs ($p<0.05$) when compare to mesemchyimal cells used as control.

In silico analysis allowed to identify potential target genes by using the specific software PicTar, TargetScan 6.2 and miRBase Targets.

The expression levels of selected miRNAs were then validated in additional 27 LMS and 32 UPS and in 10 normal tissues, using the TaqMan® Custom MicroRNA Array.

Results. Data analysis of miRNA expression profiles showed 38 and 46 miRNAs differentially expressed in UPS and LMS respectively when compared to control. 21 out of the 84 miRNA were common to the two STS subtypes. By bioinformatics analysis, 12 of the 21 miRNAs showed a common pathway involved in miRNAs biogenesis and function.

The validation data on a larger series of UPS and LMS confirmed miRNA deregulation and the Student's t-test showed a statistically significant under-expression of miR-152 and miR-212 in STS specimens when compared with normal tissue.

Conclusions. Our data suggest that miR-152 and miR-212 expression could be relevant in diagnosis and biological behavior of some STS histotypes. Integrative analysis of target genes and downstream pathways are ongoing to assess their role in tumor progression.

No conflict of interest.

128 Exome sequencing in primary melanoma provides insights into genetic alterations associated with melanoma progression

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Introduction. Melanoma is a highly aggressive form of skin cancer that originates from the malignant transformation of melanocytes; it has high metastatic propensity and it is refractory to most traditional chemotherapeutic drugs. Identification of predisposing genes implicated in melanoma progression is crucial to understand this disease and improve its treatment. The most common mutation in melanoma is the substitution V600E that occurs in the oncogene BRAF. Several whole exome sequencing studies have revealed numerous other alterations. The aggressive behavior of melanoma is highly correlated with histological features, such as the thickness of the primary tumor and the mitotic index. Here we performed whole exome sequencing of 5 thin (<1mm in thickness) and 5 thick (>4mm in thickness) primary melanomas compared to matched-normal DNA.

Material and method. We have collected 10 fresh primary melanomas from 10 untreated patients: DNA samples from melanoma tissues and peripheral blood (normal DNA) were available from all of the recruited patients. Genomic DNA was extracted from tumor and peripheral blood samples using the QIAamp DNA Minikit, (Qiagen). Extracted DNA was used for Next-Generation Sequencing analysis by Illumina.

Results and discussion. We confirmed recurrent somatic mutations in known melanoma-related genes, such as BRAF, c-KIT, EGFR, PPP6C, MLL3, STK19 and several components of the glutamate signaling. In addition, we discovered mutations in genes not previously linked to this tumor, including CSMD1, FGFR4 and components of Notch and Hedgehog (HH) signaling pathway. In particular, in a thick melanoma we found a novel mutation in one of the final effectors of the HH signaling, the transcription factor GLI1. This mutation presented the substitution of threonine at position 1045 by alanine that mimic a constitutive phosphorylation and might be a potential activating mutation. Interestingly, we identified regions of focal somatic copy-number alterations (SCNAs) that were altered at significantly higher frequency in thick compared to thin melanomas. These regions of focal SCNAs comprised several different gene families among components of pathways found altered in melanoma, including Notch, HH and Wnt/b-catenin signaling pathways, BRAF, c-MYC and its cofactor PIM1, several ADAMs, and the HOX genes.

Conclusion. Our data identify potential drivers of melanoma progression, enhancing our understanding of the genomic complexity underlying melanoma.

No conflict of interest.

129 The down-modulation of miR-23b in human hepatocellular carcinoma is mediated by DNA methylation

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Introduction. Hepatocellular carcinoma (HCC) is one of the most common human malignancy with an extremely poor prognosis. microRNAs are small non-coding RNAs that negatively regulate gene expression. miRs dysregulation frequently occurs in cancer, also in HCC, and the inactivation of some miRs is mediated by aberrant DNA methylation. In recent years, we have experimentally validated miR-23b as negative coregulator of uPA (UK) and c-met (a TKR) and miR-193a as negative regulator of uPA; the expression of these two miRs resulted down-regulated in HCC respect to peritumoral tissues (PT). Since in silicoanalysis has revealed the presence of CpG island near the sequences coding these miRs, the purpose of this study was to investigate the methylation status of miR-23b and miR-193a in HCC.

Materials and Methods. HCC cell lines were treated with the demethylating agent 5-aza-2'-deoxycytidine (5-Aza-dC). The effects on miRs expression were evaluated by stem-loop qPCR and the effects on uPA and c-met expression were analyzed by qPCR and western blotting. We profiled the expression of miR-23b and miR-193a by stem-loop qPCR in HCC and PT tissues from 59 and 67 HCC patients respectively and we verified DNA methylation status in a subset of 24 HCC cases by methylation-specific PCR (MSP) and combined bisulfite restriction analysis (COBRA).

Results and Discussion. To determine whether the down-regulation of miR-23b and miR-193a in HCC is mediated by DNA methylation, we first studied the effects of 5-Aza-dC treatment in HCC cell lines at low expression levels of miR-23b and miR-193a. 5-Aza-dC treatment increased the expression of both miRs in HepG2 cells and caused the modulation of their targets uPA and c-met. Next, we correlated the expression levels of miR-23b and miR-193a with the DNA methylation status of their relative

CpG island in HCC and PT tissues. In the cohort examined, we confirmed the down-regulation of miR-23b and miR-193a in HCC respect to PT tissues. miR-23b methylation level was significantly higher in HCC than PT tissues (p=0.05), suggesting that miR-23b is partially silenced by iper-methylation. Instead miR-193a resulted generally hypomethylated in HCC tissues.

Conclusions. Our results outline new advances in the epigenetic regulation of miRs that regulate HCC unfavourable prognostic markers (i.e. uPA and c-met) and suggest the use of hypomethylating agent in combination with/or without miR mimics to recover miRs expression in HCC cell lines.

No conflict of interest.

130 Next Generation Sequencing (NGS) approach to investigate new mutations in pediatric Glioblastoma Multiforme

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Introduction. Glioblastoma multiforme (GBM; WHO-grade IV), the most frequent primary malignant brain tumor in adults, accounts for approximately 10% of all central nervous system tumors in childhood. Adult and paediatric GBMs (pGBMs) have distinct genetic and molecular pathways of tumorigenesis and different studies, based on array-CGH analysis, reported that there are significant differences in Copy Number Alterations (CNA). Material and method: In our previous study we identified, using array-CGH, recurrent CNA in 8 pGBMs establishing minimum common regions (MCR) of duplication/amplification and deletion. Based on these results, we developed a next-generation sequencing (NGS) approach to screen the genetic profile of tumors. NGS has provided a new paradigm in biomedical research to delineate the genetic basis of human diseases. The panel was designed to cover 420 genes selected within of MCRs to try to identify new genes involved in tumorigenesis and/or progression of pGBMs. **Results and Discussion.** In 8 patients we found 18 heterozygous mutations in different genes. The same mutations were also found in DNA extracted from blood and in two cases we demonstrated the parental origin of 12 mutations. **Conclusion.**Beyond the rarity of disease and the lack of literature data, our findings may better elucidate if there is a genomic background in development and progression of pGBM. The recognition of candidate genes underlying this disease could then improve treatment strategies for this devastating tumor.

No conflict of interest.

131 Epigenetic therapy in retinoblastoma tumor: From computational system biology to pre-clinical models

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Introduction. Retinoblastoma is a very aggressive cancer of the developing retina. Initiated by the biallelic loss of RB1 gene, this cancer progresses very quickly following RB1 inactivation. Beside the RB1 deletion or mutations, multiple cancer pathways are deregulated, also epigenetically. Here the efficacy of epigenetic therapy has been evaluated through an integrated bioinformatics and pre-clinical approach.

Materials and Methods. Microarray analysis was performed on WERI-RB1 cells before and after epigenetic 2.5uM 5-Aza-2'-deoxycytidine (DAC) treatment. Gene expression profiles were analyzed using bioinformatics tools and functional annotation maps were obtained for biological processes and pathways centered on differential expressed genes, respectively. The results were validated using qRT-PCR. Cell cycle variations and apoptotic induction were assessed by cytofluorimetric analysis. In vivo, DAC was systemically used free (75 ug/dose) or delivered with an engineered magnetic erythrocyte-based carrier (EMHV, 10ug/dose DAC) to the site of action by an external biocompatible magnetic field applied on tumor mass. WERI-RB1 xenograft model of nude mice were treated I.V. biweekly and tumor mass volume was measured using a manual caliper.

Results. We identified differentially expressed genes from time course microarray experiments, which are epigenetically regulated during retinoblastoma tumorigenesis. Analyzing the gene expression profiles at 48, 72 and 96h after DAC treatment, integrated with some of the best-established retinoblastoma markers, we tracked the most interesting gene profiles affected by treatment. Validation by qRT-PCR of selected markers in treated and untreated WERI-RB1 cells reinforced the in silico results. Enrichment of subG1 phase as well as apoptotic response has been found in WERI- RB1 treated cells. Antitumor effect of DAC was reported both after using free DAC treatment and DAC loaded EMHVs in vivo suggesting potential clinical application of DAC for retinoblastoma therapy.

Conclusion. The proposed computational systems biology approach is useful for the purpose of reconciling epigenetic treatment-specific experimental evidences with gene expression measurements and could be even more useful to exploit epigenetic retinoblastoma-related makers for future therapies.

No conflict of interest.

132 Interstitial deletion of chromosome 5q in a patient with Familial Adenomatous Polyposis and mild developmental delay

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Introduction. Familial adenomatous polyposis (FAP) is an inherited condition leading to multiple intestinal polyps and predisposition to colon cancer. Most cases are caused by mutations in the adenomatous polyposis coli (APC) gene (MIM#611731), with penetrance close to 100% at the age of 40. Over 1500 mutations have already been identified. Most alterations result in truncation of the APC protein either by a nonsense or frameshift mutation with some genotype-phenotype correlations published in the literature.

Material and method. We present a 22-year-old male with suspicion for FAP. The clinical workup revealed the presence of hundreds upper GI and colonic polyps, without cancer development. The patient was presented with mild developmental delay and tendency to social withdrawal. The proband was adopted when he was 4 months old and family history was therefore unavailable. The patient was referred to our laboratory for APC genetic testing.

Genomic DNA was isolated from peripheral blood using standard protocols. Screening for larger deletions and duplications was performed by the use of Multiplex Ligation-dependent Probe Amplification (MLPA).

Results and Discussion. We found 50% peak area decrease in all specific for the APC gene probes, corresponding to heterozygous deletion of the entire APC sequence.

In order to determine the boundaries of the deletion, we performed an array CGH analysis with the Agilent Oligonucleotide Array- Based CGH (Agilent Technologies, CA, USA) according to the manufacturer's instructions. Copy number variation detection was conducted using CytoGenomics software (Agilent Technologies, CA, USA).

Our analysis showed an interstitial deletion of chromosomal region 5q that was confined to the region 5q22.2 - q23.1. The deletion, spanning about 8 Mb, encompassed the entire APC gene and other 29 genes. Interstitial 5q deletions are relatively rare and usually presented by severe phenotypes as facial dysmorphism, mental retardation and developmental delay. The milder phenotype in our case might be explained either by the extent of the deletion, or by the interaction and positive effect of modifier genes.

Conclusion. In the evaluation of this case we demonstrated the usefulness of both MLPA and array CGH in clinical practice. MLPA might be considered as the first-line analysis and if a deletion of the entire APC gene is found, the results could be confirmed and the breakpoints localized by array CGH.

No conflict of interest.

133 miRNA landscape analysis in patients with high grade serous advanced ovarian cancer receiving neoadjuvant chemotherapy

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Introduction. In the last decade platinum-based neoadjuvant chemotherapy (NACT) has been recognized as a reliable therapeutic strategy in patients with unresectable advanced ovarian cancer (AOC). However, the molecular changes induced by NACT at miRNA level, and their prognostic role has not been clarified until now.

Material and **Methods.**, 82 FIGO Stage IIIC-IV high-grade serous (HGS) tumors from AOC patients admitted to the Catholic University of the Sacred of Rome (2002 - 2011) were analyzed. patients were judged unsuitable for complete primary debulking at initial laparoscopic evaluation (i.e. Fagotti score≥8), and submitted to multiple biopsies and further carboplatin-paclitaxel NACT. After 4 courses of NACT, all received interval-debulking surgery (IDS) and tumor specimens were collected. miRNAs were purified from FFPE specimens taken at the time of diagnosis (Dg-samp), and from the IDS (IDS-samp). miRNA landscape analysis was performed using commercially available arrays. Real Time PCR was used for downstream validation. Clinical parameters were correlated in multivariate analysis with overall survival (OS) and progression-free survival (PFS). Immunohistochemistry for P-Smad2 was performed and the expression levels were assessed and scored by a dedicated pathologist as the proportion of immunostained tumor cells (at low magnification 5X) in the entire tumor area (GFZ).

Results. 369 miRNAs were differentially expressed (DEM) in FFPE samples taken before and after NACT. Of these, 125 were up-regulated and 244 were down-regulated. DEM were not scattered across the genome, but clustered into familes: miR-8 and miR-642 family members were down-regulated in the in Dg-samp compared to matched IDS-O ($p<0.05$), whereas the miR-199, let-7, miR-30 and miR-181 family members were up-regulated in the matched samples. Multivariate analysis confirmed miR-181a, an activator of the TGFB pathway, associated to OS and PFS ($p<0.01$). Immunostaining analysis confirmed an enrichment of P-Smad2 expression (a marker of TGFb activation) in tumors from patients with shorter PFS and OS. Kaplan Meyer curves derived from concomitant expression of P-Smad2 and miR-181a show significant differences in PFS and OS compared to those depicted on the expression of each biomarker alone ($p<0.001$).

Conclusion. The use of matched tumor biopsies, taken before and after NACT, is becoming an important opportunity to unravel the complex genetic background

of HGS-AOC, which can ultimately lead to the discovery of molecular events that could be predictive of tumor response and relapse. In this study we confirmed that the activation of the TGFB pathway via miR-181a deregulation is a marker of poor prognosis. Combination of miR-181a expression levels and immunohistochemical staining for P-Smad2 could improve our ability to identify those patients with poor outcome after NACT at the time of diagnosis, as previously observed in an independent cohort of patients (Parikh, A, et al Nature Communication, DOI: 10.1038/ncomms3977)

No conflict of interest.

134 The long non-coding RNA HOTAIR is aberrantly down-regulated in alveolar fusion-positive rhabdomyosarcoma

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Introduction. The pediatric soft tissue sarcoma Rhabdomyosarcoma (RMS) is an aggressive cancer of childhood characterized by the expression of myogenic markers such as MyoD and Myogenin. Conversely to normal myoblasts, RMS cells are highly invasive and unable to undergo myogenic differentiation. The alveolar RMS often expresses fusion oncoproteins such as PAX3- or PAX7-FOXO1. We and others have recently shown that the Polycomb Group protein EZH2 is aberrantly expressed in RMS. We investigated here the role of a long-noncoding RNA, HOTAIR, which can work with EZH2, in RMS.

Material and Methods. We used real time RT-PCR to detect the expression of HOTAIR and miR-196 in primary RMS samples and RMS cell lines and compared the data with those obtained in their respective controls: muscle tissues or skeletal myoblasts cultured in vitro. We treated PAX3-FOXO1 RMS cells and human myoblasts with 5-aza-cytidine (5-aza) to evaluate the expression of HOTAIR and pri-miR-196a1, a2, b and cell differentiation.

Results. We show that HOTAIR, located within the Homeobox C (HOXC) gene cluster on chromosome 12q13 region, is down-regulated in PAX3-FOXO1 RMS primary samples and cell lines compared to their respective controls. Similarly, miR-196 was reduced. In the same chromosome region is located the miR-196a-2 whose pri-miR-196a-2 expression behaved similarly appearing down-regulated together with pri-miR-196a-1 located on the 17q21 region of chromosome 17. In contrast, pri-miR-196b on chromosome 7 was not de-regulated. HOTAIR and miR-196a expression were also associated to those of HOXC10 and HOXC11. Interestingly, RMS cells treated with 5-aza showed a time-dependent induction of HOTAIR whereas pri-miR-196a-2 was not over-expressed.

Conclusions. Results from these preliminary experiments suggest that HOTAIR can be silenced by methylation in PAX3-FOXO1 RMS cells.

Future experiments will shed light on the role of HOTAIR in this soft tissue sarcoma.

No conflict of interest.

135 An integrated approach for the study of colorectal cancer: From methylome to transcriptome

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Introduction. Colorectal cancer (CRC) is the third type of most prevalent cancer in the world, with about 1.2 million new cases diagnosed each year. At least four types of genomic or epigenetic instability have been described in CRC: 1) chromosomal instability (CIN), 2) microsatellite instability (MSI), 3) CpG islands methylation phenotype (CIMP), and 4) global DNA hypomethylation. CIN tumors are characterized by the accumulation of mutations in specific oncogenes (APC, KRAS, PIK3CA, BRAF, SMAD4, TP53 etc) and tumor suppressors. Approximately 20% of CRC are also CIMP tumors. A growing number of scientific evidence suggest the predominant role of epigenetic changes, compared to genetic mutations, in determining the complexity and heterogeneity from the earliest stages of the disease, where the CIMP phenotype only refers to a restricted panel of genes.

Materials and Methods. The aim of the present study was to investigate the methylation status at more than 450k CpG loci, spread over more than 99% of the RefSeq genes, and consequently the expression profile of the transcriptome (over 47k transcripts) in 18 CRC DNA samples compared to 4 intestinal non tumoral tissues. Bioinformatics analysis of differently methylated loci and disregulated genes were conducted by DAVID and IPA tools.

Results and Discussion. Among the pathways showing more altered methylation patterns (mostly hypermethylated), were the metabotropic receptors pathways, or G protein-coupled, the receptor ligands neuroactive pathways (signaling of dopamine, serotonin, norepinephrine, GABA), glutamate receptor signaling, cAMP mediated signaling, etc. Interestingly, microarray analysis of gene expression revealed that several genes related to the pathway involved in amines degradation (serotonin, dopamine, norepinephrine) were profoundly downregulated.

Conclusions. The results indicate changes in methylation possibly leading to a reduced synthesis of molecules necessary for the degradation of the amines. These results are in agreement with the existing literature suggesting a protective role of dopamines in the gastrointestinal tract and the involvement of glutamate receptors in cancer. Further investigations are aimed to clarify whether these changes take place at early stages of CRC development (adenomas), or are rather late events in the transformation of cancer cells. Finally, the present results might contribute to the identification of new molecular targets for pharmacological interventions.

No conflict of interest.

136 Elucidating the role of SGK3 in driving poor prognosis in cancer

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Introduction. Genome instability and evasion of cell death are fundamental hallmarks of cancer. Certain cancers, including epithelial ovarian cancers (EOC), pancreatic ductal adenocarcinomas (PDAC) and a subset of sarcomas with complex karyotypes are characterized as being very genomically unstable suggesting that such tumours are driven by defects in DNA damage recognition and repair. These tumours are also characterized by a high frequency of p53 mutation, extensive intra-tumoral heterogeneity and resistance to DNA damaging chemotherapy and thus consequently carry a poor prognosis. This highlights the need for detailed molecular characterization to identify new therapeutic targets and stratification biomarkers. The aim of the study is to use a bioinformatic approach to identify commonly amplified genes, that function in DNA damage response (DDR) and apoptotic processes, across the three tumor types, which correlate with poor prognosis in these patients.

Methods. For target identification, datasets for EOC and PDAC and sarcoma were analysed for common copy number aberrations in 734 genes relating to DDR and apoptosis, and confer a poor prognosis in progression-free survival (PFS) data in EOC patients. Genes with prognostic significance ($p < 0.05$) using the log rank test were picked from the top 100 most frequently amplified loci. The targets were then functionally validated by siRNA-mediated knockdown, overexpression and/or pharmacological inhibition using apoptosis, proliferation and migration assays in intra-patient paired platinum sensitive PEA1 and resistant PEA2 and SKOV3 EOC cell lines, chemo-sensitive Aspc1, chemo-resistant TKCC6 and Panc-1 PDAC cell lines, and leiomyosarcoma cell line SK-UT-1.

Results. Four genes, SGK3, c19orf40, MRPS12 and ZBTB32 were highlighted as being commonly amplified across all three tumor types, of which only SGK3 and c19orf40 were statistically significant when examining PFS in EOC patients. SGK3, a member of the serum/glucocorticoid regulated kinase (SGK) family appeared interesting as it has similar functions and substrates to the AKT kinase family, which we have previously shown to have key roles in tumor cell survival in response to therapy. siRNA-mediated knockdown of SGK3 alone and subsequently all SGK members did not alter induction of caspase 3/7 activity in response to chemotherapy, relative to control treatments in most cell lines studied. Overnight migration assays however, revealed that over-expression of SGK3 increases cell motility suggesting SGK3's role in prognosis is via migration/tumor spread rather than response to therapy.

Conclusion. Bioinformatic analysis highlighted four genes related to DDR/apoptosis that were commonly amplified across tumors characterized by genomic instability and resistance to chemotherapy. One of these genes, SGK3, appears to have a role in poor prognosis via migration. Analysis of the molecular basis for this effect is underway and results will be summarised.

No conflict of interest.

137 Epigenetic biomarker exploration in 5-fluorouracil response against colorectal cancer

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Recently, the importance of personalized medicine therapy has been emerging. As there is a need for research on the factors that cause the difference in drug response between individuals, the study of epigenetic factors in addition to genotype affecting the diversity of drug response is required. In this study, epigenetic biomarker candidates for 5-FU, the representative anticancer drug, will be analyzed the effects on drug response in colorectal cancer (CRC) patients and cell lines. Each of thirty blood samples from normal and CRC patients (CRCP) with stage II/III was subjected to global methylation and gene expression microarray analysis to examine the genetic and epigenetic factors. It showed the distinction by DNA methylation heatmaps between normal and CRCP in global DNA methylation and the correlation between DNA methylation and tumor response grade (0 to 3) to 5-FU. From microarray results, we selected DPYD and DNAJB6 (anti-apoptosis protein) genes based on their known or potential involvement in cell growth & proliferation and cancer. To verify the function of the DPYD and DNAJB6 against 5-fu drug sensitivity, we analyzed identifications of genetic/epigenetic and gene-expression based predictors of drug sensitivity from CRCP and cell lines. Although DPYD is known as a gene predictor of sensitivity to 5-FU, there was no significant relationship from normal versus patients and drug response. In CRC cell lines, DLD-1, HT-29, and SW620 were shown insensitive to 5-FU. Interestingly, DPYD was overexpressed in DLD-1, SW48 and SW620, which was seemed genomic correlates of drug sensitivity. Nonetheless DNAJB6 as an epigenetic biomarker candidate was

not confirmed its effect on drug response. In further study, we will perform to explore applicable biomarkers from different pathway and extra putative genes obtained previously.

No conflict of interest.

Poster Session: Carcinogenesis I

139 Does Brf1 have a role in prostate carcinogenesis?

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Introduction. RNA polymerase III (Pol III) controls the expression of short untranslated/non-coding transcripts, including tRNAs, to regulate protein translation. The transcription factor IIIB (TFIIIB) recruits Pol III to its target gene promoters. Brf1 (a 90 kD transcription factor and a component of TFIIIB) directly binds to Pol III. Studies have indicated that RNA Pol III transcription products are elevated in both transformed and tumour cells. However, the significance of Brf1 (and Pol III) in prostate carcinogenesis remains unknown.

Results. Brf1 expression in a panel of human prostate cancer (PC) cell lines (PC3, PC3M, DU145, LNCaP, LNCaP-AI, VCaP) was studied by Western blot analysis. While benign RWPE1 cells expressed negligible Brf1, aggressive (invasive sub-clone or androgen independent) cells such as PC3M and DU145 showed highest Brf1 expression.

Functional significance of Brf1 in PC was studied by transient over-expression and siRNA mediated knockdown (KD) of gene expression. Using a WST1 proliferation assay, Brf1 over-expression appeared to have a mitogenic effect in DU145 LNCaP, PC3 and PC3M cells, while Brf1 KD in the same cells suppressed proliferation, along with G2/M accumulation, reduced S-phase subpopulation, and a potential increase in sub-G1 phase in cell cycle analysis. We are currently developing stable cell lines to study the effect of over-expression and KD of Brf1.

We wish to test the role of Brf1 in vivo and used a model of Pten loss which predisposes to prostate tumorigenesis. A conditional transgenic line, LSL-Brf1, with elevated Brf1 expression was crossed with the Pb(Cre) Ptenfl/fl model to generate Pb(Cre) Ptenfl/fl Brf1 (or Pten-Brf1) mice. Data from these experiments, with focus on time to clinical endpoint and prostate tumour weights, will be presented.

Lastly, BRF1 immunoreactivity was significantly higher in clinical PC when compared to Benign Prostate Hyperplasia (BPH). Collectively, data from two independent patient cohorts [Glasgow: BPH (n=21), PC (n=151); Newcastle: BPH (n=113), PC (n=365)] showed that BRF1 expression was upregulated in PC relative to BPH ($p=0.0034$). Furthermore, within the PC cohort studied, high BRF1 was associated with significantly shorter patient survival (Kaplan Meier analysis, $p<0.001$).

Conclusion. Our early data are consistent with a potential oncogenic role for Brf1 in prostate carcinogenesis, but further investigation is warranted to consolidate these findings and to define the underlying molecular mechanism.

No conflict of interest.

140 Prenatal irradiation increases T-cell lymphoma risk when mice are exposed to a chemical carcinogen in early life, but not after radiation alone

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Introduction. Although the Oxford Survey of Childhood Cancers demonstrated an increase in childhood lymphoma/leukemia after in utero radiation exposure, conflicting results in subsequent studies mean that this result remains the subject of ongoing debate. Currently, the available data do not allow precise estimation of cancer risk throughout life following in utero exposure, particularly given the combined effects of other environmental and genetic factors.

We previously reported a synergistic effect of four consecutive weekly X-ray exposures from 4 weeks of age followed by four weeks of continuous exposure to N-ethyl-N-nitrosourea (ENU) compared to either agent alone, when the radiation dose was above a threshold; while, lower radiation doses had an antagonistic effect. Here, we studied the combined effect of radiation exposure in utero with ENU treatment at various times after birth on T-cell lymphoma (TL) development using B6C3F1 mice.

Materials and Methods. Fifty female B6C3F1 mice per group were exposed to a single dose of X-rays (2.0 Gy) either at embryonic day 17 (late fetal stage) or 5 weeks old, and were then treated with ENU (125 ppm) in drinking water for four weeks from 5, 9 or 13 weeks old (intervals of 0-13 weeks), with matched sham-radiation or vehicle-only control groups. The mice were observed until moribund, when lifespan and incidence of TL were determined.

Results and discussion. X-irradiation alone at either age did not induce TL, but ENU treatment alone induced TL in 13%, 3% and 3% of mice when given from 5, 9 and 13 weeks old, respectively. After in utero X-ray exposure the incidence of TL was 26%, 10% and 14% when combined with ENU treatment at 5, 9 or 13 weeks old; while in mice irradiated at 5 weeks old TL incidence was 80%, 38% and 12%, after ENU at 5, 9 or 13 weeks old, respectively. ENU was more efficient at inducing TL with shorter intervals following irradiation, at either age. These results suggest the effects of in utero irradiation may persist, and that sub-carcinogenic doses may still pose a risk with additional environmental exposures in later life.

Conclusion. Our data demonstrates that T-cell lymphoma development is increased by ENU treatment in later life following radiation exposure in utero. Considerations of the risks associated with in utero radiation exposure should take into account any synergistic effects from additional environmental exposures in later life.

No conflict of interest.

141 MicroRNA profiling in E6/E7 human papillomavirus-transformed human keratinocytes and exosomes

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Introduction. MicroRNAs (miRNAs) are noncoding RNAs that play a key role in gene regulation by cleaving the mRNA targets or repressing their translation. Tumor cells bear a specific and altered pattern of miRNA expression.

It has been reported that the production of membrane vesicles is deregulated in cancer, indicating that microvescicles play an important role in tumors. Exosomes are small membrane vesicles that can contain proteins, RNA, miRNAs and DNA. In particular, miRNAs secreted by exosomes in the microenvironment can promote tumor growth and progression.

Viruses are able to modulate miRNAs expressed by infected cells and carried by exosomes. Keratinocytes transformed by E6 and E7 proteins from mucosal Human Papillomavirus (HPV)-16 or cutaneous HPV-38 (K16 and K38 cells) were studied to analyze the involvement of HPV oncoproteins in miRNA expression in cells and exosomes.

Materials and Methods. MiRNAs were isolated by using Mirvana Paris kit (Ambion) and analyzed by using the TaqMan® Array Human MicroRNA A Cards (Applied Biosystems), followed by Taqman-based single assay for specific miRNAs, to confirm the results. Western Blot assay was performed to study miRNA targets. In order to isolate microvescicles, K16 and K38 cell supernatants underwent differential centrifugations and exosomes were quantified by FACS analysis.

Results and discussion. MiRNA profiling of K16 and K38 cells led to the identification of a group of miRNAs deregulated in K16 and K38 cells. We analysed the involvement of HPV16 and/or HPV38 E6 and E7 single proteins in the expression of miRNAs selected on the basis of their role in the tumorigenesis. Mucosal or cutaneous HPV proteins can modify the expression of miR-18a, -19a, -34a, and -590-5p. MiRNA array analysis of exosomes isolated from K16 and K38 cells revealed that few miRNAs are carried by K16 and K38 cells. Among these, miR-320, -374-5p and -628-5p were expressed in exosomes isolated from both K16 and K38. Interestingly, in exosomes from K16 cells was identified miR-222, a key miRNA deregulated in many cancers.

Conclusions. Our results indicate that HPV infection can induce the deregulation of some miRNAs through mechanisms that appear to involve E6 and E7 oncoprotein expression. Moreover, through the action of exosomes, HPV-induced miRNA alteration can contribute to the virus oncogenic effects not only on the infected cells but also on the tumor cell microenvironment.

No conflict of interest.

143 Low-dose cisplatin-etoposide regimen (CE) for pediatric patients with diencephalic syndrome (DS): A multicenter evaluation of clinical and radiological activity

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Introduction. Diencephalic or Russell syndrome (DS) is a rare but rapidly fatal condition, usually occurring during the first year of life, as a result of a hypothalamic/chiasmatic tumor. The main clinical features are failure to thrive with weight loss leading to severe emaciation despite a normal caloric intake, hyperalertness, hyperkinesis, and euphoria.

Material and method. We analyzed the objective tumor response (OR) and the time to weight recovery (TWR) in pediatric patients with DS as a result of a hypothalamic tumor, treated with a low-dose cisplatin-etoposide (CE) regimen (10 courses of cisplatin 25 mg/sqm/day and etoposide 100 mg/sqm/day, both on days 1–3, every 4-6 weeks), in two Italian referral centers.

Results and discussion. Our study considered nine pediatric patients with DS. The median age was 8 months (range 4-60 months). Three patients had a pilomyxoid astrocytoma, three had a pilocytic astrocytoma, one had a low grade-glioma, and finally two were not histologically typed. All patients received nutritional support. During chemotherapy, all patients achieved a disease control, and a weight recovery. Four patients (45%) had a partial response (PR), two (22%) a minor response (MR), and three (33%) a stable disease (SD). The median TWR was 5 months (range 3-19). The median TWR for patients with PR was 6,5 months, for those with MR was 3 months,

and in SD was 5 months (P: not significant). For patients older than 12 months the median TWR was 4 months, for those younger it was 5,5 months (P: not significant). The median TWR was 6 months in pilomyxoid astrocytoma, and 3 months in pilocytic astrocytoma. Differences in TWR according to nutritional support types are not been documented.

Conclusions. CE in neoplastic DS is an active regimen which can produce interesting clinical and radiological benefits. In our series, TWR is not related with tumor response (clinical and radiological responses seem to be independents). Moreover, there is a favorable trend for patients older than 12 months, although it is not statistically significant.

No conflict of interest.

144 Overexpression of hexokinase 2 (HK2) in ovarian cancer contributes to cell migration, invasion and cancer stem-like cells regulation and correlates with poor patient survival

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Introduction. Altered glucose metabolism is a new hallmark for cancer. High lactate production and low glucose oxidation, regardless of the oxygen availability, known as the Warburg effect (aerobic glycolysis), are commonly found in cancers. Hexokinase 2 (HK2) converts glucose to glucose-6- phosphate, the first committed step in glycolysis. HK2 regulates glycolysis and tumorigenesis in different human cancers, yet the mechanisms remain poorly defined. In this study, we investigate the clinical significance, effects and mechanisms of HK2 on cell migration, invasion and cancer stem-like cells (CSCs) regulation in ovarian cancer.

Material and Method. Expression of HK2 was examined in 93 clinical samples including 19 benign/borderline tumors, 46 primary tumors and 28 matched metastatic foci by immunohistochemistry and correlated with clinicopathological parameters. Effects of HK2 on lactate production, migration, invasion and CSCs regulation in A2780CP cells were determined by lactate assay, migration and invasion assays and sphere formation assay, respectively. The downstream target of HK2 was evaluated by qPCR and immunoblotting. The mechanism governing HK2 deregulation was determined by immunoblotting after treatment of SKOV-3 cells with interleukin-6 (IL-6).

Results and Discussion. We found up-regulation of HK2 in ovarian cancer patients with significantly higher HK2 found in metastatic foci. High HK2 immunoreactivity was significantly associated with advanced stage (Stage 4), serous/clear cell histological subtypes and shorter disease-free survival (all $p<0.05$). HK2 was also overexpressed in ovarian cancer cell lines. Knockdown of HK2 in ovarian cancer cells decreased lactate production, inhibited cell migration and invasion, along with reduced ERK1/2 activation and (matrix metalloproteinase 9) MMP-9 mRNA expression. Inversely, ectopic expression of HK2 promoted ovarian cancer cell migration, invasion, anchorage-independent growth and sphere formation, which was accompanied by induced ERK1/2 activation and stem cell related gene NANOG protein expression. Moreover, HK2 expression can be up-regulated by IL-6.

Conclusion. Our findings suggested that HK2 was associated with ovarian cancer metastasis and CSCs regulation. A possible crosstalk between IL-6 (tumor microenvironment), HK2 (altered glucose metabolism) and ovarian cancer metastasis/CSCs regulation was revealed. HK2 could be a potential prognostic marker and therapeutic target for ovarian cancer.

No conflict of interest.

145 Prognostic value of HIF1A, HIF2A and HIF3A expression in Bulgarian patients with malignant glioma

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Introduction. Malignant gliomas are one of the most aggressive tumours which are characterized by high mortality rate. Mutations in genes coding for isocytrate dehydrogenases (IDHs) have an important role in many aspects of gliomas, including gliomagenesis and patient prognosis. The mutated IDH has a strongly decreased enzymatic activity, leading to reduced aKG levels, which promote cellular accumulation of HIF1A levels. The hypoxia-inducible transcription factors (HIFs) are the key transcription factors regulating the expression of hypoxia-induced genes that affect angiogenesis, metabolism, growth and differentiation, apoptosis and autophagy. Elevated HIFA expression correlates with poor patient survival in a large number of cancers. It has been reported that in gliomas HIF2A, but not HIF1A expression, correlates with poor patient survival, nonetheless other evidence indicates a correlation of HIF1A expression with prognosis.

Materials and Methods. In the present study we analyzed the expression of HIF1A, HIF2A and HIF3A mRNAs in 53 malignant gliomas and 8 nonneoplastic brain tissues. Gene expression levels were determined by real-time quantification PCR (RT-qPCR) and TBP was used as endogenous control gene. All samples were examined by direct sequencing for mutations in exon 4 of IDH1.

Results and discussion. HIF1A was overexpressed in 34 out of 53 tumours (64.2%) whereas up-regulation in HIF2A and HIF3A was detected in 10 (18.8%) and 5 (9.4%) of tumours, respectively. Most of the patients (33) were characterized with HIF3A down regulation. IDH1 carried genetic alterations in 14 (26.4%) tumour samples. Cox-regression analysis showed statistical correlation between overall survival and overexpression of HIF1A and HIF2A, IDH1 mutations, and age ($p<0.001$ for each). Altered HIF3A expression did not reveal a correlation with overall survival ($p=0.797$). Due to Spearman correlation between expression levels of HIF1A and HIF2A ($r=0.543$, $p<0.001$), and HIF2A and IDH1 mutations ($r=-0.424$, $p=0.003$), multivariate regression analysis was conducted independently for HIF1A and HIF2A. Age of onset (HR 1.033, $p=0.048$) and HIF1A over-expression (HR 1.232, $p=0.029$) were observed as independent prognostic factors with poor survival whereas IDH1 mutations revealed a favourable prognostic significance (HR 0.194, $p=0.014$). HIF2A expression did not show a prognostic significance as independent marker (HR 1.246, $p=0.209$).

Conclusion. Our results indicate that together IDH1 mutations and HIF1A under-expression identify gliomas with better survival and may be applied as individual prognostic biomarkers in Bulgarian patients with malignant gliomas.

No conflict of interest.

Poster Session: Carcinogenesis II

146 In utero exposure to lipotropic (methyl) nutrients suppresses mammary carcinogenesis in two generations of offspring

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Introduction. Maternal nutrition during gestation can significantly influence fetal development and programming (i.e., epigenetic imprint) that permanently shapes the body's life-long functions, including the risk of adult chronic diseases, such as cancer. Lipotropes are methyl group-containing essential nutrients (including methionine, choline, folate, and vitamin B12) that play key roles in one-carbon metabolism which provides methyl groups for DNA methylation. We hypothesized that in utero exposure to dietary lipotropes may lower susceptibility of offspring to mammary carcinogenesis via stable epigenetic alteration of gene expression.

Material and Method: Pregnant Sprague-Dawley rats were fed either the control or lipotropes supplemented diets (five times a basal level of lipotropes, except L-methionine was 1.8 times) until parturition, at which point, all dams were fed the control diet until weaning. At weaning, dams (Fo generation) were euthanized and each sacrificed dam was replaced with two of her female offspring (F1 generation). Each group of offspring was divided into two, with half used for the mammary carcinogenesis experiment while the other half was bred. Their offspring (F2 generation) were then utilized for the second mammary carcinogenesis experiment. Mammary carcinogenesis was induced in F1 and F2 generations by an intraperitoneal injection of N-nitroso-N-methylurea (NMU) at 50 days of age. Physical mammary tumor data was recorded and tumor tissues were collected for gene transcription analysis.

Results and Discussion. The F1 generation from dams fed dietary lipotropes showed significantly lower tumor incidence and multiplicity by 50% and 59% respectively, while the latency period was longer by 42% as compared with the control group. Tumor volumes were smaller by 77% in lipotropes offspring group. The F2 generation of the lipotropes ancestry showed lower tumor incidence and multiplicity by 55% and 65%, respectively at 11th week after NMU injection. The latency period was longer by 16%, and tumor volumes were lowered by 14% in lipotropes group. Further, maternal methyl nutrients decreased the transcription of histone deacetylase 1 in mammary tumor tissues of F1 and F2. In summary, intrauterine exposure to methyl nutrients resulted in suppression of mammary carcinogenesis for two generations of offspring.

Conclusion. Lipotropes-mediated epigenetic changes may be responsible for persistent reduction of susceptibility to mammary cancer.

No conflict of interest.

147 The development of cisplatin resistance in neuroblastoma is accompanied by epithelial to mesenchymal transition in vitro

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Introduction. Neuroblastoma is a challenging childhood malignancy, with a very high percentage of patients relapsing following acquisition of drug resistance, thereby necessitating the identification of mechanisms of drug resistance as well as new biological targets contributing to the aggressive pathogenicity of the disease.

Material and Methods. The SK-N-AS, Kelly and CHP212 neuroblastoma cell lines were pulsed-treated with increasing concentrations of cisplatin over a 6 month period. IC50 were examined by MTT assay. Drug resistant cells and their respective parental lines were subjected to quantitative label-free LC-MS proteomic profiling. Ingenuity Pathway Analysis (IPA) was performed to outline the most significant canonical pathways and functions in the datasets. Changes in protein expression were assessed by western blot and confocal microscopy. Invasion capacity was assessed using Growth Factor Reduced MATRIGEL Invasion Chambers.

Result and discussion. Comparative proteomic analysis using label free mass spectrometry identified 111 significant ($p \leq 0.05$, fold change ≥ 1.2) protein expression

changes in the CHP212Cis100 resistant cell line, 118 in KellyCis83, and 97 in SK-N-ASCis24, when compared to their sensitive parental lines. IPA classification of modulated proteins indicated 'cellular growth and proliferation' and 'cell death and survival' as the top scoring molecular and cellular functions. IPA for canonical pathway identified significant changes in the expression of proteins involved with pathways such as actin cytoskeletal signaling, integrin linked kinase signaling, epithelial adherens junctions signalling and remodeling of epithelial adherens junctions. All these pathways involve proteins of integrin, actin and myosin families. The elevated expression for MYH9, MYL12B and ACTN4 was validated by western blot and confocal microscopy in SK-N-ASCis24. Proteins of the actin-myosin axes play important roles in mesenchymal cell migration, and it is of interest that the SK-N-ASCis24 exhibits a very significant increase in migration potential relative to the parental line. There was no indication by mass spectrometry that ROCK1 expression was modulated in SK-N-ASCis24, although western blot and confocal microscopy of cells indicated a lower level of its expression.

Conclusion. In this study, SK-N-ASCis24 cell line acquired mesenchymal properties, including fibroblastoid morphology, increased potential for motility and invasion, which are prerequisites of epithelial-to-mesenchymal transition (EMT). The elevated levels of MYH9 and ACTN4 and reduced levels of ROCK1 contribute to the increased ROCK1-independent migratory potential of SK-N-ASCis24. The comparative proteomic profiling of cisplatin resistant neuroblastoma cell lines enabled us to identify MYH9 and ACTN4 – mediated EMT in the development of cisplatin resistance in neuroblastoma in vitro.

No conflict of interest.

148 Neurofilament (NEF-L) is overexpressed in human and rodent HCC and predicts recurrence in HCC patients

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Introduction. Neurofilament-light polypeptide (NEFL) gene has been proposed to act as a tumor suppressor gene in different types of cancer. However, no information about the role of NEFL in hepatocellular carcinoma (HCC), nor the mechanisms regulating its expression are available. To investigate the role of NEFL in HCC, in this study we analyzed its expression in human HCC and in a rat model of chemically-induced HCC.

Materials and Methods. Preneoplastic hepatic lesions and Hepatocellular carcinomas were induced in rats subjected to the Resistant-Hepatocyte (RH) model, consisting of a single dose of diethylnitrosamine (DENA) and a 2-week feeding a diet supplemented with 2-acetylaminoaminofluorene (2-AAF). Immunofluorescence, western blot and qRT-PCR were used to detect modifications of Nefl protein and mRNA levels in mouse and rat normal and pre- and neoplastic liver, human primary HCCs and in several human HCC cell lines.

Results. Using the Resistant-Hepatocyte (R-H) rat model, we found that NEFL mRNA levels were strongly increased at very early stages of the multistep process of hepatocarcinogenesis, but only in preneoplastic lesions positive for the stem/progenitor cell marker cytokeratin-19 (KRT-19). Increased mRNA levels of NEFL were also observed in HCCs, although at much lower levels. Consistent with the results in rat liver, also normal human liver tissues showed no detectable expression of NEFL, while NEFL expression was found in 90% of primary human HCCs. Furthermore, 64% of HCCs showed NEFL upregulation compared to peritumoral cirrhotic tissue and high levels of NEFL mRNA correlated with time of recurrence. In spite of the increased mRNA levels, however, NEFL protein was detected only in KRT-19+ nodules, but not in rat or human HCCs. Searching for possible mechanisms underlying NEFL enhanced expression, we found that no hypomethylation of NEFL promoter occurred in preneoplastic and neoplastic lesions.

Conclusion. Thepresent results suggest that NEFL acts as an oncogene in the liver. The finding of a similar up-regulation in both human and rat HCC supports the translational value of rat models that could be useful in the study of hepatic tumorigenesis and in the identification of new biomarkers of pre- and neoplastic stages.

No conflict of interest.

149 Improvement of tumor photoacoustic molecular images by unmixing

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Introduction. The photoacoustic (PA) technique produces multispectral images that provide important insights on tissue's metabolism and vascularization. Agents and molecules with marked multispectral response further increase the sensitivity of PA. Unmixing techniques allow for separate the signals from different optical absorbers in a photo-acoustic image, thus providing estimates of their characteristic entropy behavior, optic flow, and distribution of the apparent velocities of the molecules in a given clip recording.

The aim of this work was to provide a proof of concept that unmixing technique allows analyzing the distribution of nanosystems while lowering the physiological signals given by oxy/deoxy-hemoglobin.

Methods. Six mice were injected i.v. with a volume of 200 µl of stealth liposomes (DPPC/DSPE-PEG2000 encapsulating a 300 mM solution of the clinically approved MRI contrast agent Gadoteridol, hydrodynamic diameter 130 nm). PEGylated liposomes were labelled with near-infrared fluorophore cyanine dye (Cy 5.5). Mice bearing subcutaneous syngeneic breast cancer (TS/A) were imaged with a VEVO LAZR 2100 system (VisualSonics Inc.) equipped with a highly sensitive photo-acoustic probe at a frequency of 21 MHz.

To apply the unmixing method, we first acquired the PA spectrum of the CY 5.5 loaded liposomes in the range 680-970 nm, by using a custom developed phantom mimicking the physiological acoustic and optical attenuation of the tissues. The in-vivo PA data of the mice tumor were then acquired. By using the Unmixing Tool software we separated the signals of the CY 5.5 of the OxyHemo, and of the DeoxyHemo. The maximum flow 1 and 20 minutes after the liposomes injection was quantified by the Optical Flow Matlab code.

Results. Table 1 report the maximum flow as measured considering all the three signals (CY 5.5 + OxyHemo + DeoxyHemo) and after unmixing and considering only the CY 5.5. After unmixing of the CY 5.5 the measurement of the maximum flow changed. The increase in the measured maximum flow from 1 to 20 minutes is showed.

Conclusions. Unmixing of the PA images allows for increasing the sensitivity on the signals of specific chromophores. This could lead to improved estimation of physiological parameters in in-vivo studies.

Compounds	MAX FLOW from 1 to 10 min	MAX FLOW from 10 to 20 min
Cy 5.5 – OxyHemo - DeOxyHemo	115.5588	132.2126
Cy 5.5	129.7533	177.4399

Tab. 1 – Max flow values obtained, during two time range, before (upper row) and after unmixing (bottom row).

No conflict of interest.

150 p53 independent G1 arrest accompanied by DNA damage and apoptosis induced by dietary flavonoid fisetin in human gastric cancer cells

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Introduction. Fisetin is a naturally occurring flavonoid. It has shown anti-inflammatory, antioxidant and anticancer properties, however, its anticancer properties against gastric cancer are unknown. The aim of this study was to determine the effect of fisetin, on inhibition of cell growth and induction of apoptosis in human gastric cancer cells.

Material and Methods. Cell survival was examined by Trypan blue exclusion & MTT assay. The effects of treatments on cell cycle distribution, apoptosis and mitochondrial membrane potential were examined by flow cytometry. Western blot analysis was performed to ascertain the protein levels of gamma-H2AX, Phospho-p53 (Ser15) and total p53, MDM 2, Cyclin D, E and CDK 2,4, active caspase-3, PARP cleavage, Bax, Bcl2 and Akt.

Results and Discussion. Treatment of fisetin (25-100 µM) for 24, 48 h decreased total cell number by 4-64% and 32-79%, respectively in AGS cells and 23-45% and 65-79% in SNU-1 cells. Treatment of AGS and SNU-1 cells with fisetin also resulted in G1-phase arrest that was associated with a marked decrease in the protein expression of cyclin D1 and E and their activating partner cyclin-dependent kinases 2 and 4. Starvation-driven cell synchronization and release assay, further confirmed G1 phase of cell cycle arrest. Fisetin treatment also resulted in induction of apoptosis, poly (ADP-ribose) polymerase (PARP) cleavage, modulation in the expressions of BAX-BCL2 pathway and increased cleavage of caspase 3. Phospho-AKT (S473) and (T308) which are involved in cell proliferation and antiapoptotic pathways were suppressed after fisetin treatment. There was also change in mitochondrial membrane potential which suggested the role of mitochondria-mediated apoptosis. Fisetin also induced p53 activation through its S15 phosphorylation and increased γ-H2A.X phosphorylation (S139) in both the cell lines indicating involvement of DNA damage caused by fisetin might be one of the mechanisms through which fisetin caused cell death. P53 dependency was analyzed by using pifithrin-alfa, an inhibitor of p53, in AGS cells, which suggested that cell cycle arrest and apoptosis caused by the fisetin is p53-independent.

Conclusion. This study demonstrates for the first time that fisetin possesses anticancer potential against human gastric carcinoma AGS and SNU-1 cells and it could be developed as a novel agent for the management of gastric cancer.

No conflict of interest.

151 The IMP3-PDPN axis is associated with bone invasion and prognosis in oral squamous cell carcinoma

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Introduction. We previously reported on the specific roles of IMP3 and PDPN during cellular invasion in oral squamous cell carcinoma (OSCC). Moreover, we also verified that IMP3 stabilizes the PDPN transcript by binding to the 3'UTR of PDPN mRNA.

Materials and Methods. In the present study, we elucidated the correlation of IMP3-PDPN axis expression with bone invasion in OSCC tissue specimens, and assessed a mouse calvarium xenograft model using an IMP3- and PDPN-depleted OSCC cell line

to evaluate for the prognostic implications of the two genes as therapeutic targets against OSCC with bone invasion.

Results and Discussion. The retrospective analysis revealed that the expression of IMP3 and PDPN is significantly correlated with T stage, lymph node metastasis, and the overall survival of OSCC patients. In addition, the dual expression of IMP3 and PDPN but not the single expression of either IMP3 or PDPN was associated with bone invasion in patients with OSCC. Supportively, IMP3 or PDPN depletion inhibited the invasive capacity of OSCC cells in a three-dimensional culture system, tumorigenesis and regional bone destruction in a xenograft mouse model.

Conclusion. These results suggest that IMP3 and PDPN may have strong influence on OSCC pathogenesis, especially in bone invasion, and may serve as novel therapeutic targets with prognostic implications for bone invasive OSCC.

No conflict of interest.

152 Pyruvate dehydrogenase kinase 1 (PDHK1) regulates ovarian cancer cell migration, invasion, and chemoresistance and contributes to poor prognosis in patients

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Introduction. Pyruvate dehydrogenase kinase 1 (PDHK1) is a gate-keeping enzyme which negatively phosphorylate pyruvate dehydroagenase (PDH), leading to the conversion of pyruvate to lactate in the cytoplasm instead of further oxidation in the mitochondria, a phenomenon known as the Warburg effect. In this study, we sought to characterize the clinical and functional significance of PDHK1 in ovarian cancer.

Material and method. The expression of PDHK1 was assessed in 103 clinical samples and 9 cell lines by immunohistochemistry, qPCR and immunoblotting, and correlated with clinical outcomes. Effects of PDHK1 and DCA, a PDHK inhibitor, on lactate production, migration, invasion and apoptosis in ovarian cancer cells following cispatin exposure were evaluated by lactate assay, migration and invasion assays, and cytometry with Annexin V/PI staining respectively. The downstream target was determined by qPCR and immunoblotting. The mechanisms governing PDHK1 deregulation was determined by selective blockage of omental cytokine interleukin-8 (IL-8) and its receptor (IL-8RA) with anti-IL-8 antibody and anti-IL-8RA antibody respectively.

Results and discussion. The mRNA and protein expression of PDHK1 was found to be significantly increased in ovarian cancer clinical samples and cell lines. Significantly higher PDHK1 was found in metastatic foci than their corresponding primary carcinomas. Furthermore, high PDHK1 expression was significantly correlated with shorter overall and disease-free survival. Knockdown of PDHK1 in A2780CP, an ovarian cancer cell line, led to reduced lactate level, cell migration and invasion, along with decreased expression of urokinase type plasminogen activator (uPA), gene related to migration and invasion. Moreover, PDHK inhibitor DCA was found to reduce cell migration and invasion, and enhance the chemosensitivity of A2780 treated with cisplatin. Furthermore, we found blockage of IL-8 and IL-8RA could decrease PDHK1 protein expression in SKOV-3 cells, suggesting PDHK1 could be regulated by IL-8 through IL-8RA.

Conclusion. Our data suggested that PDHK1 was associated with ovarian cancer progression, metastasis and chemoresistance. PDHK1 could be a novel prognostic marker and potential therapeutic molecular target in ovarian cancer.

No conflict of interest.

Poster Session: Cell and Tumour Biology I

153 Mechanism of succinate dehydrogenase D mutations mediated paraganglioma

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Introduction. Gene duplication and divergence are common in evolution. The mitochondrial succinate dehydrogenase (SDH) is an essential complex of the electron transport chain and of the tricarboxylic acid cycle. Mutation in human succinate dehydrogenase subunit SDHD frequently leads to paraganglioma (PGL), but why SDHD mutations contribute to cancer formation is not completely understood. In addition to the originally discovered and assigned yeast SDHD subunit Sdh4, another conserved homolog Shh4 has recently been noticed in the budding yeast.

Material and method. SDHD expression in clinic samples was examined and yeast genetic approaches were conducted to dissect the mitochondrial functions of SDHD wild type and mutant cells. The mutator assay was applied to evaluate the deficient SDHD-mediated genomic and mitochondrial DNA mutations.

Result and discussion. We demonstrated that Shh4 is upregulated under several stresses and can substitute for Sdh4 in sdh4D cells. Mitochondrial functions and stability of SDH complex are further compromised and ROS production and mutation frequency are enhanced in sdh4D shh4D double mutants than those in sdh4D cells. Missense SDHD mutations found in cancer patients were created in Sdh4 and Shh4, and, surprisingly, mitochondrial deficiency merely appeared in the chimera of deficient human SDHD in Shh4. And this defect is due to the instability of the protein. The sequence comparison and functional analysis favor a hypothesis that human succinate dehydrogenase displays a closer genetic distance to yeast Shh4, suggesting that Shh4 is a better yeast model than Sdh4 for dissecting the tumorigenic mechanism in PGL. Furthermore, examined carotid body tumor samples from familial SDHD-mutated PGL patients all showed weak or negative staining of SDHD.

Conclusion. These findings indicate that SDHD mutation may lead to protein instability, disassembly of SDHC-SDHD complex, mitochondria damage, ROS production, genome instability and tumor formation.

No conflict of interest.

154 Study of a TOP2 inhibitor to prevent cell proliferation in ALT-type cancer cells

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Introduction. Telomere maintenance is required for chromosome stability, and telomeres are typically elongated by telomerase following DNA replication. In both tumor and yeast cells that lack telomerase, telomeres are maintained via an alternative recombination mechanism. Previous studies have indicated that yeast Sgs1 and Top3 may work together to remove highly negative supercoils that are generated from recombination. However, the mechanism by which cells eradicate highly positive supercoils during recombination remains unclear. **Material and Method.** Yeast genetic operation was used to construct top 2 mutants in telomerase-minus cells and telomere lengths were examined by Southern analysis. Human TOP2 were repressed and ALT (alternative lengthening of telomere) phenotypes were examined by FISH. Moreover, in vivo pharmacology was conducted with xenografted mouse tumors.

Results and Discussion. In the present study, we demonstrate that Top2 is involved in telomere-telomere recombination. Disturbance of telomeric chromatin by RIF1 or RIF2 deletion alleviates the requirement for Top2 in telomere-telomere recombination. In human telomerase-negative ALT cells, TOP2α or TOP2β knockdown decreases ALT-associated PML bodies, increases telomere dysfunction-induced foci and triggers telomere shortening. Similar results were observed when ALT cells were treated with ICRF-193, a TOP2 inhibitor. Importantly, ICRF-193 treatment blocks ALT-associated phenotypes in vitro, causes telomere shortening, and inhibits ALT cell proliferation in mice.

Conclusion. Taken together, these findings imply that TOP2 is involved in the ALT pathway, perhaps by resolving the highly positive supercoil structure at the front of the helicase. Inhibition of topoisomerase II may be a promising therapeutic approach that can be used to prevent cell proliferation in ALT-type cancer cells.

No conflict of interest.

155 IL-8 induces miR-424 expression and modulates SOCS2/STAT5 signaling pathway in oral cancer

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SOCS (Suppressor of Cytokine-induced Signaling) proteins consist of eight members and share a central src homology 2 domain (SH2), a C-terminal SOCS box and unique N-termini. Members of the SOCS family are negative regulators of STAT signaling pathways. Recent years, the SOCS family is found to play important roles in cancer, but there are still many functions and mechanisms need to be further explored. The down-expression of SOCS2 has been found in solid organ malignancies, such as colorectal cancer, breast cancer, lung cancer and liver cancer. Our preliminary microarray data showed that 31 (77.5%) of 40 oral cancer patients have lower SOCS2 expression profile. Meanwhile, miRNAs are 21-24 bases non-coding RNA that regulate gene expression either by degrading target mRNAs or by inhibiting their translation. We use microRNA.org and miRNAmap database to predict the putative miRNAs that can target SOCS2. We found that miR-424-5p is the only one of overlapping miRNA predicted from these two databases. And we confirmed this finding by transfection of miRNA mimic and by 3'UTR reporter assay. We also found miR-424-5p inhibitor could increase expression of SOCS2 protein and inhibits STAT5 activation in oral cancer cells. In addition, we found that overexpression of miR-424-5p could promote cell migration, invasion and STAT5 activation via downregulation of SOCS2. Taken together, our results identify a novel mechanism for mir-424-5p-mediated progression of oral cancer and establish a functional link between mir-424-5p, SOCS2, and STAT5 signaling pathway. Furthermore, we found that IL8 increased miR-424-5p expression, which activated STAT5 pathways through suppressing SOCS2 expression. We also found IL-8 promotes invasion and migration through direct induction of miR-424-5p.

No conflict of interest.

156 Mechanisms of action of 3',4',5'-trimethoxyflavonol in the inhibition of prostate cancer

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Introduction. 3',4',5'-trimethoxyflavonol (TMFol) is a synthetic flavonol with putative anti-prostate cancer activity. Both in vitro and in vivo data demonstrate growth inhibition in models of prostate cancer that is superior to the more extensively studied dietary flavonols, fisetin and quercetin. However the mechanisms by which TMFol exerts its anti-prostate cancer activity are generally unknown. Hence, the aim of this study is to elucidate the mechanisms which may mediate the anti-prostate cancer activity of TMFol.

Material and method. 22rv1 and PC-3 human prostate cancer cell lines were exposed to TMFol and the effect on proteins that are important in inhibition of cancer cell growth were determined by Western blot. Apoptosis and cell cycle distribution were analysed by flow cytometry, while biochemical assays were used to assess aconitase activity and the role of senescence in the anti-proliferative action of TMFol on prostate cancer.

Results and discussion. TMFol inhibited both 22rv1 and PC-3 cell proliferation, with IC50's of 3.7 and 4.1 μM, respectively. At 10μM TMFol did not induce apoptosis in these cells lines but caused S-phase arrest in PC-3 cells only. Although there was an increase in senescence-associated β-galactosidase activity in both cell lines, only 22rv1 cells showed corresponding increases in the senescence biomarkers, p53 and p21. Mitochondrial aconitase, a key enzyme in citrate oxidation that shows elevated activity in human prostate cancer, exhibited a significant reduction in expression and activity following TMFol treatment.

Conclusion. TMFol appears to utilise different mechanisms to inhibit the proliferation of prostate cancer cells. In 22rv1 and PC-3 prostate cancer cells TMFol induced senescence and cell cycle arrest, respectively. Furthermore, in both cell lines TMFol caused a significant reduction in mitochondrial aconitase expression and activity, which could lead to inhibition of citrate oxidation and subsequently to growth inhibition.

No conflict of interest.

157 The significance of histopathological evaluation of pancreatic fibrosis to estimate pancreas cancer progression

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Objective: To estimate the importance of the role of pancreatic stellate cells (PSCs) in pancreas cancer progression, their properties were evaluated in relation to clinical details and patient prognosis. Patients and methods: Among patients who underwent surgical treatment from 2004 to 2013, the texture of the pancreatic specimens was evaluated by histopathological measurement length (HML) of fibrosis, fibrosis grade (FG) and the intensity of PSC activity. **Results.** 1. The HML of inter- and intralobular fibrosis increased significantly with progression of FG from grade 0 to 3 (22.0±4.5 vs. 23.7±1.9 vs. 53.5±6.0 vs. 203.7±18.6 and 2.0±0.4 vs. 2.7±0.3 vs. 8.2±1.0 vs. 21.7±3.1, respectively). 2. The HML of inter- and intralobular fibrosis was also significantly longer with the increase in PSC activity score from 0 to 3 (29.7±5.9 vs. 37.4±7.2 vs. 68.4±9.5 vs. 204.7±20.5 and 3.1±0.5 vs. 5.1±1.1 vs. 9.7±1.1 vs. 21.6±3.4, respectively). 3. The HML of both inter- and intralobular fibrosis correlated with the preoperative level of HbA1c but not with pathological invasion of cancer in lymphatic vessels and the nervous system. 4. FG and PSC activity did not correlate with pancreas function and clinical factors. 5. Significantly higher rates of positive lymph node metastases were detected in the patients with high FG grade or PSC activity. 6. There were no significant differences in either FG or PSC activity in whole pancreas cancer cases, and in T3 patients only, 2-year survival rate and median survival term (MST) were similar among the different FGs. However, PSC activity in T3 patients had a significantly different effect on patient prognosis. 7. Specifically for stage II/III patients, survival rates were similar for different FGs but clearly different depending on the severity of PSC activity. When limiting the cancer site to the pancreas head, survival rate and MST in T3 patients were clearly poorer as PSC activity increased but not as FG increased. **Conclusion.** Fibrotic change as measured by the amount of fibrotic tissue present favors prediction of pancreatic function, whereas PSC activity favors prediction of cancer progression.

No conflict of interest.

158 Nonsteroidal anti-inflammatory drugs (NSAID)-induced alterations in inflammatory and metabolic stress in cancer and non-cancer cells

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Introduction. Nonsteroidal anti-inflammatory drugs (NSAIDs), including acetaminophen (APAP), have been reported to induce cytotoxicity in cancer and non-cancerous cells. The precise molecular mechanisms, however, are not well understood.

Materials and Methods. Using a mouse macrophage cell-line, J774.2 and human hepatoma HepG2 cells, we studied the dose (1-10mM) and time (0-48h)-dependent effects of acetaminophen and aspirin on inflammatory responses, metabolic and oxidative stress, mitochondrial respiratory function and xenobiotic metabolism. We also studied the effects of LPS, a bacterial endotoxin inducing inflammatory responses, and N-acetyl cysteine, an antioxidant ROS scavenger, on the cytotoxicity of NSAIDs using the above cell lines.

Results and Discussion. We observed that acetaminophen and aspirin exhibited selective but differential responses to the cell survival metabolism and apoptosis in these cells. These drugs have differentially induced cell cycle arrest, apoptosis, oxidative and metabolic stress and mitochondrial dysfunction in these two cell lines. We have demonstrated that HepG2 cells exhibit more resistance towards NSAIDs –induced toxicity than macrophages. A marked increase in apoptosis, DNA fragmentation, cytochrome c release and PARP/caspase-3 activation was seen in macrophages compared to HepG2 cells. Differential effects of APAP and aspirin on oxidative and metabolic stress appear to be associated with their differential exclusion rates from the cells and their effects on cytochrome P450s and glutathione-dependent drug metabolizing enzyme systems. Treatment of these cell lines with bacterial endotoxin LPS, which induces inflammatory responses, in the presence or absence of aspirin has suggested that the drug has enhanced the sensitivity of cells towards LPS- induced oxidative stress and mitochondrial dysfunction. The cytotoxicity induced by LPS and NSAIDs were attenuated by N-acetyl cysteine, an antioxidant and ROS scavenger.

Conclusion. These results may help in better understanding the mechanism of cell sensitivity and toxicity towards NSAIDs and LPS and also the mechanism of cytoprotection/chemoprevention by antioxidants and NSAIDs in cancer and non-cancerous cellular systems. (Supported by Terry Fox Cancer Research Funds, and funds from Sheikh Hamdan Medical Research Award and CMHS Research Committee).

No conflict of interest.

159 MiRNA-1469 targeting STAT5A promotes apoptosis in lung cancer cells

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Introduction. It was known that miRNAs are involved in crucial biological processes, including development, differentiation, proliferation and apoptosis. Recently, miR-1469 was found to be upregulated during metastasis in clear cell renal cell carcinoma and downregulated in human breast cancer MCF-7 cells after treatment ofpolyphenon-60. These results revealed deregulated miR-1469 expression in cancer cells. In this study, we found that miR-1469 was involved in apoptosis of lung cancer cells and enhances sensitivity of lung cancer cells to apoptotic induction. Further investigation demonstrated that miR-1469 targeted STAT5A transcription factor which regulates proliferation and apoptosis in many cancers, and enhanced apoptosis of lung cancer cells.

Materials and Methods. RT-PCR was used to detect miRNA and STAT5A mRNA level. MiRNA-1469 mimics and miRNA-1469 inhibitor were synthesized for transfection. Luciferase reporter assay was performed to detect STAT5A 3'-UTR reporter activity. The flow cytometry assay was performed to analyze apoptosis. STAT5A and gH2AX expression were analyzed with western blot.

Results and discussion. Abnormal expression of miRNA-1469 was observed in some cancers, but was not previously implicated in lung cancer. In this study, we firstly found that miR-1469 is dramatically up-regulated during apoptosis of lung cancer cells. Overexpression of miR-1469 in lung cancer cells including A549 and NCI-H1650increased apoptosis induced by etoposide. However, miR-1469 inhibitor reduced apoptosis. These data demonstrated that miR-1469 plays an important role in apoptotic regulation of lung cancer cells. Bioinformatics analysis indicated that transcription factor STAT5A is one of candidates targeted by miR-1469. Luciferase reporter assay showed that miR-1469 can bind to the 3'-untranslated region of STAT5A, reducing both the mRNA and protein levels of STAT5A. Thus, STAT5A is a real downstream target of miR-1469. Subsequently we found that co-expression of miR-1469 with STAT5A in A549 and NCI-H1650 cells partially abrogated the effect of miR-1469 on cellular apoptosis. Taken together, these data showed that miR-1469 downregulated STAT5A and increased apoptosis in lung cancer cells.

Conclusion. MiR-1469 functions as an apoptosis enhancer to regulate lung cancer apoptosis through targeting STAT5A and may become a critical therapeutic target for lung cancer.

No conflict of interest.

160 Role of miR-338-5p as a novel modulator of chemoresistance in esophageal cancer

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Role of miR-338-5p as a novel modulator of chemoresistance in esophageal cancer

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Introduction. Multimodal treatment incorporating surgical resection and the use of chemotherapeutic drugs is commonly used in the management of esophageal

cancer. However, resistance to chemotherapy drugs may contribute to poor treatment outcome and cancer recurrence. Increasing evidence suggests that miRNAs regulate the sensitivity of cancer to chemotherapeutic drugs. 5-Fluorouracil (5-FU) is an antimetabolite agent commonly used to treat esophageal cancer.

Material and Method. We have established 5-FU-resistant (FR) esophageal squamous cell carcinoma (ESCC) cell lines as cell models to study the mechanisms of chemoresistance. These FR cell lines overexpress thymidylate synthase, which is generally regarded as a marker of 5-FU resistance.

Results and Discussion. We found that miR-338-5p was downregulated whereas Id1, which was one of its predicted targets according to target prediction algorithms, was upregulated in the FR cells. Luciferase reporter assay confirmed that miR-338-5p could directly bind to the 3' untranslated region (UTR) of Id1. Overexpression of miR-338-5p downregulated endogenous Id1 protein level in esophageal cancer cell lines, whereas knockdown of miR-338-5p produced the opposite effect. The inhibitory effect of miR-338-5p on Id1 expression was further confirmed using Tet-on ESCC cell lines inducible for miR-338-5p expression. Flow cytometry and Western blotting were performed to study the effects of miR-338-5p overexpression and inhibition on 5-FU-induced apoptosis. The results showed that miR-338-5p overexpression restored sensitivity of the FR cells to 5-FU treatment both in vitro and in vivo, and that knockdown of miR-338-5p induced 5-FU resistance in chemosensitive esophageal cells. These effects were abolished by expression of Id1 and shId1, respectively.

Conclusion. Taken together, our data suggest that miR-338-5p can modulate the chemosensitivity of ESCC cells by regulating Id1. Since previous studies showed that Id1 is frequently upregulated in ESCC and is a marker for unfavorable prognosis, miR-338-5p and its suppressive effect on Id1 may be exploited to develop novel therapies for treatment of esophageal cancer.

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No conflict of interest.

161 ATM kinase expression regulates breast cancer stem-like phenotype

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Introduction. Recent studies have provided strong support for the cancer stem cell (CSC) hypothesis, which suggests that many cancers, including Breast Cancer (BC), are driven by a subpopulation of cells that display stem cell properties and by virtue of their relative resistance to anti-cancer drugs, contribute to treatment relapse. HER2 is a RTK overexpressed in 20-25% of BC and this overexpression seems to be associated with an increase of the fraction of CSCs pool within BC.

The role of serine threonine kinase ATM in cancer development and therapy is still largely debated. ATM may act as tumor suppressor as well as tumor promoter gene and the modulation of its activity may exert positive or negative effects in cancer therapy. Recently in our lab we identified a new tumorigenic role of ATM in HER2 positive BC. The role of ATM in regulation of CSCs has not been investigated yet, although recent studies suggest that ATM may contribute to the maintenance of the pool of normal stem cell. Based on literature and our data, the aim of this study is to identify a new possible function of ATM in the maintenance of the 'stem-like' phenotype in BC.

Material and method. Lentivirus infection to genetically interfere ATM expression; mammosphere assay; immunoblotting analysis; real time-PCR, microarray, ALDH assay.

Results and discussion. As model system for BCSCs we have isolated and characterized cell population with stem-like features from BC cell lines as MCF-7 and MCF-7HER2, by using mammosphere assay. Our data show that downregulation of ATM expression significantly reduces spheres formation and the activity of ALDH-1, a well-known marker of BCSCs features. Moreover Real-Time experiments could show that downregulation of ATM correlates very well with a modulation of the expression of genes involved in the stemness pathway. For these reasons, we will analyse different gene expression profiles obtained by microarray analysis comparing sphere versus differentiating MCF-7/MCF-7HER2 cells in presence or absence of ATM. The analysis of these data lead us to speculate new pathways regulated by ATM in BCSCs.

Conclusions. In conclusion our findings suggest a crucial role of ATM in the maintenance and in the regulation of the 'Cancer stem-like' phenotype in BC and will open up new possibilities for therapeutic intervention for eradication of BCSCs.

No conflict of interest.

162 Inhibition of c-FLIP(L) expression by miR-708 increases the sensitivity to anticancer drug in renal cancer cells

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Introduction. Dysregulation of the antiapoptotic protein, cellular FLICE-like inhibitory protein (c-FLIP), has been proven to be associated with tumorigenesis and chemoresistance in various types of human cancers. Therefore, c-FLIP is an excellent target for therapeutic intervention. MicroRNAs (miRNAs) are small non-coding RNAs that are involved in tumorigenesis, tumor suppression, and resistance or sensitivity to anticancer drugs. It remains unclear whether miRNAs can regulate the expression of c-FLIP. The goal of this study was to identify miRNAs that could inhibit the growth and induce cell death of renal cancer by targeting c-FLIP expression.

Results and Discussion. We show that c-FLIPL and miRNA-708 expressions are inversely correlated, that is, c-FLIPL is upregulated and miRNA-708 is rarely expressed in renal cancer cells. Luciferase report assay demonstrated miRNA-708 negatively regulated c-FLIPL expression via a conserved miRNA-binding site in 3' untranslated region (3'UTR) of c-FLIPL. We also show that ectopic expression of miRNA-708 increases the accumulation of sub-G1 as well as the cleavage of procaspase-3 and PARP, which were prevented by pretreatment with the pan-caspase inhibitor, Z-VAD. Furthermore, ectopic expression of miRNA-708 increases the sensitivities to various apoptotic stimuli such as tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), doxorubicin (Dox), thapsigargin (TG) in Caki cells. Interestingly, miRNA-708 specifically repressed c-FLIPL without any change on c-FLIPs expression. In contrast, inhibition of endogenous miRNA-708 by use of antago-miRNA results in increase of c-FLIPL protein expression and resistance to TRAIL, Dox, and TG treatments. We found that miRNA-708 expression was reduced in renal cell carcinoma (RCC) tissues. Inversely, c-FLIPL expression was upregulated in RCC tissues compared with normal tissues. Moreover, intratumoral delivery of miR-708 triggers to regression of tumors in xenograft model of human RCC.

Conclusion. These findings suggest that miRNA-708 should be considered as a tumor suppressor because it negatively regulates the antiapoptotic protein c-FLIPL and regulates sensitivities to various apoptotic stimuli.

No conflict of interest.

163 Stromal neuregulin-1 promotes adaptive resistance in mutant BRAF melanoma

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Introduction. RAF inhibitors are first-line treatments for melanomas that harbor BRAF V600E/K mutations; however, patients invariably progress on these targeted therapies. While durable resistance is mediated frequently by stable alterations intrinsic to the tumor cells leading to ERK1/2 pathway reactivation, the compensatory activation of growth factor receptor tyrosine kinases (RTKs) modulates the level of initial response. The composition of the tumor microenvironment likely plays a key role in these compensatory/adaptive responses to targeted inhibitors. We have previously shown that signaling downstream of the RTK ErbB3 modulates the response to RAF inhibitors; however, its ligand neuregulin (NRG1) is expressed at low levels by tumor cells.

Material and Methods. To investigate the role of NRG1 as a paracrine factor in modulating the response to RAF inhibition in BRAF mutant melanoma cells, we utilized both primary human dermal fibroblasts and cancer associated fibroblasts (CAFs) extracted from a BRAF V600E melanoma patient sample. To evaluate the effect of stromal-derived NRG1 on cancer cells, we established a co-culture system using either fibroblast conditioned medium or transwell insert. Levels of total and phosphorylated proteins were assessed by Western blot analysis; cell viability by alamar blue assay; cell growth by crystal violet growth assay; and cell death by annexin V staining.

Results and Discussion. We demonstrate that NRG1 is expressed in both fibroblasts and CAFs isolated from a mutant BRAF melanoma. Fibroblast derived-NRG1 was able to mediate the enhanced ErbB3 pathway activation in RAF-inhibited mutant BRAF melanoma cells. Furthermore, conditioned medium from fibroblasts and CAFs limited RAF inhibitor cytotoxicity in V600 BRAF-harboring melanomas. Importantly, ErbB3 and ErbB2 targeting antibodies partially reversed the protective effects of fibroblast-derived medium on ErbB3 pathway activation and cell growth properties of mutant BRAF-inhibited melanoma cells.

Conclusion. We demonstrate a requirement for NRG1 paracrine signaling in the tumor microenvironment to promote resistance to RAF inhibitor in mutant BRAF melanoma, reinforcing the idea that targeting ErbB3/ErbB2 signaling may improve the efficacy of RAF inhibitors.

No conflict of interest.

164 The anti-tumor activity of a novel STAT inhibitor OPB-51602 involves targeting mitochondrial metabolism

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Introduction. Signal transducer and activator of Transcription (STAT) proteins are ligand responsive transcription factors which help in cell proliferation, differentiation, cell survival, angiogenesis etc. Dysregulation in STAT pathway(s) leads to increase in their activity and tumorigenesis. STAT inhibitors have been proposed as a promising chemotherapeutic strategy. Here we report that OPB-51602, a novel STAT inhibitor, elicits significant inhibitory effects on STAT3 phosphorylation and growth of a variety of human tumor cell lines.

Material and **Methods.** H1975 were treated with various concentrations of OPB (Otsuka Pharmaceuticals Co.) for different durations. The effects of OPB treatment on STAT3 phosphorylation (western blot), cell proliferation (crystal violet assay), mitochondrial O2- (Mitosox), and Oxygen Consumption (SeaHorse) were determined according to the manufacturer's instructions. The expression levels of STAT3 were manipulated by transient transfection using STAT3-mutant-harboring vectors via Lipofectamine-based techniques.

Results and Discussion. Our results show that, in addition to its targeted effect on STAT3, OPB induces a significant increase in mitochondrial superoxide (O_2^-) production by inhibiting mitochondrial respiratory activity in NSCLC cell line H1975. While the inhibitory effect on cell proliferation is observed only upon 48 hours of exposure to the compound, the effect on mitochondrial O2- and oxygen consumption was detected as early as 30 minutes to 1 hour, respectively. Of note, transient expression of the pY705 or pS727 phosphorylation deficient mutants reversed the growth inhibitory effect of OPB, but only S727 mutant rescued the mitochondrial effects. Furthermore, a virtual shutdown of mitochondrial ATP production as well as a significant increase in extracellular acidification (ECAR) were observed in response to OPB, and these effects were significantly blocked by 2-deoxyglucose. Interestingly, recent findings have implicated STAT3 activation as a possible mechanism of resistance to tyrosine kinase inhibitors. In light of that, these results highlighting the STAT3 inhibitory activity as well as mitochondrial targeting potential of this novel synthetic inhibitor of STAT signalling could have potential therapeutic implications for cancer with dysregulated STAT3 network.

Conclusion. Taken together, our data indicate that this new inhibitor of STAT signalling, in particular STAT3, induces inhibition of tumor cell proliferation via mechanisms that involve targeting mitochondrial metabolism.

No conflict of interest.

165 Antiproliferative effects of a Cyclooxygenasse-2 inhibitor, Etoricoxib, alone and in combination with Cholecystokinin Antagonists in human pancreatic cancer cells

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Introduction. Pancreatic adenocarcinoma is one of the most malignant and aggressive disease that develops relatively with an unfavorable prognosis. Although numerous chemotherapeutic drugs have been tested in this malignancy, survival of advanced pancreatic cancer has not improved over the past several decades. Because of the lack of effective treatment options available for this disease, identification of novel targets and approaches has been made. Recent studies have shown an association between the gastrointestinal hormone gastrin, the expression of Cholecystokinin –B/ gastrin receptor and an increased generation of COX-2 in colon-carcinoma cells and progression of gastric cancer, suggesting that gastrin has direct stimulating potencies. However, it remains unknown whether the combination of Cholecystokinin -2 (CCK-2) receptor antagonist plus COX-2 inhibitor exerts synergistic antitumor effects on human gastric cancer. In order to identify a common pathway involved in Cyclooxygenase and Cholecystokinin we have studies protein-protein interaction databases. In the present study, we will study alone and combinatorial effect of Etoricoxib – a COX-2 inhibitor synthesized in lab and Cholecystokinin antagonists procured from Tocris on human pancreatic cancer cell lines, in which the CCK-2 receptor and COX-2 were expressed, was applied to examine whether blockade of the CCK-2 receptor and COX-2 exerts synergistic anti-tumor effects on human pancreatic cancer in vitro.

Materials &Methods. Antiproliferative effects were carried out using Cell Proliferation Kinetics, Morphological analysis and MTT Assay. Apoptotic activity was confirmed by DNA Fragmentation, Western Blot analysis and Cell cycle analysis

Result and discussion. The present study demonstrate that COX-2 enzyme is a modulator of carcinogenesis, apoptosis and angiogenesis in pancreatic cancer and is such rational target for drug development. Similarly Cholecystokinin antagonists is also a potential target for developing novel strategies for pancreatic cancer and further defines the pathway affected by CCK-BR. To study the combinatorial effect of COX-2 inhibitor and Cholecystokinin antagonist we successfully purified Etoricoxib using HPLC and further characterized by spectroscopic techniques using spectroscopy techniques. The anti-proliferative activity of Etoricoxib was checked on various cell lines and it induced proliferation arrest in Miapaca-2, Panc-I, BXPC-3 and ASPC-1 both in time and concentration dependent manner. The IC50 values were found to be 280 µM (Miapaca-2), 400µM (Panc-1), 300µM (BXPC-3) and 450 µM (ASPC-1) cells. The IC50 of CCK-2 antagonists YM-022, LY 288513 and L-365-260 was found out to be 0.002µM, 0.001µM and 0.01µM on Panc-1 respectively. Similarly, combinatorial studies was carried out on Panc-1, BXPC-3, ASPC-1 and Miapaca-2 respectively.

Conclusion. As a class NSAIDs possess analgesic, antipyretic, anti-inflammatory, and there is persuasive evidence that COX-2 inhibitor suppress cancer cell proliferation owing to their role in apoptosis, compelling evidence suggest that COX-2 over-expression promotes whereas COX-2 inhibition prevents tumor initiation and promotions. NSAIDs and COX-2 selective inhibitors may have different effects on cancer may be stage dependent therefore a better understanding of the critical COX related mechanisms of carcinogenesis, proliferation, apoptosis, and tumor invasion will help to define the potential of COX-2 blockage in cancer therapy.

No conflict of interest.

166 Simvastatin chemosensitizes spheres derived from canine mammary carcinoma cells through beta-catenin/MDR1 signaling

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Introduction. Mammary cancer is the most prevalent type of tumor in the female dogs, and shows many similarities with breast cancer in humans. Several authors have described the existence of a subpopulation of cancer cells with stem cell-like features (CSC), which have the ability to form spheres -structures that grow under harsh culture conditions-, resist conventional antitumor treatments explaining in part the recurrence of some cancers. The statins have shown antitumor effects on cancer mammary cells, however its effect have been poorly evaluated on CSC. Some of these reports indicate that simvastatin has chemosensitizing abilities on CSC, nevertheless the molecular mechanisms remain unclear. Our research group has recently published that simvastatin -a lipophilic statin- attenuated the cell proliferation and invasion of canine mammary cancer cells with characteristics of CSC, promoting apoptosis of them. Preliminar data of our laboratory support that this statin may target wnt/β-catenin pathway, which is usually activated at CSC. In addition, there is evidence that β-catenin may up-regulate multidrug resistance protein 1 (MDR-1), promoting chemo and radioresistance. The aim of this study was to investigate the chemosensitizing effects of simvastatin on spheres derived from canine mammary carcinoma cells, analyzing its effects on β-catenin/MDR1 expression.

Material and method: CF41.Mg epithelial cells from canine mammary cancer tissue were grown in DMEM high glucose supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Spheres derived from CF41.Mg were cultured in ultra-low attachment plates with serum-free DMEM/F12 in presence of EGF, bFGF, insulin, B27 and heparin. The sphere formation efficiency and cell viability (MTS reduction) assays were conducted in presence or absence of doxorubicin and simvastatin.The expression of total and phosphorylated β-catenin and MDR-1 in response to simvastatin were analyzed by immunocytochemistry and western blotting.

Result and discussion. Simvastatin induced a decrease in sphere formation ability and cell viability, incresing the effects of doxorubicin on these cells. The statin reduced total β-catenin and MDR-1 expression, and increased phosphorylation of β-catenin. These results suggest that simvastatin would facilitate the degradation of β-catenin, which may contribute in part to the antiproliferative and chemosensitizing effects of the statin.

Conclusion. Our results suggest that simvastatin could chemosensitize spheres derived from CF41.Mg cells by suppressing β-catenin/MDR-1 signaling.

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No conflict of interest.

167 Multiple anti-apoptotic BCL-2-like proteins determine antimitotic drug-mediated cell fates

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Introduction. Antimitotic drugs are among the most important chemotherapeutic agents available. However, abnormal mitotic exit mechanisms including mitotic slippage occur in antimitotic drug-treated cells and may lead to drug resistance or tumor relapse. Therefore, it is important to understand how mitotic cell death is regulated at the molecular level. The BCL-2 protein family regulates apoptosis by controlling mitochondria outer membrane integrity. This study systematically investigated the contribution of anti-apoptotic BCL-2-like proteins in mitotic cell death.

Material and Method. The expression of anti-apoptotic BCL-2 family was manipulated using RNA interference and ectopic expression in selected epithelial cell lines. Cell death and mitotic arrest were monitored with approaches including protein analysis and flow cytometry. The kinetics of mitosis and mitotic cell fates of individual cells were tracked using high-content live-cell imaging. Finally, the long-term effects of anti-apoptotic BCL-2 family on cell survival were evaluated with colony survival assays.

Results and Discussion. From a systematic screening, BCL-2, BCL-W, BCL-XL and MCL1 were identified as potent regulators of antimitotic drug-induced mitotic cell death. While the depletion of these proteins accelerated the rate of mitotic cell death, overexpression of these proteins repressed mitotic cell death, increased the

frequency of abnormal mitotic exit and enhanced long term cell survival. In particular, we identified BCL-W as one of the determinants of mitotic cell fates. The influence of BCL-W on mitotic cell death was highly related to its protein expression, which remained constant during mitotic arrest. These results revealed a role of BCL-W in setting an apoptotic threshold during mitotic arrest. Importantly, the level of endogenous BCL-W varied in different cell types, suggesting that it may be an important variable in the susceptibility of cells to antimitotic drugs.

Conclusion. This study demonstrates that multiple anti-apoptotic BCL-2-like proteins determine mitotic cell fates following treatment with antimitotic drugs. It also highlights BCL-W as a novel factor in setting the apoptotic threshold during mitotic arrest. Since BCL-2 inhibitors are being evaluated as potential anticancer agents, this study reveals the molecular basis of synergism between BCL-2 inhibitors and antimitotic drugs.

No conflict of interest.

172 M30 assay underestimates apoptosis in the case of inadequate amount of cytokeratin 18 in lung cancer cells

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Introduction. Caspase-cleaved cytokeratin 18 (M30, ccCK18), whose the production takes place only in epithelial cells, has been regarded as a pharmacodynamic biomarker of apoptotic cell death because it is released from the cells during apoptosis that is resulted from chemotherapeutic agents. Therefore, we aimed to test its performance on accurate detection of apoptosis in a panel of lung cancer cell lines.

Material and method. Three different nonsmall cell lung cancer (NSCLC) cell lines (A549, H1299, PC-3) were used. Apoptosis was detected morphologically and confirmed biochemically via well-established assays (Annexin V-FITC assay, caspase-cleaved cytokeratin 18 assay (M30 assay), Western Blottting).

Results and Discussion. Following succesful induction of apoptosis, A549 cell line yielded expected increments in M30 levels in accordance with other well-known features of apoptosis (e.g. DNA fragmentation, Annexin V-FITC positivity, pyknosis, PARP cleavage) while H1299 and PC-3 cell lines did not. Further analysis showed that H1299 and PC-3 cell lines express much lower level of cytokeratin 18 protein, compared to A549 cell line. Our results suggest that reliable detection of apoptosis via M30 assay needs proper amount of cytokeratin 18 in the cells.

Conclusion. M30 assay may cause false negative results for apoptosis in the case of inadequate amount of cytokeratin 18. Therefore, the data should be interpreted with caution.

No conflict of interest.

173 Overexpression of IGF-1Ec isoform induces aggressiveness of human breast cancer MCF-7 cells

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Introduction. Although the Insulin-like Growth Factor-1 (IGF-1) is a point of convergence for major signaling pathways implicated in breast cancer biology, the potential roles of the distinct IGF-1 isoforms in human malignancies are largely unknown. The Carboxyl (C-) terminal Ec peptide (24aa) of the IGF-1Ec variant has been suggested to have a distinct role in several pathophysiological conditions including prostate cancer. Herein, we investigated the possible role of Ec peptide in human MCF-7 breast cancer cells.

Materials and Methods. The C-terminal Ec domain of the IGF-1Ec isoform was cloned into a vector containing the CMV promoter (pIRES2-EGFP) and transfected MCF-7 cells until generation of stable clones over-expressing the Ec peptide (MCF-7 Ec cells). Using MTT and trypan blue exclusion assays along with flow cytometry (cell cycle) we compared the proliferation rates of MCF-7 Ec cells, mock transfectants (mock MCF-7 cells) and wild type MCF-7 cells (wt MCF-7 cells). In addition, we investigated the migratory capacities using the wound healing/scratch assay. Apoptosis/Necrosis rates in response to Docetaxel were also examined using flow cytometry and Annexin/PI staining.

Results and Discussion. While mock MCF-7 cells were phenotypically identical to wt cells, stable transfected MCF-7 Ec cells acquired a spindle-like phenotype and possessed an increased rate of proliferation [maximum effect 53% ($p<0.005$), 48 h], compared to mock MCF-7 and wt MCF-7 cells. Metabolic activity of the MCF-7 Ec transfectants, relative to mock MCF-7 and wt MCF-7 cells, was upregulated [maximum effect 44% ($p<0.03$), 24 h), whereas the distribution of MCF-7 Ec cells into the S phase of the cell cycle was marginally increased (by 10%, 24h). Furthermore, the migratory potential of the MCF-7 Ec transfectants was significantly increased [by 37% ($p<0.002$), 16h], whereas the rates of late apoptosis and necrosis of MCF-7 Ec cells in response to Docetaxel were slightly decreased (by 10%, 48h) as compared to mock MCF-7 and wt MCF-7 cells. These data suggested that overexpression of the Ec peptide influences the biology of the MCF-7 breast cancer cells, in vitro. Further studies on the role of this isoform may shed light into the complex progression and metastasis of the disease and identify Ec peptide as a potential target candidate in breast cancer therapeutics.

Conclusion. Our results indicate that IGF-1 Ec induces proliferation, migration and survival of MCF-7 breast cancer cells and further contribute to the notion for unique bioactivity of Ec peptide in human malignancies.

No conflict of interest.

174 A priori activation of apoptosis pathways for treatment of triple negative breast cancer

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Introduction. Dysregulation of apoptosis cell death pathways (e.g. CD95, APO-1/Fas) is a hallmark of most cancers. This aberration results in desensitizing tumor cells to chemotherapy demanding higher dose leading to systemic toxicity. This is particularly true for triple negative breast cancer (TNBC, > 65 % dysregulation), where no targeted therapy works due to the lack of biomarkers for which targeted drugs are designed. That leaves chemotherapy as the only option for treating TNBC patients. Hence, improving the clinical response to front-line therapies (e.g. doxorubicin) for TNBC patients is an unmet medical need.Here, our approach 'a priori activation of apoptosis pathways of tumors' AAAPT is to increase the therapeutic window of doxorubicin by re-activating apoptosis pathways with a targeted agent.

Material and Method. The leading candidate is a pegylated valine-citrulline conjugated a-tocopheryl succinate (VC-PA) with a cathpsin B cleavable linker. Triple negative MDA-MB-231 cells were used for in vitro studies and for in vivo studies, female Nu/Nu mice (4-6 weeks old) were innoculated in the mammary fat pad with 5x106 MDA-MB-231 cells stably expressing firefly luciferase. Several techniques including FACS, confocal microscopy for lysosomal instability, western blots for CD 95 activation, PARP inhibition, apoptosis inducing factor (AIF-1) and Bcl2-Bax ratio were used.

Results. The siginficant result was when tumor cells were preincubated with VC-PA followed by doxorubicin, IC-50 of doxorubicin was reduced from 0.5 to 0.45 mM (10 times). The mechanism of action includes data on a) activation of CD 95 pathway quantified through the expression of 43 KDa band in western plot, b) cleavage of PARP enzyme by VC-PA (85 KDa), c) translocation of AIF-1 from mitochondria to cytosol triggering irreversible cell death, d) lysosome instability through the leakege of Acridine Orange, e) enhanced influx of doxorubicin inside tumor cells and eventual reduction of BCl-2/Bax ratio. The in vitrostudies were corroborated with in vivo studies in TNBC xenograft model where tumor regression was monitored through bioluminiscence which showed tumor volume reduction by 2/3 compared to control and there was no toxicity observed for the 3 times the therapeutic dose for 2 weeks and 41 days beyond drug administration.

Conclusion. AAAPT approach sensitizes dysregulated TNBC cells and improves therapeutic window for doxorubicin which makes AAAPT as an adjuvant to chemotherapy.

No conflict of interest.

175 Upregulation of MiR-328 and inhibition of CREB-DNA-binding activity are critical for resveratrol-mediated suppression of matrix metalloproteinase-2 and subsequent metastatic ability in human osteosarcomas

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Introduction. Osteosarcomas, the most common malignant bone tumors, show a potent capacity for local invasion and pulmonary metastasis. A majority of patients with this bone tumor may harbor "micrometastasis" at the time of diagnosis, and many develop multidrug resistance during treatment. Osteosarcoma patients with metastasis have a poor prognosis, and the long-term survival rate remains at 10%~30%. It seems difficult to improve current response rates with further dose escalations to overcome drug resistance, as resistant tumor cells are able to withstand the effects of cytotoxic agents. Therefore, there is a pressing need to develop new and alternative approaches to the current medical treatment of osteosarcomas. Resveratrol (RESV), a phytochemical, exhibits multiple tumor-suppressing activities and has been tested in clinical trials. However, the antitumor activities of RESV in osteosarcomas are not yet completely defined. Here, novel molecular mechanisms through which RESV exerts its anticancer effects in osteosarcomas were investigated.

Material and methods. Human osteosarcomas cell lines, HOS, MG-63, U2OS, Saos-2, and 143B, were used as the in vitro cell models. The wound-closure assay, transwell migration and invasion assays, and cell adhesion assay were used to determine the antimetastatic effect of RESV in vitro. The Western blot analysis, zymography assay, Chromatin immunoprecipitation (ChIP) analysis, and miRNA array were used to evaluate the underlying mechanisms in RESV-mediated suppression of cell motility. The noninvasive bioluminescence system was used to monitor the antimetastatic effect of RESV in in vivo model.

Results and discussion. RESV significantly inhibited the migration/invasion in vitro and lung metastasis in vivo by suppressing matrix metalloproteinase (MMP)-2. We identified that RESV exhibited a transcriptional inhibitory effect on MMP-2

through reducing CREB-DNA-binding activity. Moreover, a microRNA (miR) analysis showed that miR-328 was predominantly upregulated after RESV treatment. Inhibition of miR-328 significantly relieved MMP-2 and motility suppression imposed by RESV treatment. Furthermore, ectopic miR-328 expression in highly invasive cells decreased MMP-2 expression and invasive abilities. Mechanistic investigations found that JNK and p38 MAPK signaling pathways were involved in RESV-regulated CREB-DNA-binding activity, miR328 expression, and cell motility. Clinical samples indicated inverse expression between MMP-2 and miR-328 in normal bone and osteosarcoma tissues. The inverse correlation of MMP-2 and miR-328 was also observed in tumor specimens, and MMP-2 expression was linked to tumor metastasis.

Conclusion. Our results provide new insights into the role of RESV-induced molecular and epigenetic regulation in suppressing tumor metastasis.

No conflict of interest.

176 Dehydroandrographolide inhibits migration and invasion by inhibition of matrix metalloproteinase-2 through modulation of SP-1 in human oral cancer

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Introduction. Oral cancer with poor prognosis is due to frequent local invasion and lymph node metastasis. Andrographolide has been reported to have anticancer activity in multiple types of cancer. However, the effects of dehydroandrographolide (DA), an andrographolide derivative, on oral cancer invasion and metastasis have yet to be evaluated.

Material and Method. In this study, we used wound healing model, Boyden chamber assays, real-time PCR and western blotting to determine the effects of DA on the migration and invasion in oral cancer cells.

Results and Discussion. DA treatment significantly inhibited migration/invasion capacities of SCC9 cell lines in vitro. The results of gelatin zymography and western blotting revealed that the activities and protein levels of the matrix metalloproteinase-2 (MMP-2) was inhibited by DA. Western blot analysis also showed that DA inhibits phosphorylation of ERK1/2, p38 and JNK1/2. Tests of the mRNA levels by real-time PCR evaluated the inhibitory effects of DA on MMP-2 expression in human oral cancer cells. DA inhibits MMP-2 expression, the inhibitory effects were associated with the up-regulating tissue inhibitor of metalloproteinase-2 (TIMP-2) and down-regulation of the transcription factors of SP-1 signaling pathways. Finally, an administration of DA effectively suppressed carcinoma-associated epithelial-to-mesenchymal transitions (EMT) in human oral cancer cells.

Conclusion.DA inhibited the invasion of human oral cancer cells and may have potential use as a chemo-preventive agent against oral cancer metastasis.

No conflict of interest.

177 Metastatic associated genes in cholangiocarcinoma

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Introduction. Metastasis is the major cause of death in cholangiocarcinoma (CCA) patients. Understanding the molecular mechanism of metastasis is necessary to inhibit this vital process. For this purpose, we first established the high (KKU-M213L5) and low (KKU-M213) metastasis CCA cell lines. KKU-M213L5 was obtained by 5 cycle-intravenous injection of lung metastasized tumor in NOD/Scid/Jak3 deficient (NOJ) mice. This study is aimed to characterize the metastatic properties of KKU-M213L5 vs. KKU-M213 CCA cells.

Material and method. The metastatic subclone KKU-M213L5 was characterized in vitro and in vivo in comparison to the parental cells KKU-M213. Cell proliferation was determined by cell counting, cell motility was observed by wound healing assay, cell invasion was performed by Boyden chamber assay. The metastatic potential of KKU-M213L5 was confirmed in the mouse model via tail vein injection. CCA metastasizing cells in the lung were analyzed for micro- and macro-metastatic cells at 13 and 21 days post-injection. Finally, the Gene expression signature between low and high metastatic cholangiocarcinoma cell lines was determined by a homemade metastatic PCR array comprising of 77 genes and verified the candidate genes by Immunohistochemistry.

Results and discussion. The in vitro experiments indicated that the metastatic cells, KKU-M213L5 have higher growth rate, migration rate and invaded cells than those of the parental KKU-M213. The numbers of micro and macro- metastases of lung colonization in mouse model at 13 and 21 days revealed significantly higher metastasis potential of KKU-M213L5 than KKU-M213. The differential expression of 22 metastatic related genes between these two cell lines were explored. Eight genes were up-regulated with 2-??cp > 1.2 and 14 genes were down-regulated with 2-??cp < 0.5. Anterior gradient protein-2 (AGR2) and KISS1 metastasis suppressor (KISS1) were the highest and the lowest expressed genes in KKU-M213L5 compared with KKU-M213, respectively. AGR2 was strongly positive in CCA tissue from patients with high tumor stage whereas KISS1 was overexpressed in low tumor stage compared with advanced stage. This may imply the association of AGR2 and KISS1 expression involved with metastatic activity in CCA.

Conclusion. In this study, high metastatic subclone of CCA cell line was first established and characterized. The metastatic cells exhibited higher metastatic phenotypes comparing to the parental cells both in vitro and in vivo. Expression of AGR2 and KISS1 was first demonstrated to be genes responsible for tumor progression in KKU-M213L5 and associated to metastatic status of CCA patients. The newly established high metastatic subclone, KKU-M213L5 and the metastatic gene profile revealed from this study may be a valuable tool for metastatic prevention or targeted therapy of human CCA.

No conflict of interest.

178 Tricetin suppresses migration/invasion of human glioblastoma multiforme (GBM) cells by inhibiting matrix metalloproteinase-2

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Introduction. Glioblastoma multiforme (GBM) can be a fatal tumor due to difficulties in treating related metastasis. Tricetin, a natural flavonoid, was demonstrated to inhibit various cancers growth, but the effect of tricetin on cancer motility is largely unknown. In the present study, we examined the antimetastatic properties of tricetin in human GBM cells.

Material and Method. Cell viability was measured using an MTT assay. The inhibitory effects of tricetin on GBM cell migration and invasion was analyzed by cell-migration assay and Matrigel invasion assay. The activities of matrix metalloproteinase (MMP)-2 were determined by gelatin zymography.

Results and Discussion. Our results showed that tricetin (20-80 μM) inhibited the migration/invasion of two GBM cell lines (GBM 8401 and U87). We found that tricetin inhibited MMP-2 activity and expression by GBM cells. Real-time PCR and promoter activity assays indicated that tricetin inhibited MMP-2 expression at the transcriptional level. Such inhibitory effects were associated with suppression of specificity protein 1 (SP-1) DNA-binding activity and SP-1 expression. Moreover, blocking of the ERK pathway also inhibited MMP-2-mediated cell motility, and further enhanced the antimetastatic ability of tricetin in GBM 8401 cells.

Conclusions. SP-1 is an important target of tricetin for suppressing MMP-2-mediated cell motility in GBM cells, and a combination of tricetin and an ERK inhibitor might be a good strategy for preventing metastasis of GBM.

No conflict of interest.

179 Cantharidic acid suppresses the proliferation and induces apoptosis involving MAPKs and caspase-8/-9/-3 signals in human hepatocellular carcinoma cells

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Introduction. Cantharidic acid, a lipophilic structural analog of cantharidin, is widely used to study the role of protein phosphatase in the regulation of various physiological functions. However, the effect of cantharidic acid on hepatocellular carcinoma (HCC) remains unclear. Here, the molecular mechanism by which cantharidic acid -induced apoptosis effects in human HCC cells was investigated.

Material and Method. Cell growth was measured using an MTT assay. Cell cycle analysis and Annexin V/PI double staining were estimated on flow cytometry. Caspase-3, -8, -9 and mitogen-activated protein kinase (MAPK) pathway were evaluated using western blotting.

Results and Discussion. The results showed that cantharidic acid suppressed cell proliferation in various types of HCC cell lines. Moreover, cantharidic acid induced cell-cycle arrest of SK-Hep1 HCC cells at the Go/G1 phase. Furthermore, cantharidic acid effectively induced apoptosis of SK-Hep1 cells through caspases-8, -9, and -3 activation concomitantly with a marked induction of Erk1/2 and p38 MAPK activation, but without affecting expression levels of Bcl-2.

Conclusions. Our results suggest that cantharidic acid inhibited SK-Hep1 cell proliferation through inducing cell-cycle arrest and apoptosis and could serve as a potential additional chemotherapeutic agent for treating HCC.

No conflict of interest.

180 Hispolon attenuates metastasis via Akt pathways in human nasopharyngeal carcinoma cells

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Introduction. Hispolon is an active phenolic compound of Phellinus igniarius, a mushroom that was recently shown to have antioxidant and anticancer activities in various tumors. This study investigated the mechanisms by which Hispolon inhibits the invasiveness of human nasopharyngeal carcinoma cells.

Material and Method. Western blot was used to analyze matrix metalloproteinases (MMP)-2 protein levels and quantitative real time RT-PCR was used to measure

mRNA expression. The specific pharmacological inhibitors LY294002 were used to block Akt pathway. The transcriptional factor specificity protein-1 (SP-1) binding activity was confirmed by using chromatin immunoprecipitation.

Results and Discussion. In this study, we demonstrate that Hispolon attenuated HONE-1 and NPC-39 cell migration and invasion in a dose-dependent manner. The anti-metastatic activities of Hispolon occurred at least partially because of the down-regulation of MMP-2 gelatinase activities and the down-regulation of protein expression. The expression and function of MMP-2 were regulated by Hispolon at a transcriptional level, as shown by quantitative real-time PCR and reporter assays. Binding of the transcriptional factor specificity protein-1 (SP-1) to the MMP-2 promoter diminished at the highest dosage level of Hispolon. Hispolon did not affect the mitogen-activated protein kinase signaling pathway, but did inhibit the effects of gelatinase by reducing the activation of serine-threonine kinase Akt.

Conclusion. These results demonstrate that Hispolon may be a potent adjuvant therapeutic agent in the prevention of nasopharyngeal carcinoma cells.

No conflict of interest.

181 Polyphyllin G induced apoptosis and autophagy in human hepatocellular carcinoma

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Introduction. Chemotherapeutic agents target proliferating cells and induced cell death, mostly via caspase-mediated apoptosis involving both intrinsic and extrinsic pathways. However, the role of autophagy in cancer development and in the response to therapy is still controversial. This study investigated the effects of polyphyllin G, a steroidal saponin in Paris polyphylla, on cell growth, apoptosis, autophagy, and the underlying mechanisms in human hepatocellular carcinoma (HCC) cells.

Material and Method. Cell growth was tested by trypan blue staining and MTT assay. Cell autophagy was examined by acidic vesicular organelles (AVO) staining and LC3-II western blot assay. Cell apoptosis was checked by FITC Annexin-V and PI labeling, tunnel staining and caspase 3 western blot assay.

Results and Discussion. Our results showed that around 40% of cells survived after a treatment of polyphyllin G at 5 μM by MTT assay. Since trypan blue staining indicating cell death, microscopic cell counting revealed a dramatic decrease in viable cell numbers in polyphyllin G-treated HCC cells. Polyphyllin G delayed cell cycle progression and increased percentage of cells displaying phosphatidyl serine (PS) externalization in polyphyllin G-treated HCC cells. In addition, polyphyllin G also increased LC3-II and beclin-1 protein expression in a dose-dependent manner. Polyphyllin G caused induction of AVO in HCC cells indicated that the induction of autophagy was dose- and time-dependent.

Conclusion. These results suggested that the polyphyllin G -induced autophagy process and apoptosis. Taken together, polyphyllin G may act as a new and potential anticancer agent for human HCC.

No conflict of interest.

182 Dehydroandrographolide, an iNOS inhibitor, induces autophagy in human head and neck cancer

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Introduction. Autophagy, which is constitutively executed at the basal level in all cells, promotes cellular homeostasis by regulating the turnover of organelles and proteins. Dehydroandrographolide has been reported to have a wide range of biological activities, such as those that are anti-inflammatory and anti-cancer activities. However, the effect of Dehydroandrographolide on Head and Neck cancer (HNC) remains unclear. Here, the molecular mechanism by which Dehydroandrographolide-induced autophagy effects in human HNC cells was investigated.

Material and Method. Cell growth was measured using an MTT assay. Cell autophagy was examined by acidic vesicular organelles (AVO) staining. LC3-II and mitogen-activated protein kinase (MAPK) pathway were evaluated using western blotting.

Results and Discussion. In this study, we demonstrated that Dehydroandrographolide induces HNC cell death by activating autophagy but not apoptosis. Dehydroandrographolide significantly increased LC3-II and acidic vesicular organelle expression in HNC cells. Treatment with autophagy inhibitors inhibited Dehydroandrographolide-induced human HNC cell death. However, Dehydroandrographolide failed to reduce cell viability in the presence of the VZV-G pseudotyped lentivirus–shRNA system or siRNA knockdown autophagy-related gene, LC3 or beclin-1. Conversely, treatment with autophagy enhancers increased Dehydroandrographolide-induced cell death. In addition, Dehydroandrographolide increased LC3-II expression and reduced p53 expression in a time- and concentration-dependent manner. Dehydroandrographolide -induced autophagy was triggered by the activation of ERK1/2 and JNK1/2 and inhibition of Akt and p38.

Conclusion. This is the first study to reveal the novel function of Dehydroandrographolide in activating autophagy, suggesting that Dehydroandrographolide could serve as a new and potential chemopreventive agent for treating human HNC.

No conflict of interest.

183 A high TRAIL-receptor clustering is able to overcome TRAIL resistance in pediatric bone sarcoma models

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Introduction. Osteosarcoma (OS) and Ewing's sarcoma (EWS) are the two most common pediatric bone tumors. OS and EWS patients have not seen major therapeutic advances these last thirty years and the survival rate of 70 % at five years for a localized tumor falls to around 20 % in the case of metastatic tumor or resistance to chemotherapy. The pro-apoptotic cytokine TNF-Related Apoptosis Inducing Ligand (TRAIL) can selectively kill tumor cells and could therefore represent a promising therapeutic approach for patients at high risk. However, the transfer to clinics seems limited because several OS and EWS cell lines show resistance towards TRAIL sensitivity in vitro.

Methods. In vitro and in vivo approaches allow us to identify several molecular mechanisms involved in TRAIL resistance in these particular pathologies: death (DR4 and DR5) and decoy receptors (DcR1, DcR2, Osteoprotegerin) expression, involvement of inhibitory proteins of apoptosis (cFLIP; IAP1/2)...

Result and discussion. Even if OS and EWS exhibit similar clinical features, these pathologies differ in response to TRAIL pro-apoptotic effects: a TRAIL-receptor agonist antibody induces MAPK pathway activation in OS cell lines, showing even a protumoral effect in vivo in a OS xenograft model. Accumulated evidences indicate that TRAIL, besides its pro-apoptotic effects can also induce the activation of another signaling pathway involving NF-kB, MAPK, PI3K/Akt via binding to the same receptors, but leading to increased tumor cell proliferation, survival, migration and invasion. The key regulator of this kinase network is the RIPK1 protein. We hypothesize that an efficient TRAIL-receptors clustering could raise resistance of tumor cells and trigger apoptosis instead of proliferation. To this aim, two different approaches were used: trimeric TRAIL presentation at the surface of carrier Mesenchymal Stem Cells (MSC) stably transfected with full length human TRAIL and a novel TRAIL-receptor agonist able to bind 6 receptors (APG880). We validate in vitro that coculture of tumor cells with MSC-TRAIL or use of APG880 can induce apoptosis even in initial resistant cell lines. In vivo, intratumoral injection of untransfected MSC accelerates tumor development in both EWS and OS models, whereas MSC-TRAIL inhibit tumor progression in EWS models but not in OS models.

Conclusion. For these models, APG880 may represent a good compromise between the induction of receptor clustering and the lack of pro-proliferative effect of MSC by themselves.

No conflict of interest.

184 The inhibitory effects of an ethanol extract of Annona atemoya seeds on tumor-induced angiogenesis in hypoxia conditions

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Introduction. Annona atemoya was first produced by Wester by crossing A. squamosa and A. cherimola in 1907. Crude extracts of A. atemoya seeds can potentially be used in botanical insecticides, and annonaceous acetogenins isolated from A. atemoya, has been reported to induce apoptotic cell death in the various cancer cell line. However, the effect of the ethanol extract of A. atemoya seeds (EEAA) on angiogenesis and its underlying mechanism remain unknown. In the current study, we investigated the anti-angiogenic potential of EEAA under hypoxia conditions in vitro.

Material and method. Dried A. atemoya seeds were extracted in 70% (v/v) ethanol and the yield of the final extract was 10.49% (w/w). The expression of hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF) was investigated using reverse transcription-polymerase chain reaction, immunoassays, and western blotting. The anti-angiogenic potential of EEAA was evaluated using various in vitro tumor-induced angiogenesis models, including cell proliferation, migration, and tube formation by Human umbilical vein endothelial cells (HUVECs) in the concentrated NCI-H460 culture medium (CM).

Results and discussion. EEAA down-regulated the expression of VEGF and HIF-1alpha/2alpha at the mRNA and protein levels, respectively, in cancer cells under hypoxic conditions. Also, the EEAA-treated CM significantly inhibited these angiogenic properties mediated by HUVEC. Because EEAA was able to down-regulate the expression of VEGF under hypoxia, we investigated whether the suppression of cell growth, migration, and tube formation via H460-CM-EA could be reversed by VEGF supplementation. As expected, H460-CM-EA supplemented with 3 ng/mL VEGF was able to relieve the inhibitory effects of EEAA on HUVEC-mediated angiogenic properties. These results indicate that EEAA prevented angiogenesis primarily by down-regulating VEGF production in NCI-H460 tumor cells.

Conclusions. Taken together, our findings demonstrated that EEAA exhibits anti-angiogenic potentials via suppression of tumor-mediated angiogenesis, including down-regulation of VEGF and HIF signaling in tumor cells. Therefore, we suggest that EEAA may be a valuable herbal source for the development of tumor therapeutic agent.

No conflict of interest.

185 Molecular and functional traits of ovarian cancer stem cells

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Epithelial Ovarian Cancer (OC) is one of the leading causes of death for women, and no significant therapeutic progress has been made in the last decades.

Recent data suggest that drug resistance and/or disease recurrence are driven by a subpopulation of cells in human tumors with stem-like characteristics (cancer stem cells, CSCs). CSCs are defined as a small subpopulation of cells within the tumor bulk that possess the capacity, on one hand, to self-renew and, on the other hand, to give rise to all heterogeneous cancer cell lineages that compose the tumor of origin. The CSC hypothesis provides an attractive cellular mechanism to explain the therapeutic refractoriness, dormant behavior, and relapse of the disease.

Our study aims at assessing ovarian cancer stem cells (OCSC) as causal players in OC etiology and progression and at defining their molecular and functional traits. Specifically, we are pursuing these objectives through the accomplishment of the following milestones: 1) collection of normal and pathological samples; 2) identification of OCSC based on functional properties; 3) comparison of gene expression profiles between cancer stem cells and their normal counterpart; 4) characterization of novel genes/pathways involved in OCSC function (clonogenicity, tumorigenicity, quiescence, chemoresistance, etc.).

This workflow is being applied to a series of fresh surgical samples of OC as well as to normal ovarian surface epithelium (OSE) and fallopian tube epithelium (FTE), namely the tissues of origin of OC. Thus, we obtained a collection of primary cells that recapitulate many traits of the original tissue both in vitro and in vivo. The gene expression profiles of sphere-forming SC has been compared to that of adherent, parental cells, aimed at identifying stemness-associated genes. This screening has been extended to OC, OSE, and FTE, resulting in a set of genes that are differentially expressed in OCSC as compared to their normal counterparts.

A subset of such genes has been selected for the subsequent validation, both as OCSC biomarkers that could have clinical applications and as drivers in the biological and pathogenic function of OCSC.

Our study might set the stage for innovative therapeutic approaches aimed at the selective elimination of OCSC, thus preventing tumor recurrence and chemoresistance, namely the two main causes of ovarian cancer lethality.

No conflict of interest.

186 L1CAM: A new driver in tumor vasculature

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Introduction. Anti-angiogenic therapy for tumor treatment, best exemplified by the anti-VEGF drug bevacizumab, has displayed a remarkable potential in certain cancer types. However, it is clear that novel vascular targets are needed to improve the efficacy of current anti-angiogenic strategies as well as to circumvent the resistance/evasion mechanisms that have emerged in different experimental models and in cancer patients.

The neural immunoglobulin-like cell adhesion molecule L1CAM, that plays a crucial role in CNS development and plasticity, is aberrantly expressed in cancer-associated vessels, while it is not found in normal vasculature. However, the functional role of L1CAM in endothelial cells, and its contribution to tumor angiogenesis, remain elusive.

Materials and Methods. To study the role of L1CAM in tumor vessels, we combine the endothelial-specific ablation of L1CAM in conditional knockout mice model with orthotopic, syngeneic models of cancer.

In addition, the molecular mechanisms underlying the vascular function of L1CAM are studied in appropriate endothelial cell models.

Results. Our results implicate L1CAM in the control of tumor vasculature, due to its ability to regulate both angiogenesis and vascular maturation. We also observed that the inactivation of endothelial L1CAM represents a viable option for novel anti-angiogenic treatments, resulting in reduced tumor growth and progression.

At the molecular level, L1CAM emerges as a master regulator of the endothelial transcriptional activity and, as such, is capable of orchestrating multiple functions of endothelial cells, thus accounting for the aberrant pathophysiology of cancer vessels. Our recent data provide mechanistic insights into such a new role of L1CAM in tumor vasculature.

Conclusions. Besides shedding light on the molecular mechanisms that underlie the formation and function of cancer vasculature, our data may pave the way for innovative vascular targeting strategies in the context of tumor therapy.

No conflict of interest.

187 Dual CXCR4 and e-selectin pharmacogical inhibition reduces tumour growth and increases the sensitivity to docetaxel in experimental bone metastases of prostate cancer

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Background. Prostate cancer (PCa) patient morbidity can be attributed to bone metastases posing a significant clinical obstacle. Therefore, a better understanding of this phenomenon is imperative and might help to develop novel therapeutic strategies. PCa cells preferentially roll and adhere on bone marrow vascular endothelial cells, where abundant E-selectin and stromal cell-derived factor 1a (SDF-1a) are expressed, subsequently initiating a cascade of activation events that eventually lead to the development of metastases. This suggests that agents able to suppress this signaling pathway may be used as pharmacological treatments of bone metastatic disease. In addition it has been suggested that chemotherapy have scarce success when administered to patients with bone metastases since in this site the CXCR4 activation may determine protection vs cell death.

In this preliminary study we investigate if the dual E-selectin/CXCR4 inhibitor (GMI1359) plays a role in the reduction of bone growth of PC3 cell line collaborating with docetaxel in the control of tumor burden and osteolysis. We compare GMI1359 with the sole E-selectin (GMI1271) and CXCR4 (plerixafor) inhibitory activity by using intratibial injection of PC3M-luciferase cells.

Results. PCa cells with aggressive phenotype able to determine visceral and bone metastases express high levels of CXCR4 and E-selectin ligands than normal or not aggressive/non metastasizing PCa cell lines. In vitro we demonstrated that bone microenvironment sustains CXCR4 and SDF1-a expression determining increased efficacy toward CXCR4 pharmacological inhibition. Additionally, SDF-1a induced tumor cell migration and invasion, as well as MMP-9, MMP-2 and uPA expression, were reduced by CXCR4 inhibition. In vivo we demonstrated that the percentage of tibiae positive by X-ray and the size of osteolytic lesions were reduced by treatments mainly when we used Plerixafor and GMI1359 whereas GMI1271 effects were not significant when compared to controls. The amounts and the size of bone metastases were significantly reduced when mice were treated with Plerixafor, GMI1359 and GMI1271 in combination with docetaxel. The effects were more marled with plerixafor and GMI1359 when compared with GMI1271. The reduced intra-osseous growth of PC3M cells, as a result of treatments, correlated with decreased osteolysis and serum levels of both mTRAP and type I collagen fragments. **Conclusions.** Our report provides novel information on the potential activity of CXCR4 inhibitors as compounds able to increase/restore docetaxel sensitivity of bone metastatic lesions and supports a biological rationale for the use of these inhibitors in men at high risk to develop clinically evident bone lesions who undergone taxane-based chemotherapy as first line of therapy. However, further experiments will be necessary to indicate the effective role of CXCR4 and/or E-selectin in the metastatic process of PCa.

No conflict of interest.

188 Hypoxia modulates the expression of aldehyde dehydrogenases in colon cancer cells with ALDH7A1 emerging as a key enzyme whose functional involvement is dependent on the tumour microenvironment

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Introduction. Most solid tumours generate hypoxic regions as a consequence of poorly developed and incomplete neovasculature. It is well known that hypoxia is associated with an aggressive cancer phenotype, causing resistance to both radiotherapy and chemotherapy. The aldehyde dehydrogenase (ALDH) superfamily, which belongs to the class of phase 1 drug metabolising enzymes, is thought to be involved in drug resistance. However, their regulation and expression within the tumour microenvironment is poorly understood. Accordingly, we have initiated an investigation to understand the role of ALDHs in tumour tissues and explored the impact hypoxia might have on the expression of these enzymes in colon cancer.

Material and method. Colon cancer cell lines (HT29, DLD-1, SW480 and HCT116) were grown under normoxic or hypoxic conditions (0.1% O2) for 6, 24 and 48h. HT29 and DLD-1 cells were also grown in spinner flasks until multicellular spheroids (MCS) were obtained (diameter ≈600μm). The hypoxic regions of the MCS were detected using the hypoxia marker, pimonidazole, and isolated using sequential trypsinisation. Gene expression analysis of ALDH isoforms (1A1, 1A2, 1A3, 1B1, 2, 3A1 and 7A1) in monolayer cells and MCS was carried out using quantitative RT-PCR. The protein expression was evaluated using Western blot and immunohistochemistry.

Results. The gene analysis data of monolayer cells showed that hypoxia exerts upregulation of ALDH(1A1, 1A2 and 7A1) in DLD-1 and HT29, ALDH1A3 in SW480 and all investigated ALDH in HCT116 with the exception to ALDH(2 and 7A1). However, on the protein level, only ALDH7A1 was upregulated in HT29 and DLD-1 and ALDH1A3 in HCT116 and SW480. Cells residing in the hypoxic region of HT29 and DLD-1 MCS showed upregulation of ALDH7A1 compared to surface layer cells and monolayer cells at both gene and protein levels.

Conclusion. Our results reveal that tumour hypoxia has impact on the expression of ALDHs in colon cancer cells at both gene and protein levels. An understanding of how these enzymes are affected by hypoxic conditions and their location within the

tumour microenvironment will elucidate the role of these enzymes in colon cancer progression and drug resistance. Our data suggests that ALDH7A1 is increased by exposure to hypoxia and current studies are focussed on understanding how this enzyme may be linked to HIF-1 and/or metabolic signalling pathways. The data from these studies will also be presented at the meeting.

No conflict of interest.

189 Effect of mechanical properties of tumor microenvironment on breast cancer behavior

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Introduction. The local microenvironment of breast cancer (BC) plays an important role in its development and progression, and the extracellular matrix (ECM) elicits different biochemical properties that affect tumor cell behavior. Tissue mechanics have recently emerged as a player that cooperates with ECM biochemical cues. We used new in vitro tools to analyze the effect of mechanical signals on BC cells and on progression of human BCs.

Material and method. Human BC cell lines MDA-MB-231 and MCF7 were cultured on genipin-crosslinked gelatin hydrogel scaffolds of different stiffness (2, 15 and 80 kPa) and evaluated for migration potential and then for RNA profile on the Illumina platform. Mechanical properties of 46 frozen tumor samples from BC patients were investigated by analysis of the elastic modulus through compression tests and RNA was profiled on the Illumina platform.

Results and discussion. MDA-MB-231 cells showed a 2- to 3-fold increase in migration potential as a function of increased stiffness, and MCF7 cells were induced to migrate when cultured on the stiffest scaffolds. GSEA analysis of cell expression profiles showed a significant enrichment of gene sets belonging to glycosaminoglycan, chondroitin sulfate, heparan sulfate and glycan biosynthetic pathways ($p<0.05$, FDR<25%) in cells grown on scaffolds with highest stiffness. Consistent with in vitro mechanically induced aggressiveness, analysis of the elastic modulus of human samples showed that patients with higher stiffness had lower relapse-free survival rates than did the low-stiffness group ($p<0.05$); the same gene sets up-modulated in in vitro cells were also significantly enriched in highly stiff tumors ($p<0.05$, FDR<25%).

Conclusion. Our results indicate that tumor tissue rigidity increases BC aggressiveness and contributes to plasticity of the tumor microenvironment through regulation of ECM composition.

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No conflict of interest.

190 Inhibition of SP1-DNA-binding activity is critical for melatonin-mediated suppression of matrix metalloproteinase-9 and subsequent metastatic ability in human head and neck squamous cell carcinoma

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Introduction. Head and neck squamous cell carcinoma (HNSCC) mortality has increased during the last decade due to the difficulties in treating related metastasis. Melatonin is an indoleamine molecule produced primarily by the pineal gland and other organs. A number of studies have reported it to have anti-inflammatory and anti-cancer effects. However, the effect of melatonin on metastasis of HNSCC has yet to be fully elucidated. Here, we investigated the anti-migration activity of melatonin on human HNSCC cell lines (HONE-1 and NPC-39) in vitro and its underlying mechanisms.

Material and Method. Cell viability was examined by MTT assay, whereas cell motility was measured by migration and wound healing assays. Zymography, reverse transcriptase polymerase chain reaction (PCR), and promoter assays confirmed the inhibitory effects of melatonin on matrix metalloproteinase-9 (MMP-9) expression in oral cancer cells. The transcriptional factor specificity protein 1 (SP1) binding activity was confirmed by using chromatin immunoprecipitation.

Results and discussion. We established that various concentrations (0-2 mM) of melatonin inhibited the 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced migration capacities of HONE-1 and NPC-39 cells and caused no cytotoxic effects. Zymography and western blot analyses suggested that melatonin inhibited TPA-induced MMP-9 gelatinolytic activity and protein expression. Mechanistic investigations found that JNK signaling pathways were involved in melatonin-regulated SP1-DNA-binding activity and cell migration.

Conclusion. Melatonin inhibits MMP-9 expression and HNSCC cell metastasis and, thus, has potential use as a chemopreventive agent. Its inhibitory effects are associated with downregulation of JNK signals pathways and SP1-DNA-binding activity.

No conflict of interest.

191 MiR-141 not only modulates anoikis resistance of ovarian cancer cells but also alters pre-metastatic niche for ovarian cancer cell metastatic colonization

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Introduction. Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy worldwide. This disease is generally called the 'silent killer' because there are no symptoms and thus, the majority of patients are found in advanced stages accompanied by extensive metastasis. Most deaths from this cancer are attributed to metastatic progression. The cancer metastasis is determined by the priming of metastatic niche and the intrinsic properties of cancer cells to adapt the microenvironemntal stresses. However, the associated molecular mechanisms remain unclear.

Material and method. The miRCURY™ LNA, cDNA, and proteomic array profilings in combination with a series of biochemical and functional analyses were performed to identify miR-141 as a putative miRNA in regulating anokis resistance of ovarian cancer cells through KLF12/survivin signaling. Both in vitro cell culture and in vivo mouse model showed miR-141 is an secretary miRNA, while immunofluorescent, qPCR, Proteome Profiler Human XL Cytokine Array Kit and functional analyses demonstrated miR-141 was able for cell-to-cell communication to alter host cells as a premetastatic niche.

Results and discussion. Using miRCURY™ LNA Array profiling in combination with a series of biochemical and functional analyses, we identified Hsa-miR-141 (miR-141) was highly expressed in advanced serous subtype ovarian cancers. The overexpressed miR-141 could enhance cell survival of ovarian cancer cells against anoikis by targeting Krüppel-related zinc finger protein AP-2rep (KLF12). Restoration of KLF12 in miR-141-expressing cells remarkably reduced, or knockdown of KLF12 similar to miR-141 overexpression augmented, anoikis resistance of ovarian cancer cells through alteration of survival-associated factor, survivin. Luciferase reporter assay using survivin promoter luciferase plasmid (luc-survivin) indicated that survivin could be transcriptionally inhibited by KLF12. Intringuingly, miR-141 was found to be secreted from ovarian cancer cells and taken up by hFF-1 fibroblast cells. Ovarian cancer cells cultured in miR-141-expressing fibroblast cell medium displayed increased cell growth and cell migration in the presence of GROa and EMMPRIN chemokines.

Conclusion. MiR-141 not only plays a key role in altering cancer cell plasticity against anoikis but also can reprogram stroma to be a pre-metastatic niche facilitating the ovarian cancer metastatic colonization.

No conflict of interest.

194 Docosahexaenoic acid induces colon cancer cell death through AMPKa/FOXO3A/miR-21 pathway

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Introduction. Docosahexaenoic acid (DHA, C22:6) is an n-3 polyunsaturated fatty acid with anti-cancer activities. Recently microRNAs (miRs) have been discovered as new regulators of various cellular functions and might contribute to the DHA anti-cancer action. Indeed, some miRs (i.e. miR-21) as small non-coding RNAs that mediate post-transcriptional gene silencing, exhibits oncogenic properties. The aim of this study is to precise the role of miR-21 and the regulation of its expression in DHA-mediated apoptosis of colon cancer cells.

Materials and Methods. The human colon cells lines (HCT-116, HCT-8) were treated with DHA at concentrations of 50, 75 and 100 µM. Cell death was evaluated by AnnexinV-7AAD staining analyzed by flow cytometry. The regulation of miR-21 expression was assessed by RT-qPCR. We analyzed Foxo3a subcellular distribution by immunofluorescence staining. Overexpression of miR-21 expression was performed by oligonucleotides mimic-21 transfection while inhibition of AMPKα and Foxo3a expressions was performed by siRNA or pharmacological inhibitors.

Results. We reported that DHA induced apoptosis was associated with down-regulation of miR-21 expression in colon cancer cells. Conversely, overexpression of miR-21 inhibited the apoptotic effect of DHA. This effect of DHA is mediated by Foxo3a, as we observed that DHA inhibited nuclear translocation of this transcription factor and repressed miR-21 expression. Indeed, the change of Foxo3a subcellular distribution depended on AMPKα activation by DHA. Finally, we evidenced that inactivation of AMPKα and Foxo3a signaling curtailed the pro-cytotoxic effect of DHA in colon cancer cells.

Conclusion. The present study highlights the involvement of AMPKα/Foxo3a/miR-21 signaling in DHA-induced apoptosis in colon cancer cells.

No conflict of interest.

195 The H3K9 methyltransferase G9a is a marker of aggressive ovarian cancer that promotes tumor invasion and metastasis

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Introduction. Ovarian cancer (OCa) peritoneal metastasis is the leading cause of cancer–related deaths in women with limited therapeutic options available for treating it and poor prognosis, as the underlying mechanism is not fully understood. Epigenetic dysfunction plays a central role in the pathology of OCa. Atypical modification of histones and dysregulated expression of histone-modifying

enzymes have been found in OCa. G9a, a histone methyltransferase for lysine 9 of histone 3 (H3K9), was originally identified as a key histone methyltransferase (HMT) that mediates euchromatin gene silencing and is essential for early embryogenesis through regulating developmental gene expression. G9a has since been found to cooperate with transcription factors to regulate gene expression, and G9a-dependent H3K9 methylations have been shown to mediate epigenetic silencing of several tumor suppressor genes including DSC3, MASPIN, andCDH1. In the present study, the association between histone methyltransferase G9a and ovarian cancer (OCa) progression was investigated.

Material and method: The clinicopathological correlation of G9a expression was assessed in tumor specimens of ovarian cancer patients. Knockdown or overexpression of G9a in ovarian cancer cell lines was analysed with regard to its effect on adhesion, migration, invasion and anoikis-resistance. In vivo biological functions of G9a were tested by i.p. xenograft ovarian cancer models. Microarray and quantitative RT-PCR were used to analyze G9a-regulated downstream target genes.

Result and discussion. G9a was expressed in OCa cell lines and tumor tissue. A significant correlation between G9a expression and patient survival was observed. Repression of G9a expression suppressed peritoneal metastasis by inhibiting several critical steps required for peritoneal dissemination, including anoikis-resistance, adhesion, migration and invasion. G9a depletion significantly attenuated the development of ascites and tumor nodules in a peritoneal dissemination model of OCa. Importantly, microarray analysis of G9a-depleted cells revealed that G9a regulates a cohort of anti-metastatic genes, including DUSP5, SPRY4 and PPP1R15A in OCa.

Conclusion. G9a is a pro-metastatic histone methyltransferase in OCa that acts at multiple steps in the peritoneal dissemination cascade by repressing a cohort of anti-metastatic genes. G9a may be an attractive target for therapeutic intervention in advanced OCa.

No conflict of interest.

196 Smad7 knockdown-induced colon cancer cell growth inhibition is mediated by protein kinase RNA-driven eIF2a hyperphosphorylation

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Background. Up-regulation of Smad7, an inhibitor of Transforming Growth Factor (TGF)-b1, occurs in sporadic colorectal cancer (CRC) and knockdown of Smad7 inhibits CRC cell growth, a phenomenon that associates with decreased expression of CDC25A and arrest of cells in S-phase of the cell cycle. These findings were observed in CRC cells unresponsive to TGF-b1 thus suggesting that Smad7 controls CRC cell growth through a TGF-b1-independent mechanism.

Aim. To investigate the molecular mechanisms by which Smad7 controls CRC cell growth.

Methods. Phosphorylation of eIF2α, an attenuator of CDC25A protein synthesis, was evaluated in CRC cell lines (i.e. HCT116 and DLD-1) either untreated or treated with Smad7 sense or antisense (AS) oligonucleotide, by Western blotting and immunofluorescence. Activation of up-stream kinases, which control eIF2α phosphorylation [(i.e. Protein Kinase RNA (PKR), GCN2 (General Control Non-derepressible 2) and PERK (Protein kinase RNA-like Endoplasmic Reticulum Kinase)], was assessed by Western blotting using commercial antibodies that recognize active forms of such proteins. EIF2α phosphorylation was also evaluated in CRC cells treated with Smad7 AS in the presence or absence of PKR inhibitor. Since eIF2α phosphorylation is also controlled by the complex GADD34 (growth arrest and DNA damage protein)/protein phosphatase-1 (PP1), and it is known that GADD34 can interact with Smad7 in other systems, by immunoprecipitation and immunoblotting we finally evaluated whether, in CRC cells, Smad7 interacts with GADD34/PP1 and knockdown of Smad7 prevents interaction of GADD34/PP1 with eIF2α.

Results. Inhibition of Smad7 increased eIF2α phosphorylation and this was preceded by activation of PKR but not of GCN2 and PERK. Inhibition of PKR activation reduced but not abolished Smad7 AS-induced eIF2α phosphorylation, thus suggesting the existence of further mechanisms that control eIF2α phosphorylation. Indeed, in CRC cell lines, Smad7 was co-expressed and interacted with PP1 and GADD34 and Smad7 knockdown inhibited association of GADD34/PP1 with eIF2α.

Conclusions. Data indicate that Smad7 is a key regulator of multiple steps regulating cell cycle in CRC cells and reveal a novel TGF-beta independent role of Smad7 in the control of CRC cell growth.

No conflict of interest.

197 A role for 6-phosphofructo-2-kinase in the epithelial-mesenchymal transition of tumor cells

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Introduction. An epithelial-mesenchymal transition (EMT) is a biologic process that allows epithelial cells to undergo multiple biochemical changes that enable them to assume a mesenhcymal cell phenotype. Induction of an EMT program is associated with increased expression levels of several transcription factors including Snail, Slug, and ZEB1, which are powerful repressors of the gene encoding E-cadherin (CDH1), an established marker for epithelial cells. A subtype of the EMT process that is specific to carcinoma cells is recognized as a key inducer of invasiveness, stemmness, and resistance to apoptosis and chemotherapeutics. The family of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) comprise four bifunctional isozymes which are well established regulators of glycolysis via their synthesis of fructose-2,6-bisphosphate (F2,6BP), a potent allosteric activator of 6-phosphofructo-1-kinase (PFK1). Given that PFKFB3 is induced by HIF-1α and and Akt, and suppressed by PTEN, which are established regulators of EMT, we hypothesized that PFKFB3 may be involved in the regulation of the EMT program in tumor cells.

Material and Method. Silencing of PFKFB3 was achieved using siRNA molecules. Localization of E-caderin was assessed by confocal microscopy. mRNA and protein expressions of EMT markers were determined by Real-time qPCR and Western blot, respectively. Invasion assay was performed on Boyden chambers.

Results and Discussion. Silencing of PFKFB3 in HeLa, HCT116 and PANC1 cell lines led to marked decreases in expressions of Snail, ZEB1, and vimentin. Increased E-cadherin expression on the cell membrane was visualized upon PFKFB3 depletion. Intriguingly however, depletion of PFKFB3 was associated with increased expression of Slug. We next tested the effect of PFKFB3 on in vitro invasion and found that PFKFB3 knockdown almost completely prevented the invasion of A549 cells. Taken together, these data indicate that PFKFB3 promotes the EMT of tumor cells and contributes to traits such as invasion associated with high-grade malignancy. We then analyzed the effect of recombinant TGF-β1, a master regulator of EMT, on the expression levels of PFKFB isoforms in PANC1 and A549 cells, and found that PFKFB3 and PFKFB4 mRNA expressions were markedly induced by TGF-β1 in PANC1 and A549 cells, respectively (fold-changes compared to control: PFKFB3, 2.5±0.5 in PANC1; PFKFB4, 4.6±1.0 in A549). We also noticed that the induction kinetic of PFKFB3 by TGF-β1 exhibited a similar kinetic to that of Snail, a well-known target for TGF-β1. We further showed that the steady-state level of PFKFB3 was required for the full induction of Snail by TGF-β1.

Conclusion.PFKFB3 is a novel regulator of the EMT and may serve a role in TGF-β1-induced malignant traits. This study was supported by The Scientific and Technological Research Council of Turkey - TUBITAK (#113Z776).

No conflict of interest.

198 Suppression of PPARß, or DHA treatment, inhibit NaV1.5 and NHE-1 proinvasive activities

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Introduction. Peroxisome proliferator-activated receptor β (PPARβ) and NaV1.5 voltage-gated sodium channels have independently been shown to regulate human breast cancer cell invasiveness. The n-3 polyunsaturated docosahexaenoic acid (DHA, 22:6n-3), a natural ligand of PPAR, is effective in increasing survival and chemotherapy efficacy in breast cancer patient with metastasis. DHA reduces breast cancer cell invasiveness and it also inhibits PPARβ expression. We have shown previously that NaV1.5 promotes MDA-MB-231 breast cancer cell invasiveness by potentiating the activity of Na+/H+ exchanger type 1 (NHE-1), the major regulator of H+ efflux in these cells.

Material and method. Human breast carcinoma cell line MDA-MB-231 was cultured in DMEM Medium supplemented with 5% fetal calf serum. Cell migration and invasiveness were analyzed in 24-well plates receiving cell culture inserts of polyethylene terephtalate membranes of 8 µm pore size, covered (invasion), or not (migration), with a film of Matrigel™ matrix. Lipids were extracted following the Bligh and Dyer method and total phospholipids were purified by thin layer chromatography and transmethylated to produce fatty acid methyl esters that were separated, identified and quantified by gas chromatography. Reagents used were: PPARβ antagonist GSK0660 dissolved in 100% DMSO, DHA methyl ester (22:6n-3), oleic acid methyl ester (OA; 18:9n-1), siRNA (sc-37007 for siCTL, sc-36305 for siPPARβ), shCTL (sc-108080), shPPARβ (sc-36305-V), NaV1.5 antibodies (S0819), PPARβ antibodies (sc-7197).

Results and Discussion. We report here that DHA inhibited NaV1.5 current and NHE-1 activity in human breast cancer cells, and in turn reduced NaV1.5-dependent cancer cell invasiveness. For the first time, we show that antagonizing PPARβ, or inhibiting its expression, reduced NaV1.5 mRNA and protein expression and NaV1.5 current, as well as NHE-1 activity and cell invasiveness. Consistent with these results, the DHA-induced reduction of both NaV1.5 expression and NHE-1 activity was abolished in cancer cells knocked-down for the expression of PPARβ (shPPARβ).

Conclusion. The present study demonstrates a direct link between the inhibition of PPARβ expression and the inhibition of NaV1.5/NHE-1 activities and breast cancer cell invasiveness. This provides new mechanistic data advocating for the use of natural fatty acids such as DHA to block the development of breast cancer metastases.

No conflict of interest.

199 Colon-derived tumor initiating cells display an altered expression and function of the pro-apoptotic kinase HIPK2

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HIPK2 is an evolutionarily conserved kinase involved in the regulation of cell survival and proliferation during development and in response to genotoxic damage. HIPK2 phosphorylates an extensive variety of targets, including transcriptional regulators and signal transducers. In the DNA damage response (DDR), HIPK2 modulates the activity of several proteins directly or indirectly related to apoptosis, including the

tumor suppressor p53. Reduction of HIPK2 expression was shown to impair apoptosis and/or proliferation and induce resistance to different anticancer treatments. Besides, a few mechanisms of HIPK2 dis-regulation have been identified in human cancers, suggesting that HIPK2 may act as a tumor suppressor.

A growing body of evidence is supporting the idea that malignancies originate from a small fraction of cells endowed with self-renewal ability defined as tumor initiating cells (TICs) that are capable of initiating tumor growth and are responsible for resistance to therapy. We observed that, if compared to several colon cancer cell lines, colon TICs exhibit aberrant expression of HIPK2 isoforms and display an impaired HIPK2 response to DNA damage. Moreover, in the context of a mutational screening, two extremely conserved adjacent aminoacids of HIPK2, a phosphorylated site and an ubiquitinated site, have been found mutated in colon TICs. The characterization of these HIPK2 mutants is currently ongoing. Preliminary results indicate that both mutants display an increased stability but a reduced pro-apoptotic activity when overexpressed in cancer cell lines. Future experiments are aimed at verifying whether HIPK2 defects may have a causative role and could be considered as predictive markers in colon cancers.

No conflict of interest.

201 Serum biomarkers identification by nanoparticle technology in Giant Cell Tumor of Bone

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Introduction. Giant Cell Tumour (GCT), is a benign bone tumour that has a relatively high local aggressiveness, while development of lung metastases is a rare event, occurring in 2%-5% of cases. Since proteomic studies can early detect the malignant phenotype and identify specifically expressed molecules and/or deregulated pathways, we conducted a protein study with the aim of selecting candidate circulating biomarkers differentiating GCT patients from healthy donors and to assess their prognostic role in a series of GCT with different progression of disease.

Material and Method. Proteomic technologies are used for global profiling and identification of disease-associated markers in biological fluids, such as serum. The low-molecular-weight proteome (<30 kDa) is considered as a rich source of new potential biomarkers, that often can escape the detection because of the presence of thousands of very abundant proteins in serum. In order to determine whether low-abundant serum proteins can be measured and useful as new markers predictive of aggressive behavior, we analyzed twelve patients who developed lung metastasis and nine who remained disease free for a minimum of 3 years using poly(N-isopropylacrylamide-co-vinylsulfonic acid) hydrogel core-shell nanoparticles with incorporated Cibacron Blue F3G-A, that selectively entrap low molecular weight proteins. A set of ten sera by healthy donors were used as control.

Results. Circulating protein expression profiling identified differentially abundant candidate peptidome biomarkers that appear to be specific for detection of GCT, revealing the potential utility for this methodology. In fact, the level of a panel of circulating proteins was differentially expressed between tumor and control groups ($p<0.005$) and statistical analysis are now ongoing. The preliminary results indicates Clathrin heavy chain 1, Alpha enolase, Serum amyloid A 4 protein and Fibrinogen beta chain as potential circulating markers for GCT.

The prognostic significance and the evaluation of clinical impact of these proteins will be validated by western blotting and by Tissue Microarray on a wider cohort of paraffin-embedded sections of primary GCT samples.

Conclusions. The statistical correlation between protein expression and clinical parameters could define a proteomic profile useful to discovery diagnostic and prognostic factors helpful in identifying higher risk GCT patients for closer follow-up and medical adjuvant therapy.

No conflict of interest.

202 Studying the contribution of putative platinum drug uptake channels as drug resistance mechanism in Brca1-mutated mammary tumor cells

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Introduction. The use of platinum-based chemotherapy has recently been shown as effective treatment for BRCA1/2-mutated breast cancer. Unfortunately, despite good initial responses, many patients are not cured and eventually develop drug resistance. Although restoration of BRCA1/2 function due to secondary mutations has been identified to cause clinical drug resistance, this does not explain all cases of resistance. Here, we investigated the relevance of specific membrane proteins on platinum uptake and drug resistance. In particular we hypothesize that genetic depletion of candidates will reduce the platinum hypersensitivity of Brca1/2-deficient mouse mammary tumors.

Materials and Methods. A genome-wide loss-of-function screen using insertional mutagenesis was performed in haploid KBM7 cells to select cells resistant to 7μM carboplatin. Deep sequencing and mapping of gene trap insertions before and after carboplatin selection were performed to identify genes more frequently mutated in carboplatin-resistant cells. To validate the effect of these gene depletions, independent gene knockouts were introduced in the haploid KBM7 and HAP1 cells as well as in p53-/-, p53-/-;Brca1-/-, and p53-/-;Brca2-/- mammary tumor cells. For this purpose, CRISPR/Cas9-mediated gene editing was carried out in these cell lines.

Results and discussion. In our carboplatin resistance screen we identified a significant increase of insertions in genes that encode subunits of the volume-regulated anion channel. Validation of these hits using various independently mutated KBM7 or HAP1 clones yielded a 3-4 fold increase in the carboplatin and cisplatin IC50. This correlated with a decrease in intracellular Pt concentrations early after treatment. Like in the haploid cells, we found that the genes of interest are also expressed in the platinum drug hypersensitive Brca1/2-deficient mouse mammary tumor cells. In addition to the absence of functional BRCA1/2-associated DNA repair, the presence of these membrane proteins may be required for the observed platinum drug efficacy. To test this hypothesis, we introduced specific gene deletions in the Brca1/2-mutated cells, and we will present their effect on drug sensitivity.

Conclusion. Loss-of-function genomic screens using haploid cells and target validation by CRISPR/Cas9-mediated gene editing yielded components of the volume-regulated anion channel as mediators of platinum drug resistance.

No conflict of interest.

203 Extracellular superoxide dismutase supports tumorigenesis through autocrine-paracrine switch

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Introduction. Mesenchymal stromal cells (MSC) are a major component of tumor stromal microenvironment. Elevated oxidative stress in tumor stroma may influence MSC metabolic pathways supporting carcinogenesis. Even though reactive oxygen species (ROS) are essentially involved in stroma supported cancer growth the sources of ROS are not well characterized. Extracellular superoxide dismutase (SOD3), which catalyzes the dismutation of superoxide anion to hydrogen peroxide at cell membranes, regulates RAS-ERK1/2 signal transduction and the cellular growth in a dose-dependent manner. At low expression levels SOD3 stimulates non-transformed and transformed cell proliferation. In the current work we studied the effect of MSC secreted SOD3 on cancer cell growth and migration.

Materials and Methods. We isolated primary mesenchymal stromal cells from papillary thyroid cancer patients. As control cells we used MSCs isolated from the healthy part of the thyroid tissue. As cancer cells we used papillary thyroid cancer TPC1 cell line. The phenotypic and functional characteristics were studied using flow cytometry, differentiation assays, qRT-PCR, protoarray, Western blotting, matrigel migration, lentiviral SOD3 over-expression, lentiviral SOD3 RNAi, BrdU DNA replication assay, and growth curve analysis.

Results and discussion. According to the differentiation and flow cytometry assays the stromal cells isolated from thyroid and papillary thyroid cancer showed mesenchymal stem cell phenotype. The expression assays suggested higher expression of redox enzyme SOD3 in cancer MSCs as compared to normal thyroid MSCs suggesting autocrine-paracrine transfer of SOD3 during tumorigenesis. Migration assay showed that papillary thyroid TPC1 cancer cells had higher migration potential towards normal thyroid MSCs than towards papillary thyroid cancer MSCs. Growth analysis indicated that normal MSCs reduced cancer cell proliferation where as cancer derived MSCs stimulated the growth. Moderate SOD3 over-expression in normal thyroid MSCs mimicked the growth stimulatory effect of cancer MSCs. In line, SOD3 RNAi in cancer derived MSCs reduced their ability to support cancer cell proliferation therefore suggesting that stromal cell derived SOD3 supports carcinogenesis.

Conclusions. Based on our data mesenchymal stromal cell derived SOD3 is a paracrine factor influencing cancer cell migration and proliferation. Therefore, SOD3 is a potential drug target to reduce tumorigenesis.

No conflict of interest.

204 SOX2 and Hedgehog signaling: Partners in melanoma tumorigenesis

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Introduction. Melanoma is an aggressive skin cancer, marked by heterogeneity and resistance to chemotherapy. A subpopulation of cells with a stemness signature has been identified in melanoma. These Melanoma-initiating cells (MICs) are characterized by unlimited self-renewal, multilineage differentiation and high tumorigenicity. Recent findings show that the Hedgehog (HH) signaling is required for melanoma growth and for MIC self-renewal. HH signaling has been shown to regulate the expression of stemness genes, including SOX2. In melanoma, SOX2 is critical for the maintenance and survival of MICs. Here we address the interplay between SOX2 and HH pathway in melanoma and a possible approach to target SOX2 using Gentian violet®, that has been recently proposed to treat cutaneous melanoma metastases.

Material and method. MICs were enriched by tumor sphere assay. Modulation of HH signaling was performed by silencing of GLI1 and SMO (HH inhibition) and of PTCH1 (HH activation). Functional studies of SOX2 were performed by stable overexpression and knockdown using lentiviral vectors.

Result and Discussion. We find that HH pathway regulates SOX2 expression and that SOX2 acts as an important mediator of HH signaling in controlling melanoma cell proliferation and survival, as well as MIC self-renewal. In turn, SOX2 regulates HH signaling. We detect putative SOX2-binding sites within the core proximal promoter region of GLI1, the downstream effector of HH pathway, and we show that SOX2 directly regulates GLI1 by binding to its promoter in melanoma cells. Our results highlight the existence of a positive regulatory loop between HH and SOX2 and provide evidences for the importance of SOX2 as a therapeutic target in melanoma. Gentian violetâ significantly inhibits melanoma cell proliferation, viability and MIC self-renewal by selectively affecting the expression of SOX2, but not that of the other stem-specific transcription factors OCT4 and KLF4.

Conclusion. Our data highlight the role of SOX2 as a mediator of HH signaling in melanoma and MICs. In addition, SOX2 directly regulates HH signaling, contributing to create a HH-GLI-SOX2 regulatory loop in melanoma. We suggest Gentian violet ® as a potent inhibitor of melanoma cell growth and MIC self-renewal acting, at least in part, by selective targeting of SOX2. Taken together these findings identify SOX2 as a potentially therapeutic target in melanoma and MICs.

No conflict of interest.

205 A potential novel metabolic symbiosis between acidic mesenchymal stem cells and melanoma cells

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Introdution. Mesenchymal stem cells (MSC) are recruited into developing tumor stroma and several evidence suggests that they play a role in facilitating cancer progression, promoting an epithelial-to-mesenchymal transition (EMT) program and a metastatic phenotype. Thus, understanding MSC-tumor cell interactions is required to determine their role in tumor progression and disclose new therapies.

MSC-tumor cell interaction could probably occurs in a low pH regions of tumor stroma, resulting from glycolysis pathway adopted by tumor cells even in a plenty of oxygen tension (Warburg effect).

The aim of this study is to elucidate whether acidity affects the metabolic profile of MSC, suggesting a metabolic symbiosis with tumor cells.

Methods MSC were cultivated in acidic (pH 6.7) or non-acidic medium. 24 hour after, media were removed and MSC grown in standard medium for additional 24 hours, which represent conditioned media used to grow human melanoma cells. Analysis of metabolic pathways, EMT markers and drug exposure were performed in MSC and melanoma cells.

Results We found that MSC exposed to an acidic medium change their metabolic profile to oxidative phosphorylation (OxPhos). Indeed, they reduce glucose transporter (GLUT) 1 and 3 expression, up-regulate ratio between monocarboxylate transporter (MCT) 1 and 4, reduce lactate dehydrogenase A (LDH-A) and PKM2 expression. Reduction of c-Myc and p-Akt transcription factors are other indicators of glycolysis inhibition in acidic MSC. Melanoma cells grown in low pH-MSC conditioned medium express a mesenchymal phenotype with an up-regulation of GLUT 1 and 3, reduction of MCT1 and increased levels of MCT4, LDH-A and p-Akt transcription factor. Metformin, a well-known antidiabetic drug also used in tumors, when added to the acidic medium used to grow MSC, reverts the induced OxPhos metabolism and melanoma cells exposed to conditioned medium fail to express an up-regulation of glycolysis and EMT markers.

Conclusions Acidity promotes in MSC a state of activation instrumental of EMT induction in melanoma cells associated with an OxPhos profile in MSC and an up-regulation of glycolysis in melanoma cells. It is possible that acidic MSC switch to OxPhos in order to remove lactic acid generated by cancer cells, attenuate acidosis and support a glycolysis-dependent proliferative activity of tumor cells. This possible metabolic symbiosis might sustain proliferation of tumor cells even when tumor microenvironment becomes acidic.

No conflict of interest.

206 Biological evaluation of MRT-92, a novel antagonist of the SMOOTHENED receptor

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Background – The SMOOTHENED (SMO) receptor is an essential component of the Hedgehog (HH) signalling pathway that plays a critical role in embryonic development and patterning, in preserving stem cell compartment as well as in tissue repair and maintenance. Several studies have highlighted the role of the Hedgehog pathway also in different types of human cancer. In this scenario SMO antagonists draw particular attention for their potential therapeutic value. Acylguanidine derivatives recently developed by our group emerged as interesting SMO inhibitors. MRT-92, a member of this class of molecules, has been recently demonstrated to bind to the 7-transmembrane domain of SMO. Here we show that MRT-92 drastically reduces growth of a number of commercial and patient-derived cancer cell lines and attenuates the activity of the HH pathway.

Materials and Methods. – A ligand-based pharmacophore model was built starting from a set of active compounds and it was used to perform a virtual screening of commercial libraries. A set of putative SMO antagonists was identified and MRT-92 was selected. Human patient-derived and commercial tumour cell lines of different origin were treated with increasing doses of MRT-92 at different time points. Dose-response curves were carried out to assess the efficacy of MRT-92 in terms of viability. Western blot and quantitative real-time PCR were performed to determine the expression of GLI1, the readout of HH pathway activation. HH pathway was inhibited by knocking down SMO by means of specific short interference RNA (shRNA).

Results and Discussion. – A significant dose- and time-dependent reduction in viability of melanoma, medulloblastoma, breast and prostate cancer cell lines was obtained after treatment with MRT-92 at nanomolar doses. Accordingly, GLI1 mRNA and protein levels were significantly reduced after MRT-92 administration. MRT-92 had no significant effect on the growth of cancer cells silenced for SMO. All together these data confirm that MRT-92 acts on SMO receptor, negatively modulating the HH pathway.

Conclusions – MRT-92 appears to be a good candidate to negatively modulate HH pathway and, as a consequence, to attenuate growth of HH-dependent human cancers. Further studies are needed to elucidate the mechanism underlying cell death following MRT-92 treatment in order to optimize its formulation for therapeutic application.

No conflict of interest.

208 A novel small molecule IMP1338 induces apoptosis of human cancer cells by inducing p53-independent- S and G2/M cell cycle arrest

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Introduction. Although a target-specific based screening takes obvious advantages to help to identify novel anticancer agents, a phenotypic screen is still the mainstay of drug development. We aimed to identify novel small-molecules that that increase cancer cell death by a phenotypic screening using cell-based viability assay system. In this study, we suggest that IMP1338 could be a potent anticancer agent in human cancer cells.

Materials and Methods. To identify small molecules that inhibit cancer cell proliferation in HCT116 human colon cancer cells and A549 human lung cancer cells, we screened a chemical library containing 2,080 compounds using cell viability assay system with Cell Counting Kit-8 (CCK-8) in a 96-well plate format. A potent hit was validated by cell cycle distribution, Annexin V/PI staining and immunoblotting.

Results and Discussion. Using a cell based viability screen of 2,080 compounds, we found a novel acetamide derivative, N-(4-{[(9,10-Dioxo-9,10-dihydro-1-anthracenyl)sulfonyl]amino}phenyl)-N-methylacetamide (designated IMP1338), significantly inhibit of HCT116 and A549 cancer cells in a dose-dependent manner. Moreover, treatment of IMP1338 selectively inhibits HCT116, HT29 and A549 cancer cell proliferation compared to Beas2B normal like epithelial cells. To understand the cellular mechanism of IMP1338, we examined the effect of IMP1338 on cell cycle distribution and cell death in cancer cells. IMP1338 treatment significantly arrested at S and G2/M phases through DNA damage response and led to apoptotic cell death, which determined by FACS analysis using Annexin V/PI double staining and increased caspase-3 and PARP-1 cleavages by immunoblotting in wild-type p53 (p53+/+) and p53 knockout (p53-/-) HCT116 human colon cancer cells. A marked induction of phosphorylation of ATM, histone H2AX and Chk1 was observed after treatment of IMP1338 in the both cell lines. In addition, combination treatment with IMP1338 and 5- Fluorouracil (5-FU) inhibited cell viability of p53+/+ and p53-/- HCT116 cells, compared to 5-FU alone.

Conclusion. Our findings indicated that IMP1338 induced p53-independent apoptosis through S and G2/M phases arrest with DNA damage response. To assess its potential as a promising anticancer agent requires further investigation.

No conflict of interest.

209 The chemokines GRO-a and IL-8 secreted form omentum promote aggressiveness of ovarian cancer cells

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Introduction. Metastatic cancer progression is the major cause of the high mortality of ovarian cancer. Omental metastasis is the most common route in ovarian cancer metastatic progression. Our previous studies have found that TAK1/NFκB signaling cascade is required for ovarian cancer cell aggressiveness in omental metastasis. Here, we further reported that the chemokines secreted from omentum promote the aggressiveness of ovarian cancer by activating TAK1/NFκB signaling cascade.

Material and method. Omentum condition media (OCMs) were prepared by incubating fresh human normal or cancerous omentum tissues in culture medium. Chemokine array profiling was conducted to identify chemokines secreted from omental tissues. Effects of OCMs and chemokines found in OCMs on ovarian cancer cell lines were studied by functional assays, western blots and NF-κB luciferase reporter assays. A pair of ovarian cancer cells isolated from a patient's ovaries and omentum was included to investigate the differential effects of OCMs and chemokines towards primary and metastatic ovarian cancer cells. TAK1 inhibitor, (5Z)-7-oxozeaenol, was used to study the effects of OCMs and chemokines in activation of TAK1.

Results and discussion. We found that OCMs could significantly promote ovarian cancer cell migration, invasion and soft-agar colony formation. The oncogenic effect was stronger in metastatic ovarian cancer cells than primary ovarian cancer cells,

indicating that OCMs contain oncogenic factors enhancing the aggressiveness of metastatic ovarian cancer cells. Chemokine array profiling revealed that chemerin, growth-regulated oncogene α (GRO-α) and interleukin 8 (IL-8) were remarkably upregulated in OCMs. Functionally, GRO-α and IL-8 but not chemerin promoted the similar oncogenic effects as OCMs on ovarian cancer cells, while such effects were stronger in metastatic ovarian cancer cells. Moreover, OCMs and the two chemokines remarkably elevated NF-κB reporter luciferase activity, as well as the expression levels of p-TAK1S412, p-IKK and p-IκBα. However, co-treatment of TAK1 inhibitor (5Z)-7-oxozeaenol abrogated the oncogenic effects of OCMs and both chemokines in ovarian cancer cells, suggesting GRO-α and IL-8 secreted from omentum play as activators of TAK1/NFκB pathway in ovarian cancer cells.

Conclusion. GRO-α and IL-8 are the dominant chemokines secreted from omentum tissues for promoting ovarian cancer cell aggressiveness by activating TAK1/NFκB pathway.

No conflict of interest.

210 Role of microRNAs in regulating Met protein expression and invasiveness in papillary thyroid carcinoma

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Purpose: MET, the tyrosine kinase receptor for hepatocyte growth factor, is frequently overexpressed in papillary thyroid carcinoma, however, the MET regulatory mechanism is not well known. A new mechanism of regulation of protein involves MicroRNAs, a class of small noncoding RNAs. Recent studies identified five miRNAs (miR-34b, miR-34c, miR-1, miR-199a and miR-410), which negatively regulate the expression of MET. In this study, we aimed to evaluate the possible role of these miRNAs in regulating Met protein expression and invasiveness in papillary thyroid carcinoma.

Experimental design: the expression of Met, miR-34b, miR-34c, miR-1, miR-199a and miR-410 was evaluated by Real Time PCR in 18 matched pairs of laser microdissected frozen tissues of PTC and nontumoral surrounding tissue. The expression levels of miRNAs were also evaluated on one cell lines of PTC (K1) and on primary cultures of PTC. The biological role of miR-34b, miR-34c, miR-1, miR-199a and miR-410 in controlling MET expression and biological activity was assessed in K1 cell line either by their forced expression with synthetic premiRNAs or by AntagomiR-mediated inhibition. MET mRNA levels in transfected cells were evaluated by real-time PCR and protein expression by Western blot and immunocytochemistry. The role of miRNAs in influencing K1 biological activity was evaluated testing invasion invasive, adhesive and proliferative tumor cells ability in vitro.

Results and **Conclusions.** all the analyzed miRNAs were downregulated in the most cases of tumor tissue respectively, miR-1 and miR-199a were dowregulated in 17/18 cases (94%), miR34c and miR34b in 5/18 cases (28%) and miR410 in 5/6 cases (83%). Consistent with a suppressive role of these miRNAs their forced in vitro expression in TPC cancer cells reduced MET RNA and protein levels and impaired MET-induced invasive capacity. Our findings suggest a possible role of the investigated miRNAs, as a tumor suppressor in human papillary carcinoma of the thyroid by controlling MET expression.

No conflict of interest.

211 OXPHOS inhibition and pentose phosphate pathway induction are early events priming preneoplastic lesions towards HCC development

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Introduction. A shift towards Warburg metabolism in which aerobic glycolysis is increased has long been associated with cancer cell transformation. However, whether the switch from OXPHOS to glycolysis can occur at early stages of cancer development in hepatocellular carcinoma (HCC) remains elusive.

Material and method. Hepatocarcinogenesis can be precisely monitored in the Resistant-Hepatocyte (R-H) rat model, from preneoplastic lesions formation to HCC. Notably, it is established that the highly proliferating KRT19+ preneoplastic lesions are the HCC precursor. Metabolic characterization of R-H rat lesions or cells derived from normal, pre- and neoplastic rat tissues, were performed using an array of different biochemical techniques.

Results and discussion. Here we show that Warburg phenotype acquisition is a very early event in rat HCC development. Preneoplastic lesions showed a concomitant increase in glycolytic activity and inhibition of OXPHOS, revealed by the inhibition of succinate dehydrogenase (SDH) by the chaperone tumor necrosis factor receptor-associated protein 1 (TRAP1) and an increase in the expression and activity of citrate synthase (CS). Additionally, their metabolism is reprogrammed towards the Pentose Phosphate Pathway (PPP) as indicated by enhanced expression and activity of glucose-6-phosphate dehydrogenase (G6PD). In keeping with PPP induction, we also observed increased CS expression and activity, inhibition of pyruvate kinase activity, due to oxidation of the isoform M2 and induction of the fructose-bis-phosphatase 2 enzyme, TIGAR. G6PD increased expression was observed exclusively in KRT19+ preneoplastic lesions and was associated with low levels of miR-1, a miRNA known to target G6PD. Accordingly, ectopic miR-1 transduction reduces G6PD expression in HCC cells. PPP induction is considered one of the mechanisms by which deregulated NRF2-KEAP1 signaling promotes cellular proliferation and tumorigenesis. We previously demonstrated that KRT19+ preneoplastic lesions show a sustained NRF2/KEAP1 pathway activation. Crucially, NRF2 silencing in HCC cells decreases G6PD and increases miR-1 expression, consequently inhibiting PPP and PKM2 oxidation. Finally, an inverse correlation between miR-1 and its target G6PD was found in a cohort of human HCC patients.

Conclusion. Our results demonstrate that Warburg metabolic deregulation and PPP induction are early events in HCC development. Crucially, TRAP1 and NRF2 are key regulators of this metabolic reprogramming in preneoplastic hepatocytes

No conflict of interest.

212 CXCR4 role in ovarian cancer dissemination

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Introduction. Epithelial ovarian cancer presents a 29% survival rate at five years of advanced disease. Most patients (80%) have already exhibed disseminated neoplasia in diagnosis indicating how important are movement to the disease progression. The cytokine receptor CXCR4 has been implicated in metastasis for different tumor types. Our aim was to analised the role of CXCR4 in the ovarian carcinogenesis progress.

Material and methods. To assay the in vivo effect of CXCR4 on tumor progression, we have chosen two models of human ovarian tumor orthotopically grown in nude mice : OVA17 ; 50% CXCR4 positive tumor cells and OVA15; 5% CXCR4 positive tumor cells . Both orthotopic models were treated with the CXCR4 inhibitor AMD3100 and the effect on tumoral growth and dissemination was evaluated. Western blot, immunohisto analysis , Real Time PCR and cell cytometry technique were used during this study.

Results. AMD3100 treatment significatively inhibited a 60% of primary tumor growth in OVA17 and 30% in OVA15. Treatment clearly increased apoptosis, decreased vessels number and induced hypoxia. OVA17 implantation produced peritoneal dissemination while we never detected any dissemination nodule in OVA15 models. Blood analysis showed EpCAM/CXCR4 positive circulating tumor cells in OVA17 model in contrast of OVA15 models where we never found them. Treatment with AMD3100 completely blocked dissemination and metastasis, in correlation with the complete block of circulant human EPCAM/CXCR4 positive cells.

Conclusion. Our results showed CXCR4 is playing an important role in ovarian cancer dissemination, on consequence it is a possible agent for the alternative treatment of epithelial ovarian cancer patients expressing CXCR4.

No conflict of interest.

213 The TS3R domain of thrombospondin-1 affects tumor vascularization and improves response to chemotherapy

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Introduction. The tumor microenvironment is a critical determinant of malignant progression and response to therapy. Structural and functional abnormalities of the tumor vessels, stroma and extracellular matrix constitute a barrier to drug delivery and promote drug resistance. There is increasing interest for the development of compounds that act on the tumor microenvironment and particularly on the vasculature, to normalize the tumor architecture and improve the delivery and efficacy of chemotherapy. Our previous studies identified a new antiangiogenic, FGF-2-binding site in the type III repeats domain (TS3R) of thrombospondin-1, a major endogenous inhibitor of angiogenesis. Aim of this study was to investigate the effect of TS3R on tumor growth, vasculature properties and response to chemotherapy.

Materials and Methods. The human ovarian carcinoma cell line A2780-1A9 was transfected to express and secrete the TS3R domain or a truncated form lacking the active site. Tumorigenicity and response to therapy were evaluated by grafting cells in nude mice, treated or not with paclitaxel (10 mg/kg, i.v.) or cisplatin (4 mg/kg, i.v.). Changes in the tumor vasculature were assessed by IHC and drug distribution by HPLC and MALDI imaging mass spectrometry analysis on tumors collected 4h after a single administration of paclitaxel (60 mg/kg, i.v.).

Results and Discussion. Tumor cells expressing the TS3R domain had a decreased tumorigenicity compared to controls. Moreover, TS3R-expressing tumors showed a greater sensitivity to paclitaxel and cisplatin, the standard-of-care drugs for ovarian carcinoma. A truncated form of TS3R lacking the FGF-2 binding site did not affect tumor growth and response to therapy, confirming that inhibition of FGF-2 was responsible for this activity. In vitro, cell response to cytotoxic drugs was not affected by the expression of TS3R, indicating that the domain did not directly sensitize tumor cells to chemotherapy. The TS3R-expressing tumors showed evidence of vasculature reorganization: increased number of CD31 positive vessels and decreased vessel area and diameter. In agreement with the observed vasculature modifications, the concentration of paclitaxel was higher and more homogeneously distributed in tumors expressing TS3R.

Conclusions The TS3R domain of TSP-1 has the potential to affect the tumor microenvironment and improve tumor drug delivery, setting the basis for new therapeutic strategies.

No conflict of interest.

215 The telomeric protein TRF2 regulates tumor angiogenesis by affecting the secretome of cancer cells

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Introduction. The key telomeric protein TRF2, which is at the heart of the molecular events that maintain telomere integrity, is overexpressed in many different types of human cancers and contributes to carcinogenesis in mice. However, very little is known about its exact role in tumorigenesis. Recently, our group showed that a reduced dosage of TRF2 impairs tumorigenicity in the absence of overt activation of DNA damage response (DDR), without promoting any intrinsic anti-proliferative program and in a natural killer (NK)-cell-dependent manner. Notably, our observation of a significantly reduced neovascularization in tumors from TRF2-compromised cells, led us to hypothesize an additional role of TRF2 in the regulation of tumor microenvironment.

Material and Method. In order to demonstrate the role of TRF2 in tumor angiogenesis, the intracellular levels of TRF2 were modulated in tumor cells of different histotypes and the conditioned medium (CM), deriving from these cells, was tested on HUVEC endothelial cells, by evaluating the effects of TRF2 on cell proliferation, migration and invasion. Moreover, the angiogenic potential of the CM was evaluated both in vitro, by vasculogenic mimicry, and in vivo, by matrigel plug assays. Finally, a large-scale analysis of the secretome of tumor cells either overexpressing or interfered for TRF2, will be performed in by applying a targeted high-throughput strategy based on multiplexed luminex XMAP technology.

Results and Discussion. The data so far collected, demonstrate a role of TRF2 in regulating tumor angiogenesis. Indeed, our experiments evidenced a dramatic impairment in endothelial cell proliferation, chemotaxis and vessels formation, in response to CM deriving from tumor cells of different histotypes, interfered for TRF2 by two different shTRF2s. In contrast, TRF2 overexpression produces opposite effects, by favouring the formation of new blood vessels in vivo. Strikingly these effect were not accompanied by an overt telomere deprotection, since were obtained in tumor cells in which telomeres are protected against TRF2 dysfunction offering us the opportunity to exclude any effect due to DDR activation.

Conclusion. This study discovers a new mechanism through which TRF2 favours the tumor progression by positively affecting the surrounding environment. The molecular mechanism through which TRF2 regulates angiogenesis is, at the moment, under investigation.

No conflict of interest.

216 Effects of tumor-secreted miR-9 on human breast fibroblasts

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Introduction. Carcinomas are composed of neoplastic cells admixed with supportive cells, which constitute the tumor stroma. Fibroblasts are one of the most active cell types forming the basic cell component of the extracellular matrix and the interaction with tumor cells promotes their conversion into Cancer-Associated Fibroblasts (CAFs). However, how such conversion occurs is not well understood. There is evidence that cancer-secreted miRNAs may play a crucial role in regulating various cellular components of the tumor microenvironment and participate in cancer metastasis. Since miR-9 resulted up-regulated in human breast cancer and was previously described as a pro-metastatic miRNA, we aim to investigate if tumor-secreted miR-9 participates in the intercellular communication that promotes the conversion of breast normal fibroblasts (NFs) toward a CAF-phenotype.

Methods. To understand whether a different expression of miR-9 could play a role in the conversion of NFs to CAF-phenotype, the expression of mature miR-9 was evaluated by Real-Time PCR in NFs and CAFs. The functional role of miR-9 in NFs has been initially investigated performing migration and invasion assays after transiently transfection with miR-9 or control. To evaluate the functional role of the tumor-secreted miR-9 on NFs, NFs were treated with exosomes isolated from MDA-MB-231 and MCF-7 transiently transfected with miR-9 (exo+miR-9) or control (exo+miR-neg). First, the uptake of miR-9 by recipient NFs was assessed measuring the miR-9 intracellular levels by Real-Time PCR. Second, the effect of the exosome-mediated delivery of miR-9 has been investigated performing migration and invasion assays.

Results. The analysis of the miR-9 expression level in NFs versus CAFs revealed higher levels of the miRNA in CAFs. The overexpression of miR-9 in NFs resulted in enhanced cell motility and invasion. NFs treated with exo+miR-9 showed higher cellular level of miR-9 than the control. Moreover, this internalization resulted in a stronger capability in migration and invasion.

Conclusion. Our data indicate that miR-9, up-regulated in CAFs versus NFs, is secreted by cancer cells and transferred via exosomes to NFs where it enhances cell migration and invasion, thus suggesting a potential involvement in the transition to a CAF-phenotype.

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No conflict of interest.

217 Hypoxia mediates the up-regulation of the plasminogen receptor S100A10 in cancer cells

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Introduction. A key characteristic of a cancer cell is its ability to escape the constraints imposed by the neighbouring cells, invade the surrounding tissue and metastasize to distant sites. During initial tumour development cancer cells adapt to survive in a hypoxic environment as a consequence of their proliferation in conjunction with a restricted blood supply, limiting nutrients and oxygen. The hypoxia response leads to the activation of multiple signalling pathways that promote cancer cell invasion and metastasis. The serine protease plasmin is a key protease that participates in fibrinolysis, extracellular matrix degradation, invasion and angiogenesis (the development of blood vessels). As a component of the annexin A2 heterotetramer (AIIt), S100A10 is an important plasminogen receptor that contributes significantly to plasmin activation at the surface of a number of different cell types, including cancer cells. However, the regulation of S100A10 in cancer cells during hypoxia has not been investigated.

Materials and Methods. I have used several cancer cell lines, namely MDA MB231 breast cancer cells, HT1080 fibrosarcoma cells and A549 lung cancer cells and compared the protein expression levels of S100A10 in normoxic versus hypoxic conditions by western blotting. I have analysed the cell surface expression of S100A10 protein in MDA MB231 cells under normoxic versus hypoxic conditions using immunofluorescence microscopy. I have investigated annexin A2 and S100A10 gene transcription by qRT-PCR in order to discern how S100A10 is regulated resulting in an increased expression of S100A10 during hypoxia. Finally I have investigated the capacity of hypoxic cancer cells to activate plasmin in the presence or absence of S100A10, using S100A10 knockdown cells.

Results and discussion: My results revealed that when exposed to a hypoxic environment there is an increase in transcription of S100A10 gene that resulted in the up-regulation of S100A10 protein expression and the translocation of the AIIt complex to the cell surface. My plasmin assays showed an increase in plasmin activation in the control cancer cells (expressing normal levels of S100A10) which was not observed when cells were depleted of S100A10; these results indicate that S100A10 plays a significant role in the production of plasmin at the surface of cancer cells during hypoxia.

Keywords. Annexin A2 heterotetramer, hypoxia, plasmin, S100A10.

No conflict of interest.

218 The AAA+ ATPase Ruvbl1 is a major regulator of liver metabolism and promotes hepatocellular carcinoma progression through the mTOR pathway

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The AAA+ ATPase Ruvbl1 is overexpressed in several human cancers, including hepatocellular carcinoma (HCC), in which high Ruvbl1 expression correlates with a poor prognosis. A growing body of data from in vitro models show that Ruvbl1 is involved in many cancer related processes, including beta-catenin and c-myc signalling, telomerase and p53 activity, making this protein an attractive target for anti-cancer therapies. However, the actual role played by Ruvbl1 in HCC is largely unknown, and whether it actively participates in the oncogenic transformation and cancer progression in vivo remains speculative. To challenge this questions we realized an hepatocyte-conditional Ruvbl1 hemizygous mouse and evaluated the tumor onset and progression by the DEN model. Ruvbl1 hemizygous mice were obtained by crossing Ruvbl1-floxed with Albumin-Cre mice. The male offspring were subjected to a single i.p. injection of DEN (5mg/kg) to induce liver cancer, and were monitored up to one year after. Conditional liver-hemizygous mice had a nearly 50% reduction of Ruvbl1 protein expression and showed no obvious phenotype with respect to liver size, viability and liver function. However, they had significantly higher glucose, triglycerides and cholesterol serum levels, and an increased body weight. Despite an initial delay in the onset of liver cancer, Ruvbl1+/- mice eventually developed significantly larger tumors than control mice. Expression of Ruvbl1 within the tumor mass was similar in Ruvbl+/- and WT mice. We found that Ruvbl1+/- mice had reduced hepatocyte turnover and impaired mTOR signalling, which likely causes both the metabolic alterations and the delayed onset of HCC. Consistently, silencing Ruvbl1 in HCC cell lines reduced mTOR levels and cell proliferation rate. 2D-proteomic and G.O. analysis highlighted a significant alterations of the carboxylic acids metabolic processes in Ruvbl1 silenced cells. In conclusion, this is the first report describing a role of Ruvbl1 as a major regulator of hepatic metabolism. Contrary to our expectations, we found that although in the hepatocyte-conditional Ruvbl1+/- mice the onset of HCC is delayed, its progression is accelerated. The underlying molecular mechanisms are under investigation, nevertheless this report highlights the potential risks of prolonged Ruvbl1 inhibition in a intact mammalian organism. Acknowledgement: this research is founded by the Italian Ministry of Health through grant GR-2009-1600315.

No conflict of interest.

219 Significance of Nrf2-Keap1 pathway in regulating RCC chemoresistance

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Introduction. Evidence accumulates that the metabolic features of cancer cells are predominately increased the activity of aerobic glycolysis (Warburg effect), which produced high levels of reactive oxygen species (ROS). In addition to its genotoxicity, ROS can also serve as a signaling transduction inducer in regulation of cancer cell proliferation, differentiation and survival through multiple pro-oncogenic signaling pathways. The transcription factor, nuclear factor E2-related factor 2 (Nrf2), regulated by Kelch-like ECH-associated protein 1 (Keap1) is response for combating oxidative and electrophilic stress. In this study, we aimed to determine the role of Nrf2-Keap1 pathway in modulating RCC cell proliferation and chemoresistance.

Material and Method. The transformed human proximal tubular cell line, HK-2, a mouse RCC cell line, Renca and 4 human RCC cell lines were used in this study. Nrf2 and Keap1 knockdown RCC cell lines were established by using lentivirus-based RNA interference technology. Furthermore, 5-Fluorouracil (5-FU) and vincristine were used to testify the ability of chemoresistance.

Results and Discussion. There was less differences of Nrf2 and CUL3 mRNA expression levels between RCC cell lines and HK-2 cell, while Keap1 mRNA was significantly increased in RCC cell lines. The Nrf2-Keap1 pathway regulated genes, such as HO1, Mrp1, Mrp2 and NQO1 were all significantly higher increased than HK-2 cell. The protein expression levels of Nrf2 and HO1 were both expressed higher in RCC cells, in addition, higher Nrf2 expression was presented in both the nuclear and cytoplasmic fractions. Silencing Nrf2 expression suppressed the expression of HO1 and NQO1, while silencing Keap1 increase the expression. However, silencing either Nrf2 of Keap1 had less effect of cell proliferation. Keap1 knock-down cells had increased the resistance to 5-FU and vincristine, while increased the sensitivity in Nrf2 knock-down cells. In addition, silencing Keap1 increase ABCG2 mRNA expression, this may contribute to drug-resistance.

Conclusion. In all, our findings indicate the importance of Nrf2-Keap1 pathway in RCC chemoresistance, and suggest Nrf2 is a potential target for RCC treatment.

No conflict of interest.

221 Tumor-associated macrophages-driven modulation of 5-fluorouracil effects on C26 colon carcinoma cells

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Introduction. Tumor-associated macrophages (TAMs) are pivotal players in tumor progression via modulation of tumor angiogenesis, inflammation, metastasis, and oxidative stress. Moreover, TAMs influence the response of cancer cells to various cytotoxic drugs. Therefore, the aim of the present study was to provide more insight into the impact of the interaction TAMs-C26 murine colon carcinoma cells on the response of C26 cells to 5-fluorouracil (5-FU) treatment.

Material and Method. The anti-proliferative effects of different concentrations of 5-FU ranging from 0.125-16 μM on C26 cells in standard cultures as well as in co-culture with murine peritoneal macrophages were assessed by using BrdU-colorimetric immunoassay. Further on, the impact of the microenvironment generated by TAMs on the response of C26 cells to 5-FU administration was investigated by quantification of oxidative stress markers (HPLC analysis of malondialdehyde levels, spectrophotometric assessment of non-enzymatic antioxidants and catalase activity) and of angiogenic/inflammatory molecules by Western blot and protein array.

Result and discussion. Our results showed that 5-FU inhibited strongly the growth of C26 cells (by 80%) under both culture conditions, compared to controls. Moreover, among all processes responsible for tumor development modulated by TAMs, tumor angiogenesis and inflammation were mainly affected by the interaction of macrophages with C26 cells in the co-culture model. Thus, the expression of most of the angiogenic/inflammatory proteins tested was much higher in the co-culture model compared to their expression in the standard C26 cell culture. However, a TAM-driven chemosensitivity of C26 cancer cells to 5-FU treatment was suggested by significant inhibition of the levels of NF-κB (by 80%) and pro-angiogenic/pro-inflammatory factors (by 44%). Nevertheless, 5-FU administration in the co-culture model activates TAM-mediated production of IL-1ß and TNF-? and inhibits the expression of TIMP-2 and PF-4 that further enhance the metastatic potential of C26 cells.

Conclusion. These results pointed out that TAMs display a dual role in the response of C26 cells to 5-FU administration via modulatory effects on tumor angiogenesis and inflammation. Thus, 5-FU treatments might be combined with therapeutic approaches based on the reduction of TAM-mediated chemoresistance of colon cancer cells to this drug.

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No conflict of interest.

222 LECT2 inhibits the intra-hepatic vascular invasion and metastasis of HCC by directly binding c-Met and disrupting its activation

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Introduction. Leukocyte cell-derived chemotoxin 2 (LECT2) is a multifunctional secreted protein and has been shown to act as a tumor suppressor in hepatocellular carcinoma (HCC). However the corresponding receptor and underlying mechanism remain largely unclear.

Material and c-Methods. LECT2-based affinity chromatography and receptor tyrosine kinase array were used to identify c-Met as the functional targets of LECT2. Co-immunepreciptation and site-directed mutagenesis were used to investigate the activation and binding status between LECT2 and c-Met. The dependency of c-Met on tumor-suppressive effects of LECT2 was determined by cell invasion assays in vitro and orthotopically xenograft mice model in vivo. The clinical correlations between LECT2 expression and c-Met phosphorylation were examined by immunoblotting, Rank correlation test, and Kaplan-Meier survival analysis in HCC specimens.

Results and discussion. LECT2 directly interacted with the α-chain of c-Met and suppressed its phosphorylation and subsequent signal activation through recruiting protein tyrosine phosphatase 1B (PTP1B) instead of competing HGF binding. The antagonistic effect of LECT2 on c-Met activation also contributes to the blockage of vascular invasion and metastasis of HCC in mouse model. Serial deletions and mutations of LECT2 revealed a c-Met-inhibitory motif (HxGxD) within LECT2 protein. The protein expression levels of LECT2 were inversely correlated with c-Met phosphorylation levels and associated with vascular invasion and overall survival in HCC patients.

Conclusion. Our findings reveal a novel and specific inhibitory function of LECT2 in HCC via the direct binding and inactivation of c-Met, opening a potential avenue for treating c-Met-related cancer.

No conflict of interest.

223 Resistance to hormone therapy alters both migration pattern and mechanical properties of breast cancer cells

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Introduction. Breast cancer is considered to be, the most frequent malignancy in women worldwide. The majority of breast tumors (75%), are estrogen receptor positive (ER+) and their growth is stimulated by estrogens. Therapies based on anti-estrogens, represent the mainstay treatment in clinical practice using Fulvestrant (Fulv) and Tamoxifen (Tam) as the key drugs for the manipulation of ER+ breast cancer patients. However, in many patients resistance has been observed, either acquired or de novo that must be addressed. Our aim is to study the profile of breast cancer cells with acquired hormone resistance regarding cell motility and mechanical properties.

Materials and Methods. We developed clones of breast cancer cell line MCF-7, resistant to Fulv and Tam named hereafter as MCF-7/Fulv, MCF-7/Tam. Scanning electron microscopy (SEM) was applied to observe cell morphology and 3D cell culture in soft agar was performed to estimate cells' growth. Furthermore, scratch-wound assay and modified Boyden chamber assay were applied for studying cell migration and invasion, respectively. Micropipette method was used to determine the mechanical properties of cells prior and after resistance to hormone therapy. The evaluated parameters were elastic shear modulus (G) and viscosity (η) that indicate cell stiffness and rheology, respectively.

Results and Discussion. Resistance to endocrine therapy stimulated both clones' growth in 3D soft agar. In addition, the resistant cells exerted increased ability to migrate and invade. More specifically, MCF-7/Fulv were more aggressive regarding migration compared to MCF-7/Tam, whereas MCF-7/Tam found to be more invasive compared to MCF-7/Fulv. Regarding the mechanical parameters of cells, elastic shear modulus was found to be elevated in both resistant clones compared to untreated cells, in accordance with the migration pattern. Furthermore, the viscosity of clones was decreased in MCF-7/Fulv and increased in MCF-7/Tam. SEM analysis was in line with the changes in cell viscosity whereas MCF-7/Fulv demonstrated monolayers while MCF-7/Tam formatted multilayers.

Conclusions. Our data show that the resistance of MCF-7 cells to hormone therapy provoked a more aggressive phenotype and altered their mechanical behavior in a way that favors elevated cell migration, while changes in cell viscosity determined which clone was more aggressive.

No conflict of interest.

224 A novel biomarker for fighting malignant pleural mesothelioma

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Introduction. Malignant pleural mesothelioma (MPM) is a deadly tumor whose treatment remain very challenging. We demonstrated that CD157 GPI-anchored protein is expressed in MPM and predicts poor clinical outcome. In biphasic MPM

histotype, CD157 promotes cell growth, migration, invasion and survival ultimately increasing tumor aggressiveness. In this study we investigated the potential impact of CD157 in the modulation of response to platinum-based therapy.

Materials and Methods. CD157 expression was determined by immunohistochemistry and quantified by histological score (H-score) in tissue sections from surgically resected MPM from patients receiving chemotherapy and compared with survival (n = 41) and with the activation of -mTOR and p70S6K (n = 81). MPM cell line models engineered to overexpress (MSTO) or knockdown (CG98) CD157 were used to investigate the association between CD157 expression and activation of the mTOR pathway and to perform drug efficacy studies by conventional in vitro assays.

Results and Discussion. CD157 influenced survival of patients with biphasic MPM treated postoperatively with platinum-based chemotherapy. Indeed, patients expressing high-levels of CD157 showed a trend toward a shorter survival (albeit not statistically significant, log-rank test p=0.062) than patients with low or absent CD157. In vitro, both CD157-positive CG98 and MSTO cells showed reduced sensitivity to cisplatin and carboplatin treatment compared to the corresponding CD157-negative cells. Moreover, CD157 expression was accompanied by increased activation of the mTOR pathway and phosphorylation of STAT3 transcription factor both in vitro and in vivo. Notably, pretreatment with Rapamycin (a specific mTOR inhibitor) or with Stattic (a specific STAT3 inhibitor) reduced the ability to growth in CD157-positive cells and restored their sensitivity to cisplatin and carboplatin.

Conclusion. Evidence obtained by correlative clinical studies in MPM patients and from in vitro experiments suggests that CD157 is a promising predictive marker of response to platinum-based therapy, potentially useful to select patients that may benefit from particular chemotherapeutic approach that may include mTOR or STAT3 inhibitors.

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No conflict of interest.

225 Resistance to DNA damaging chemotherapy in cancer is associated with specific alterations in the nuclear protein compartment identified by Stable Isotope Labelling of Amino acids in Cell Culture (SILAC) proteomics

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Introduction. Chemotherapy resistance is a major obstacle in successful cancer treatment and adversely affects prognosis. Recent data indicated that non-canonical subcellular localisation of key proteins alters treatment response. The oncogenic serine/threonine kinase, AKT, has been reported by us to be activated by DNAPKcs (PRKDC) in response to DNA damage in the nucleus of DNA damage resistant cancer cells and it's inhibition restores therapeutic response in vitro, in vivo and clinically (Stronach et al, 2011; Blagden et al 2014). Further cytoplasmic/nuclear signalling changes have been observed indicating the importance of nuclear signalling in chemoresistance. We describe here a proteomic approach to capturing novel nuclear signalling changes associated with chemo-resistance.

Method and Materials. Stable isotope labelled populations of intra-patient paired ovarian cancer cell lines PEA1 (platinum sensitive) and PEA2 (resistant) and the chemo-resistant pancreatic cancer cell line Panc-1 were prepared, seeded overnight and treated with cisplatin (25μM) or doxorubicin (1.5μM) for 24 hours with and without an inhibitor of DNA-PK (10μM NU7441; prevents DNA damage induced AKT activation). Treatment and control cell populations were mixed and fractionated to collect nuclear and cytoplasmic fractions for Stable Isotope Labelling of Amino acids in Cell culture (SILAC)/mass spectrometry analysis.

Results. Following established statistical filtering methods, common alterations in protein families were observed in nuclear fractions of drug treated resistant cells. Seven proteins were differentially expressed, some belonging to serine/arginine rich splicing factor protein family (1.3-2.4 fold increase), nuclear ribonucleoprotein family (1.2 fold increase) and DEAD box protein families. Protein changes in PRKDC were also observed in the resistant cell lines, which were absent in the sensitive PEA1 cells.

Conclusion. The application of SILAC proteomics has identified novel protein changes in subcellular compartments that correlate with response/resistance to chemotherapy. Functional validation of identified candidate proteins is underway.

No conflict of interest.

226 Crosstalk between TGF beta and IGF signaling pathways in ovarian cancer orthotopic mouse models

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Introduction. Ovarian cancer is the leading cause of cancer deaths among women. It is generally considered chemosensitive although resistant clones evolve in the majority of the cases; new potential therapies to sidestep this resistance are need as its high mortality rate has not been reduced significantly over the last 30 years. A good candidate is Transforming Growth Factor Beta (TGFβ) signaling pathway as is highly activated in ovarian cancer, independently of tumor type. In addition, treatment against it presented high efficiency on other solid tumors.

Material and Methods. We used different models of human ovarian cancer orthotopically grown in nude mice. Animals implanted with these orthotopic tumors were treated with LY2109761 inhibitor (TGFβRI&II inhibitor) and the effect on tumoral growth and its molecular changes were evaluated. Furthermore, these studies were complemented with in vitro studies using different ovarian cancer cell lines to confirm the in vivo results. In addition, a Tissue Micro Array (TMA) with human ovarian tumor samples from patients was analyzed.

Results and Discussion. Here we show significant tumor size reduction after treatment with TGFβ inhibitor LY2109761 in different ovarian orthotopic mouse models. TGFβ blockage significantly reduces tumor size due to decrease on cell proliferation rate. We describe a crosstalk between TGFβ and Insulin Growth Factor (IGF) signaling pathway in order to control tumor cell proliferation, with a positive regulation of active IGF1R by TGFβ. Treatment with LY2109761 inhibitor downregulate IGF1R levels by post-traductional mechanisms, mainly by changes in its life span. This crosstalk between TGFβ and IGF1R signalling pathways is clinically relevant as IGF inhibitors have been used in clinical ovarian cancer trials.

Conclusion. These results suggest an important role of TGFβ signaling pathway in ovarian tumor cell growth through the control of IGF signaling pathway. Moreover, it elucidates new possible therapy for ovarian cancer patients.

No conflict of interest.

227 SerpinB3 and HIF2a expression in liver cancer cells: Mechanisms and prognostic significance

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Introduction. SerpinB3 (SB3) is a serine-protease inhibitor undetectable in normal liver but overexpressed in hepatocellular carcinoma (HCC) and proposed as an early biomarker of liver carcinogenesis. SB3 can be released as a paracrine mediator and has been proposed to be up-regulated by hypoxia through a HIF2α-dependent mechanism. In this study, we have investigated the selectivity, the mechanism(s) involved in the paracrine action of SB3 and the prognostic relevance of HIF2α and SB3 expression in human HCC.

Materials and Methods. Molecular and cell biology techniques and morphological analysis have been used on normal and genetically manipulated HepG2 cells (to overexpress either SB3, HIF1α or HIF2α, respectively), transgenic mice overexpressing SB3 and liver specimens from a cohort of 67 HCV cirrhotic patients carrying HCC.

Results and Discussion. SB3 up-regulation under hypoxia was selectively abolished only by siRNA versus HIF2α (not HIF1α) with only HIF2α being able to bind to SB3 promoter (ChIP assay). Moreover, up-regulation of SB3 was detected only in HepG2 manipulated to overexpress HIF2α and hypoxic conditions were unable to affect SerpinB4 isoform expression.

Experiments performed to investigate mechanism(s) involved in the paracrine action of SB3 outlined that: i) SB3 can induce stabilization and increased nuclear translocation of HIF2α and up-regulation of HIF2α-related genes in liver cancer cells; ii) increased HIF2α protein levels are unrelated to increased transcription or inhibition of proteasome activity but rather to the induction of HIF2α selective neddylation by NEDD8 and consequent stabilization which is independent on hypoxia. Finally, HIF-2α and SB3 co-localize in liver cancer cells (immunohistochemistry) and a positive correlation ($p<0.01$) exists between HIF-2α and SB3 transcript levels in HCC specimens with the highest levels of HIF-2α and SB3 transcripts being detected in the most aggressive subset of HCC cases and correlating with early tumor recurrence.

Conclusions. In addition to mediate selectively SB3 up-regulation by hypoxia, HIF2α, following neddylation and stabilization, also mediates the action of SB3 when operating as a paracrine mediator on liver cancer cells. Expression of HIF2α and SB3 correlates in HCC specimens and has prognostic implications.

No conflict of interest.

228 Phosphatidylcholine-specific phospholipase C as new target for inhibiting proliferation and survival of tumor initiating cells in squamous carcinoma

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Introduction. Despite progress in clinical oncology, cervical cancer, including the squamous carcinoma, is the second most common cancer among women up to 65 years of age and is the most frequent cause of death from gynecological malignancies worldwide. Therefore, improved targeted therapies and new strategies aimed to increase both drug and radiation sensitivity are essential for reducing the mortality of this malignancy. One emerging model suggests the existence within the tumors of a pool of self-renewing malignant cells (TIC) that can generate the full repertoire of tumor cells. Stemness and metabolic alterations were recently identified as hallmarks of tumor cells, but, at present no direct relation between TIC and altered metabolism has been reported. Different studies have shown that an

abnormal choline phospholipid metabolism is a common feature of cancer cells and we contributed in this field identifying an upmodulation of phosphatidylcholine-specific phospholipase C (PC-PLC) in breast and ovarian cancer. Purpose of this study was to investigate the role of PC-PLC in two adherent (AD) squamous carcinoma cell lines (A431 and CaSki) and in their TICs sub-population, in a model developed in our laboratory of non-adherent spheres (SPH).

Materials and Methods. Expression, activity and overall content of PC-PLC were monitored by confocal laser scanning microscopy, enzyme assays and western blot analyses in all tumoral cell lines, compared to non-tumoral keratinocyte HaCaT cell line. Tricyclodecan-9-yl-xanthogenate (D609) was used as a PC-PLC inhibitor and the proliferation rate, sphere forming efficiency, expression/modulation of stem/differentiation markers were evaluated.

Results and Discussion. Analyses of PC-PLC expression and activity showed that tumor cells expressed high levels of PC-PLC, compared to HaCaT cells. The PC-PLC inhibitor reduced to different extent the proliferation of all the analysed cell lines, exerting a more pronounced cytostatic effect on A431 tumor cells and long lasting consequences on all cell lines. In A431-SPH and CaSki-SPH D609 induced both cytostatic and cytotoxic effects at a 16-fold lower dose. Moreover, treatment of A431-AD and CaSki-AD cells with D609 affected the sphere-forming efficiency, and induced down-modulation of stemness markers mRNA levels (such as Oct4, Nestin, Nanog and ALDH1).

Conclusions. Altogether these results suggest a role for PC-PLC in stem cells maintenance and may open the way to new molecular therapeutic strategies aimed at selective interference with TIC proliferation and survival.

No conflict of interest.

230 Preclinical study of afatinib in esophageal squamous cell carcinoma (ESCC)

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Introduction. Squamous esophageal cancer (ESCC) is endemic to North East Asia and EGFR is commonly overexpressed in this cancer.

Material and method. Five ESCC cell lines, HKESC-1, HKESC-2, KYSE510, SLMT-1, and EC-1, were exposed to increasing concentrations of afatinib and the effect on growth inhibition was determined by MTT assay. The total and phosphorylated forms of HER2, HER3, MAPK, AKT and S6K signaling were determined at basal condition by western blot. The selected cell lines were further evaluated for the change in protein level. Cell cycle and apoptotic effect were evaluated via the flow cytometry and cell death detection ELISA kit.

Result and discussion. ESCC cell lines were sensitive to afatinib with IC50s values ranging from nanomolar to micromolar (72hour incubation: HKESC-1 = 2.3nM, HKESC-2 = 1.8nM, EC-1 = 108nM, KYSE510 = 1090nM and SLMT-1 = 1160nM) with a maximum growth inhibition of over 95%. Basal expression of HER3 was strong in HKESC1, HKESC2 and SLMT-1 while HER2 expression was weak in all cell lines. Meanwhile, the ErbB family downstream effectors such as pMAPK and pAkt were strongly detected in all cell lines.

HKESC-2 (stronger HER2 and HER3 expression) and EC-1 (weakest HER2 and HER3 expression) were sensitive to afatinib and they were selected for the following mechanistic studies. Treatment of afatinib with doses around their IC50 concentrations for 24 and 48 hours resulted in partial inhibition of pMAPK, pAkt and pS6 in both cell lines. The MAPK phosphorylation was strongly suppressed in HKESC-2 after treatment of afatinib, but moderately reduced in EC-1. The phosphorylation of Akt was slightly decreased in both cell lines, but the phosphorylation of S6 protein was almost completely abolished. On the other hand, a higher concentration of afatinib would also effectively suppress S6 protein expression in EC-1 cells. Afatinib can strongly induce G0/G1 cell cycle arrest in HKESC-2 in a dose- and time-dependent manner and it can also induce G0/G1 cell cycle arrest in EC-1. Interestingly, G2/M arrest was observed in EC-1 at a higher dose of afatinib treatment. Apoptosis was found in both cell lines at 24 hours after exposure to afatinib as determined by cell death ELISA assay and the presence of cleaved PARP

Conclusion. Afatinib can inhibit ESCC cell growth effectively by inducing cell cycle arrest and apoptosis and it warrants further studies.

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H.H. Deng and C.H. Wong contributed equally in this study

No conflict of interest.

231 Preliminary study to evaluate the role of SERPINA1 (AAT) in OC and EC progression

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Introduction. Protein homeostasis, regulated by proteases and their inhibitors, has a significant impact on cancer progression. Emerging evidence suggests that α1-Antitrypsin (AAT or SerpinA1), a 52-kDa serine-protease inhibitor, has also anti-inflammatory and tissue-protective properties. It is known that an inflammatory microenvironment may promote tumor progression and the pro-inflammatory cytokine IL1β has been shown to be important in promoting tumor angiogenesis and progression by directly affecting tumor cell invasion through the induction of the expression of some matrix metalloproteinases, including MMP9.

Aim: The aim of this study was to characterize the effect of SerpinA1 overexpression in some ovarian (COV318, OVCAR3) and endometrial (EFE184, MFE280) cancer cell lines on biological properties known to contribute to cancer progression.

Material and Methods. SerpinA1 was cloned in pUSEamp(+) plasmid and its overexpression was obtained by transfection in human OC and EC cell lines. The transfected cells were used for the in vitro scratch assay in normoxia and hypoxia and the scratch images were analyzed by ImageJ freeware. Paclitaxel and Cisplatin dose-response curves were also plotted and the IC50 values were calculated by ED50 plus v1.0 freeware. SerpinA1 overexpression effects on mRNA expression of VEGFA, KDR, TSP1, HIF1A, PHD2, MMP9, IL1β were analyzed by qPCR.

Result and discussion. SerpinA1 overexpression induced a downregulation of the expression level of pro-angiogenic factors KDR and HIF1A, IL1β and MMP9 in COV318 cell line while TSP1 and IL1β were upregulated in OVCAR3. A weak but not significant decrease of IC50 in transfected cell lines was found. The ability to migrate of SerpinA1 overexpressing OC cells seems lower than the control both in normoxia and hypoxia (Tab.1).

Conclusion. We hypothesize that SerpinA1 could have a beneficial effect to hinder cancer progression in OC by down-modulating factors known to contribute to tumorigenesis. Our preliminary data support the possibility that the anti-inflammatory effect of the AAT therapy could be used as a potential additive treatment in OC, expanding the clinical applications of AAT therapy.

	Normoxia		Hypoxia	
	Ctrl	SerpinA1	Ctrl	SerpinA1
0hrs	100%	100%	100%	100%
6hrs	84%	98%	91%	99%
12hrs	80%	97%	87%	97%
24hrs	76%	97%	83%	94%

Tab.1 Percentage of open wound area after scratch in transfected OVCAR3

No conflict of interest.

232 Characterization of a human colon carcinoma HCT-8 cells with MDR phenotype

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Introduction. The major cause of treatment failure in cancer chemotherapy is the development of multidrug resistance (MDR), therefore, the characterization of biological factors involved in MDR and the development of new strategies able to prevent or evading this phenomenon are particularly needed. The MDR phenotype is multifactorial and partially unknown; it involves several processes including DNA repair, cell cycle and apoptosis dysregulation, alterations in drug targets or enzymes that control their metabolism, and the increased activity of P-glycoprotein (P-gp).

Materials and Methods. A doxorubicin resistant HCT-8/R clone was selected from sensitive parental cells HCT-8/S and characterized analyzing its cell cycle phase distribution, apoptotic activity, presence, distribution and functionality of the P-gp pump, the response to other chemotherapy agents, its ultrastructural features and invasiveness. Moreover a whole gene expression analysis was performed to identify an expression profile associated with the MDR phenotype.

Results and Discussion. HCT-8/R cells showed a peculiar S phase distribution characterized by a single pulse of proliferation after 48 hours after seeding and displayed resistance to drug mediated apoptosis induction. HCT-8/R showed also a greater cell volume and the presence of several intra cytoplasmic vesicles, respect to HCT-8/S. The resistant clone is characterized by a significant increase of P-gp presence and functionality, by a cross-resistance to other cytotoxic drugs and a greater capacity for migration and invasion, compared to HCT-8/S. The trascriptomic analysis identified a large number of differentially expressed genes including the up-regulation of ABCB1, encoding for P-gp and other proteins involved in the onset of resistance such as RRM1 and 2 and the staminality marker CD44. It is interesting to note that the most up-regulated gene in HCT-8/R cells was the carbonyl reductase 1 (CBR1), a NADPH dependent enzyme that catalyzes a large number of endogenous and pharmacological substrates, reported to be upregulated at the transcriptional level by HIF-1α and able to protect the cells against hypoxia and anticancer drugs such as cisplatin and doxorubicin, by reducing oxidative stress.

Conclusion. HCT-8/R cells may represent an useful tool not only to understand the biological basis of MDR phenotype, but also to test novel therapeutic strategies to overcome this phenomenon.

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233 The Anp32C oncogene is a novel client of Hsp90 and enhances cellular sensitivity to PU-H71

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Introduction. The acidic nuclear phosphoproteins (ANP32 A-E) are a family of evolutionary conserved proteins with high sequence identity yet diverse cellular functions. While ANP32A is a tumor supressor ANP32C/D are known to be oncogenic. ANP32C is upregulated in prostate and breast cancer, can stimulate ras- and myc-induced transformation and shows tumorigenicity in vivo when stably transformed into NIH3T3 cells. We have previously shown that a functional mutation in ANP32C comprising a Y140H substitution is associated with the rapid growth of cancer cells and promotes resistance to the apoptotic drug FTY720. In this work we investigated ANP32C interacting proteins and show for the first time an association between ANP32C and Hsp90.

Materias and methods. ACHN or HeLa cells were transfected with lentivirus encoding ANP32A, B, C, C-YH, D, or E genes. ANP32C expressing cells were cross-linked using DSP and subjected to fractionation, immunoprecipitation and Peptide mass fingerprinting of interacting proteins. Cells were treated with the Hsp90 inhibitor PU-H71 and growth characteristics determined using CCK-8 and colony growth assays.

Results and discussion. Our results show that ANP32C is present in several high molecular weight complexes in cancer cell lines and that these complexes contain the molecular chaperones Hsp90 and Hsp70. Using cell lines individually expressing each of the ANP32 family members we demonstrated that only the oncogenic family members ANP32C/D interact as novel clients with Hsp90. ANP32C/D also demonstrated a high degree of instability with half-lifetimes <1 h emphasizing their dependence on Hsp90 in their folding, stabilization and degradation, which is not required for other family members.

Furtheremore ANP32C was found to be associated with oncogenic Hsp90 complexes by virtue of its ability to interact and be immunoprecipitated by the Hsp90 inhibitor PU-H71. Cells overexpressing ANP32C or its mutant showed enhanced sensitivity to treatment with PU-H71 as demonstrated by CCK-8 and colony formation assays. Our results highlight that certain malignancies with ANP32C/D overexpression or mutation might be specifically targeted using Hsp90 inhibitors.

Conclusions.

- ANP32C/D are novel clients of Hsp90.
- Hsp90 helps mediate the oncogenic activities of ANP32C/D.
- Hsp90 inhibitors potentially could be successful in targeting malignancies expressing ANP32C/D.

No conflict of interest.

234 Delta-tocotrienol induces ER stress-mediated apoptosis in human melanoma cells

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Introduction. Malignant melanoma is an aggressive tumor with increasing incidence and still limited therapeutic outcome in advanced disease. Tocotrienols (TT), Vitamin E derivatives, were reported to exert anticancer activity in some tumors. The aim of this study was to elucidate whether δ-TT might induce endoplasmic reticulum (ER) stress-mediated apoptosis in human melanoma cells.

Material and method. In human melanoma cell lines (BLM, WM115, WM 1552, IGR39, A375) the effect of different doses (5-20 μg/ml) δ-TT on cell viability was evaluated by MTT Assay. In BLM melanoma cells: the effect of δ-TT on cell death was evaluated by Trypan blue exclusion assay; the expression of apoptosis-related and ER stress-related proteins was assessed by Western blot and fluorescence microscopy assay.

Results and discussion. The results obtained demonstrate that δ-TT exerts a significant and dose-dependent cytotoxic effect on different human melanoma cell lines (MTT assay). These results were further confirmed in BLM melanoma cells by using the trypan blue exclusion assay. Moreover, in BLM cells, the expression of cleaved form of caspase 3 and of cleaved PARP was increased after δ-TT treatment (20 μg/ml), confirming that this compound exerts an apoptotic effect on melanoma cells. Since chronic ER stress has been shown to be involved in apoptotic cell death, we analyzed the expression of specific markers of ER stress in BLM cells after δ-TT treatment. We observed that, after 18 or 24 hours of treatment, δ-TT significantly increases the expression of the ER stress mediators Bip, IRE1a, PERK and CHOP. Moreover, δ-TT induced the cytoplasmic-to-nuclear translocation of CHOP (as evaluated by immunofluorescence analysis), indicating its activation. To demonstrate that ER stress mediates the apoptotic effect of δ-TT, we performed MTT and Western blot assays in the presence or absence of the ER stress inihibitor salubrinal. In this setting, we could demonstrate that salubrinal significantly counteracts the cytotoxic effect of δ-TT on the expression levels of CHOP and of the cleaved form of caspase 3.

Conclusion. These results demostrate that δ-TT exerts a proapoptotic effect on human melanoma cells and this effect is mediated, at least partially, by the activation of the proteins involved in the ER stress.

* contributed equally to this work. (Supported by Fondazione Banca del Monte di Lombardia).

No conflict of interest.

235 Role of exosome-associated miR-939 in breast cancer metastatic process

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Background. During metastatic spread, cancer cells induce changes that disrupt endothelial cell junctions and enable transmigration through blood vessels. There is evidence that some secreted miRNAs modulate adhesion molecules to enable tumor cell transendothelial migration during the metastatic process. miR-939 is predicted to target Ve-cadherin (Ve-cad), which plays a critical role in maintaining endothelial barrier stability and integrity, although analysis of its potential role in cancer has been limited to biomarker studies in lung adenocarcinomas. We investigated whether miR-939 might function in breast cancer dissemination through regulation of Ve-cad expression.

Methods. miR-939 expression in human breast carcinomas was analyzed in the TCGA publically available dataset. RNA from formalin-fixed, paraffin-embedded triple-negative breast cancer (TNBC) samples was analyzed for miR-939 expression by qPCR. Ve-cad expression was analyzed by Western blot and immunofluorescence in human umbilical vein endothelial cells (HUVEC) transfected and treated with exosomes purified from MDAMB231 cells transiently transfected with a miR-939 mimic. Effects of miR-939 on the endothelial monolayer were assessed by permeability and transendothelial migration assays.

Results. Analysis of the TCGA dataset revealed higher miR-939 levels in TNBC than other subtypes. miR-939 expression in a cohort of 59 TNBC samples significantly interacted with lymph node status in predicting disease-free survival: tumors with high miR-939 levels and positive lymph nodes were 7.5 times more likely to relapse than negative tumors. In vitro, miR-939 was secreted via exosomes, suggesting its extracellular effect on endothelial cells. HUVEC treated with exosomes showed increased intracellular levels of mature miR-939, but not of pre-miR-939, indicating miR-939 uptake but not new synthesis. Moreover, both Ve-cad protein expression and endothelial monolayer stability were decreased after miR-939 transfection or exosomes treatment: miR-939 efficiently destroyed adherens junctions and integrity of this barrier, leading to increased monolayer permeability and favoring transendothelial migration of MDAMB231-GFP cells.

Conclusion. Our data identify miR-939 as a key player in the entry of cancer cells into the blood circulation and provide a potential mechanism underlying the higher rates of recurrence and mortality in TNBC.

No conflict of interest.

236 Characterization of the role of LMW-PTP in tumor onset

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Introduction. The PTP superfamily comprises almost 70 enzymes that, share a common CX5R active-site motif and an identical catalytic mechanism. LMW-PTPs are a group of cytosolic enzymes of 18 kDa that are widely expressed in different tissues. They are represented by two most abundant isoforms, named fast and slowaccording to their electrophoretic mobility. Several works have shown how LMW-PTP overexpression is associated with human tumorigenesis. In fact, results obtained with a wide array of human carcinomas indicate a significant increase in the expression of LMW-PTP in tumor tissue and a correlation between higher expression and worse prognosis. Moreover, LMW-PTP acts as a positive regulator of tumor onset and growth in a in vivo animal model. It remains unclear which mechanisms and pathways in the cell are involved in the tumorigenic effect of LMW-PTP. By silencing technique we found important changes in several phenotypic characteristic, relevant for tumorigenesis: such as ability to migrate and chemoresistance.

Material and Method. Human A375 Melanoma cells were cultured in DMEM supplemented with 10% FBS. The cells were transiently transfected with siRNA directed against ACP1 gene, using HiPerFect. Apoptosis analysis were performed using Annexin-V-FLUOS Staining Kit.

Results and Discussion. Phenotypic analysis of silenced cells revealed a key role of LMW-PTP in the formation of cell-cell adhesion and migration. Detachment and adhesion test, have shown that without the phosphatase action, cell are less able to establish connection, and so adhesion is significantly reduced. In the prostate human carcinoma, Wound healing assay show that silenced cells have an increased migratory capacity with respect to control. By citofluorimetric analysis we evaluated the resistance to anoikis and chemotherapy. Our results show, after LMW-PTP silencing, an increased sensitivity to anoikis, but above all a dramatic increase in apoptosis infact after chemoterapic treatment the rate of apoptotic cells in the silenced sample is about 40%, in contrast to the control when 5Fluorouracil has no effect.

Conclusion. There are numerous characteristics acquired by tumor cells, especially those necessary to become more aggressive: among these the ability to anchor themselves strongly, and to form aggregated masses play a crucial role. It seems clear that LMWPTP overespression, which strengthens cell adhesion, is a mechanism used by these cells to acquire a metastatic phenotype, and so to start first periferical

dissemination, and then implanting in a different tissue. Even more important for tumor cells is to gain resistance towards chemoterapics. This difference appears even more marked under silencing conditions.

No conflict of interest.

237 Identification of novel putative Bcl-2 interacting proteins by mass spectrometry

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Introduction. Bcl-2, the most studied member of the bcl-2 family proteins, plays a critical role in resistance to antineoplastic drugs by regulating the mitochondrial apoptotic pathway. Moreover, it is also involved in other relevant cellular processes such as tumor progression, angiogenesis and autophagy. Deciphering the network of bcl-2 interacting factors should provide a critical advance in understanding the different functions of bcl-2.

Material and Method. Bcl-2 immunocomplexes (IM), obtained from H1299 human lung adenocarcinoma cells overexpressing FLAG-bcl-2 protein were separated by SDS-PAGE gel and analyzed by mass spectrometry for protein identification. Identified proteins were analyzed by Ingenuity Pathway Analysis (IPA).In vitro validation of bcl-2 binding to its novel putative interacting protein, SLIRP, was performed by co-immunoprecipitation (co-IP) experiments in both H1299 and MDA-MB-231 human breast adenocarcinoma cell lines overexpressing FLAG-bcl-2. Cellular proteins co-localization was investigated by immunofluorescence (IF). Mitochondrial genes expression was assessed by quantitative Real-time PCR.

Results and discussion. Analyzing by bioinformatics tools the identified proteins obtained by bcl-2 IM, we evidenced that mitochondrial associated proteins represent a significant part of the total identified proteins. In accordance with this observation, mitochondrial dysfunction and oxidative phosphorylation were among top canonical pathways found. These evidences prompted us to validate the identified interacting proteins starting from SLIRP (SRA stem-loop interacting RNA-binding protein), a protein mainly associated to mitochondria and involved in maintaining mitochondrial mRNA homeostasis. Co-IP of SLIRP with bcl-2 was strengthened in FLAG-bcl-2 IM obtained from both H1299 and MDA-MB-231 cell lines overexpressing FLAG-bcl-2 by using antibodies against both FLAG epitope and endogenous SLIRP. As expected, SLIRP subcellular localization was mitochondrial in both H1299 and MDA-MB-231 cell lines. Interestingly, partial co-localization of bcl-2 and SLIRP was observed in the transfectants overexpressing FLAG-bcl-2. Modulation of mitochondrial mRNAs in presence or absence of bcl-2, prior and after SLIRP silencing, is under evaluation.

Conclusion. We identified by mass spectrometry a list of putative novel bcl-2 interacting proteins. Among them SLIRP may be a promising partner of bcl-2 in regulating its mitochondrial associated functions.

No conflict of interest.

238 SCD1 as a prognostic marker and a therapeutic target for adenocarcinoma of the lung

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Introduction. Distinctive features of CSCs are their capacity of self renewal, increased resistance to chemotherapy and the ability to form three dimensional non adherent spheroids in appropriate conditions. We have demonstrated that cell cultures derived from malignant pleural effusions of patients with adenocarcinoma of the lung efficiently form spheroids in non-adherent conditions supplemented with growth factors. By expression profiling, we identified 18 genes whose expression is significantly upregulated in lung tumor spheroids versus adherent cultures. Among the upregulated genes we found five genes involved in lipid metabolism. SCD1, the enzyme involved in the conversion of saturated into monounsaturated fatty acids, was one of the most strongly upregulated gene. We also demonstrated that SCD1 inhibition leads to lung cancer spheroid collapse and to the selective apoptosis of ALDH+ cells, a marker enriched in cells with stem like properties. Furthermore, SCD1 inhibited spheroids were strongly impaired in their in vivo tumorigenicity.

Material and Method. H460 lung cancer cell line and the MPE-derived o/11 primary culture were grown in three dimensional non adherent spheroids culture to enrich for cells with cancer stem cell-like traits. Spheroid cultures were subjected to cell sorting using ALDEFLUOR assay kit to isolate respectively cells with high and low ALDH activity. Sphere formation assays were performed using sorted cells in the presence or not of Cisplatin and MF-438 inhibitor. Bioinformatic analysis was carried out using public datasets from lung cancer.

Result and discussion. We demonstrate by cell sorting that ALDH positive cells are more sensitive to SCD1 inhibition than ALDH negative cells and that treatment with an SCD1 inhibitor increases sensitivity of spheroids cells to Cisplatin. Furthermore, we observe that SCD1 inhibited spheroids are strongly impaired in their in vivo tumorigenicity. Using public datasets from lung cancer we conducted a bioinformatics analysis to define the prognostic role of SCD1 for the overall outcome of patients affected by lung cancer. The Kaplan-Meier curves show that the survival rate of patients expressing SCD1-high levels is significantly lower than that of the patients expressing low levels of SCD1 in lung adenocarcinoma. Although a trend was noted toward shorter overall survival for patients affected by squamous carcinoma expressing high levels of SCD1, we found no significant differences between patients with tumors having high or low SCD1 expression. We found that the correlation of SCD1 and stemness markers (nanog, CD44, CD24) selects the patients with bad prognosis. We are currently investigating the regulation of SCD1 expression and the interplay between SCD1 and the intracellular pathways important in cancer stem cells.

Conclusion. These results overall, indicate that SCD1 could be considered a prognostic markers for lung adenocarcinoma and a potential target for therapeutic intervention.

No conflict of interest.

239 Use of prostate cancer cells as in vitro model for discovering miRNAs released by cells that developed docetaxel resistance

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Introduction. One of the major problems in androgen-independent prostate cancer (PCa), nowadays defined castration resistant prostate cancer (CRPC), is the development of resistance to docetaxel (DCT). Considering the availability of new therapeutic agents it would be of great interest discovering predictive biomarkers of the early onset of a DCT resistance. In recent years circulating miRNAs have been validated as potential non-invasive biomarkers in several cancers, including PCa. So far, only few studies explored the connection between the modification of intra/extracellular miRNAs and the development of a resistance to drug therapy. In this work we exploited PCa cells as in vitro model to identify miRNA involved in the resistance to DCT.

Material and methods. Cells of the androgen-independent prostate cancer cell line DU-145 were treated with 3nM DCT. After 48h cells and culture medium were harvested and used to extract intra/extracellular RNA. The RNA was used to quantify extra/intracellular miRNA by qRT-PCR and by smallRNA-seq using TrueSeq smallRNA sample preparation kit and MiSeq sequencer (Illumina). DU-145 DCT resistant clones were isolated after about 30 days of continuous treatment with 3nM DCT.

Results and discussion. We detected miRNAs in cells and growth media before and after treatment of DU-145 with a concentration of DCT sub inhibitory for cell proliferation. The dot plot of the intra and extracellular miRNAs ratios showed that specific miRNAs were differentially expressed/released by DU-145 DCT treated cells (DU/DCT-miRNAs). In addition, we showed that DU/DCT-miRNAs are specific of DU-145 cells as none of them were differentially expressed/released by PC-3, LNCaP and 22Rv1 prostate cancer cell lines treated with DCT. Finally, we report that DU-145 clones resistant to DCT differentially released 3 DU/DCT-miRNAs, suggesting that some of the miRNAs that change their release after an acute treatment may early indicate the presence of DCT resistant cells.

Conclusion. Overall results indicate that the analysis of immediate post-treatment modifications may represent a useful strategy to discover miRNAs specifically released by PCa cells resistant to DCT. In perspective, these miRNAs may be candidates to be tested as predictive biomarkers of an early DCT resistance in CRPC patients candidate to DCT therapy.

No conflict of interest.

240 ANGPTL1/integrin a1ß1 axis represses SLUG–mediated cancer cell invasion and metastasis through miR-630

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Introduction. Metastasis is the most important contributor to the mortality of patients with cancer. Recently, a family of proteins structurally similar to the angiopoietins was identified as angiopoietin-like proteins (ANGPTLs), which comprise 8 proteins, ANGPTL1–8. ANGPTLs do not bind to the angiopoietin receptor Tie2, which indicates that the functional mechanism of ANGPTL proteins may be different from that of angiopoietins. Several studies show that ANGPTL proteins involve multibiological properties, such as angiogenesis, cancer cell invasion, lipid metabolism, hematopoietic stem cell activity, and inflammation. ANGPTL1 has been reported as an antiangiogenic protein by inhibiting the proliferation, migration, tube formation, and adhesion of endothelial cells as well as tumor growth; however, little is known about whether ANGPTL1 can influence the malignant properties of cancer cells and the exhaustive mechanisms in cancer progression.

Material and methods. Human lung cancer cell lines were used as the in vitro cell models. The transwell migration and invasion assays were used to determine the anti-metastatic effect of ANGPTL1 in vitro. The Western blot analysis, quantitative

PCR (qPCR) analysis, Chromatin immunoprecipitation (ChIP) analysis and Immunoprecipitation (IP) analysis were used to evaluate the underlying mechanisms of ANGPTL1-mediated suppression in cancer cell motility. The Immunohistochemistry (IHC) analysis was used to determine the clinical significance of ANGPTL1 expression in patients with cancer.

Results and discussion. Here, we show that ANGPTL1 expression inversely correlates with invasion, lymph node metastasis, and poor clinical outcomes in cancer patients. We also found that ANGPTL1 significantly suppresses the migratory, invasive, and metastatic capabilities of cancer cells through downregulation of SLUG. In addition, our results indicate that ANGPTL1-mediated suppression of the SLUG protein is due to the induction of miR-630 transcripts in the Sp-1–dependent ERK pathway. Furthermore, we show that ANGPTL1 interacts with integrin $\alpha 1\beta 1$ and represses the downstream signaling FAK/ERK. These findings indicate that ANGPTL1 inhibits cancer cells invasion and metastasis by inducing the mesenchymal-epithelial transition via the integrin $\alpha 1\beta 1$-FAK-ERK-Sp-1-miR-630-SLUG signal cascade.

Conclusion. In this study, we reveal a novel mechanism involved in ANGPTL1-mediated suppression of invasion and metastasis of cancer cells.

No conflict of interest.

241 Studies on epithelial-mesenchymal transition and cancer stem cell's role in cisplatin response in ovarian cancer xenografts

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Introduction. Epithelial ovarian cancer affects more that 100,000 people dying per year in western countries. The lack of screening tests results in 75% of patients being diagnosed at late stages, and even if about 70% of patients is responsive to a first-line chemotherapy (cisplatin-DDP+taxol), most of them relapse with a resistant disease. The molecular basis of resistance to therapy is multifaceted, and recently, the presence of cancer stem cells (CSCs) and the activation of Epithelial-Mesenchymal Transition (EMT) have been advocated as possible mechanisms. In the present work, by using ovarian cancer xenografts we have investigated the involvement of EMT and CSCs in the response to a DDP-therapy by gene expression analysis.

Material and method. Ovarian cancer xenografts were injected s.c. in nude mice (Harlan s.p.a.) and treated with a first DDP cycle q7dx3 at the dose of 5 mg/kg. A second cycle was given to the regrowing tumors. Antitumor activity is expressed as T/C%. By RT-PCR (Qiagen, RT2 ProfilerTM PCR Array System) the expression of EMT and CSCs genes was evaluated in untreated and DDP-treated xenografts. Statistical analysis were performed by DataAssist Software v2.0.

Results and discussion. The ovarian xenografts showed different sensitivity to the first DDP cycle, with Very Responder-VR, Responder-R, and Low Responder-LR xenografts, respectively in which cures, stabilization with/without regressions, and tumor growth inhibition were observed. Interestingly enough, all the tumors were less sensitive to the second cycle, mimicking clinical situations. We investigate if the expression of some EMT and/or CSCs genes could be predictive of DDP response. An increased expression of genes known to induce EMT (CAMK2N1, TCF3), to favour cell growth (EGFR, IGHFBP4), to be involved in self-renewal pathways such as Notch (DLL1, NOCH2), Hedgehog (SMO), Hippo (SAV1, LATS1) and an upregulation of CSCs markers (ABCG2, ATXN1, GATA3, MS4A1, ITGA6) were found to be inversely correlated with DDP in vivo response.

We then analyzed if DDP treatment could modulate the expression of EMT and CSCs genes. Preliminary analysis suggests an increased expression of MMP9, ALCAM, CD44, and ITGA2 in DDP- treated versus no-treated xenografts.

Conclusion. The validation of the increased expression of some of the described genes could lead not only to the identification of biomarkers of resistance to a DDP based therapy, but also envisage new therapeutic strategies to overcome it.

No conflict of interest.

242 miR-26a targets identification in prostate cancer cell lines using miRNA pull-out assay

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Introduction. The re-expression of tumor suppressor (TS) miRNAs in cancer cells in which they are downregulated (miRNA replacement therapy) has been evaluated as a promising approach to inhibit tumor proliferation both in in vitro and in vivo models. As a single miRNA simultaneously targets hundreds of genes, it derives that the TS-miRNA re-expression gives rise to a broad inhibition of pro-tumorigenic genes and pathways. In this work we investigated the molecular mechanism at the basis of the antiproliferative potential of the TS-miR-26a by identifying all its targets in prostate cancer (PCa) cell lines using the miRNA pull-out assay.

Material and methods.miR-26a was transfected using the appropriate transfectant for each tumor cell lines. Cell proliferation was detected with crystal violet staining. miRNA pull-out assay was performed using biotinylated synthetic version of miR-26a and the miRNA/target complexes isolated with streptvidine sepharose high performance (GE Health care). RNA-seq was performed with TruSeq stranded total RNA sample preparation kit and sequenced with HiSeq 2000 (Illumina).

Results and discussion. We first demonstrated that miR-26a was downregulated in several tumor cell lines (including PCa cell lines). By overexpressing miR-26a in some of these tumor cell lines we established its tumor suppressor activity in that it was able to inhibit cell proliferation. In particular, it was able to affect cell proliferation in both PC-3 and DU-145 PCa cells. To identify the miR-26a targets in PCa, the miRNA pull-out assay was performed in DU-145 cells. Using this approach we were able to isolate the miRNA/target complexes that we sequenced using high-throughput technology. We obtained 1423 transcripts and we found that 85% of them presented canonical miRNA binding sites predicted by more than one predictive algorithms, suggesting that the miRNA/targets isolation was successful. These results were reinforced by the fact that some of the identified transcripts were miR-26a targets already validated in other biological contests. Finally, the isolated targets were significantly enriched of transcript belonging to biological processes relevant for cancer proliferation.

Conclusion. The results indicate that the TS-miRNA pull-out assay protocol may be useful for the identification of TS-miRNA targets involved in key anti-tumorigenic processes and for that possible targets for anticancer therapy.

No conflict of interest.

243 miR-28-5p showed a tumor suppressive activity in DU-145 prostate cancer cells and regulated E2F6

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Introduction. It is now well known that the dysregulations of miRNAs expression is one of the cause or contributory causes of cancer development. Several evidences have clearly demonstrated both that the downregulation of tumor suppressors (TS) miRNAs favors the expression of oncogenes and tumor growth and that the re-expression of TS-miRNAs in defective tumor cell lines reduces their proliferation. We have demonstrated that miR-28-5p induces apoptosis and senescence in mouse embryo fibroblasts (MEF) and that it is strongly downregulated during MEF immortalization. In this work we analyzed the role of miR-28-5p in human tumor cells and we investigated the miR-28-5p targets in prostate cancer (PCa) cell lines using the miRNA pull-out assay.

Material and methods. miR-28-5p was transfected using the appropriate transfectant for each tumor cell lines. Apoptosis was measured by annexin assay and by the detection of PARP cleavage. miRNA pull-out assay was performed using biotinylated synthetic version of both miRNAs and the miRNA/targets complex isolated with streptvidine sepharose high performance (GE Health care).

Results and discussion. We evaluated the miR-28-5p expression level in several PCa as well as in other tumor cell lines and we found that it was strongly downregulated. Moreover, the miR-28-5p re-expression inhibited cell proliferation. In particular, miR-28-5p was able to cause a G1 arrest and to affect the colony forming ability of DU-145 PCa cells. To discover the miR-28-5p targets we performed the miRNA pull-out assay and we measured the enrichment of miR-28-5p targets already validated in other biological context using qRT-PCR. We found that not all validated targets were enriched by the pull-out procedure. The most enriched was E2F6. By overexpressing miR-28-5p we demonstrated that it was able to inhibit E2F6 also in DU-145 cells. Since it has been demonstrated that E2F6 plays an antiapoptotic role, we investigated and found that miR-28-5p overexpression induce apoptosis in DU-145 cells. Moreover, we showed that miR-28-5p re-expression enhanced the apoptosis induced by docetaxel even if at the same level of the miR-28-5p induced apoptosis.

Conclusion. The overall results indicate that: i) the miR-28-5p plays a TS role in DU-145 cells; ii) its re-expression in DU-145 cells induces apoptosis through E2F6 even if it is not the only mediator. In addition, we reinforce the concept that miRNAs regulate different targets depending on the biological context.

No conflict of interest.

245 Novel peptide inhibitors of CXCR4 directly affect the subset of lung cancer initiating cells and prevent their metastatic dissemination

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Introduction. We identified within the subset of CD133+ lung cancer initiating cells (CICs), a subpopulation of CD133+CXCR4+ cells endowed with high dissemination and metastatic potential that represent lung metastasis initiating cells (MICs).

Evidence in different solid tumors indicates that activation of CXCR4 pathway by its natural ligand CXCL12 regulates tumor cells motility and can sustain stemness features. Here we investigate the ability of a newly developed cyclic peptides CXCR4 antagonists (R, S, I) to affect lung CICs and to impair their metastatic spread.

Methods. Lung cancer cell lines and ex vivo cultures termed cancer tissue originated spheroids (CTOS), established from primary lung cancers and patients derived xenografts (PDXs), were treated with the CXCR4 antagonist peptide R, S and I or with CXCL12 and tested through in vitro invasion and migration assays and by FACS analysis to assess CICs modulation. Xenografts established in SCID mice by the injection of primary lung adenocarcinoma cell line (LT73) and metastatic lung cancer cell line

(H460) were treated with peptide antagonists alone or in combination with cisplatin and effects on tumor growth, lung tumor dissemination and modulation of CICs, in particular of CD133+CXCR4+ subset, were evaluated by FACS and IHC.

Results. Peptide R, S and I significantly inhibited CXCL12 and FBS-dependent migration and invasion of lung tumor cell lines. Ex vivo CTOS cultures treated with peptides showed a drastic reduction of CD133+ CICs (S p=0.004; R p=0.006; I p=0.01) with a concomitant down-regulation of stemness genes. Conversely activation of CXCR4 by CXCL12 ligand resulted in an expansion of CICs (p= 0.01), that can be counteracted by peptides R and S.

In vivo treatments of LT73 xenografts with peptide R and S delayed tumor growth and decreased CD133+CXCR4+ MICs, functionally ensuing in a reduction of tumor dissemination to murine lung.

Finally combination treatment of H460 xenograft with peptides R and S and cisplatin was able to specifically prevent the enrichment for CD133+CXR4+chemoresistant and metastatic subset induced by cisplatin and to impair their metastatic dissemination.

Conclusion. We show that a novel class of cyclic peptide CXCR4 antagonists was able to affect CICs and to block their dissemination; the ability of CXCR4 inhibitors to counteract the relative enrichment and metastatic spread of lung MICs induced by chemotherapy, suggests their potential use in combination therapy for a more effective treatment of lung cancer.

No conflict of interest.

246 GREB1 is an estrogen-regulated gene which promotes ovarian cancer cell proliferation and tumour progression in mouse models

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Introduction. Estrogenic hormone replacement therapy increases the risk of developing ovarian cancer, and 17-β estradiol (E2) accelerates tumour initiation and progression in mouse models, but little is known about the mechanisms underlying these observations. Growth regulation by estrogen in breast cancer 1 (GREB1) is an estrogen receptor alpha (ESR1)-upregulated protein which we propose mediates some of these estrogenic effects. GREB1 is required for hormone-driven proliferation of several breast and prostate cancer cell lines, which may be related to its only identified function: acting as a transcriptional cofactor with ESR1. The role of GREB1 in ovarian cancer is unknown, and most studies have examined its actions in vitro only. We therefore examined GREB1 expression and function in ovarian cancer cell lines and mouse models.

Materials and Method. GREB1 upregulation by E2 was discovered in a microarray comparing tumours from E2- vs. control-treated mice injected with ovarian cancer cells, and confirmed by QPCR in these tumours and in ovarian cancer cell lines. To investigate the actions of GREB1 in ovarian cancer, lentiviral constructs were used to overexpress or knock down GREB1 in several ovarian cancer cell lines. The effects of GREB1 knockdown and overexpression were evaluated both in vitro and in vivo (by injecting cells into immune-compromised mice). To examine GREB1 expression in human ovarian cancer, we measured mRNA levels by QPCR and protein levels by immunohistochemistry in epithelial ovarian tumours of several histological subtypes.

Results and Discussion. In a mouse ovarian cancer cell line, proliferation was decreased by GREB1 knockdown and increased by overexpression, indicating that GREB1 promotes proliferation. Cell migration in a scratch wound assay was also increased by GREB1 overexpression. GREB1 knockdown in cells injected into mice slowed tumour growth, prolonging median survival by 1.8-fold. In human ovarian tumours, GREB1 was expressed in all histological subtypes examined and correlated with ESR1 expression by both QPCR and IHC.

Conclusion. Determining the actions of GREB1 and other E2-target genes will elucidate the mechanism of E2 action in ovarian cancer and may explain how estrogenic hormone therapy increases the risk of ovarian cancer. It could also help clarify the negative clinical results observed with anti-estrogens in ovarian cancer, despite their efficacy in hormone-responsive breast cancer.

No conflict of interest.

247 Prostate cancer: Chemotherapy effects in cancer stem cells

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Prostate cancer is one of the most incident cancers in the world and a leading cause of cancer death in men. It is known that the prostate tumor is sensitive to the presence of testosterone and many of its cells´ proliferation depend on this hormone. Despite side effects, hormone therapy has been proven as effective in preventing tumor growth. The disease gets a worrying prognosis when it begins to show resistance to castration. On this stage, the mainly treatment is based on chemotherapy. Similarly as many other types of cancer, the tumor frequently develops resistance to the drugs which will eventually lead to a lethal stage. In this study, we investigated the relation between cancer stem cells (CSC) and drug resistance, in vitro. Aldehyde dehydrogenase enzyme (ALDH) might be directly involved in mechanisms of resistance to chemotherapy and radiotherapy. Higher activity of ALDH has been used as a marker for identifying tumor initiating cells in various types of cancer, such as colon, lung, breast, among others.

Materials and Methods. PC3 and DU145 cell lines were exposed in vitro to two chemotherapeutic agents already commercially available and used in the clinics: the antimitotic docetaxel and the inhibitor of methyl transferase 5- azacytidine. Subsequently, the presence of stem-like characteristics in the remaining cells was evaluated by using ALDEFLUORTM and clonogenic assay. Bicalutamide, an analogue of testosterone, was used as negative control.

Results and discussion. In summary, the results indicate that after treatment with docetaxel, PC3 and DU145 cells had a significant increase in the population of cells with high expression of ALDH activity (sometimes up to 20% increase). Similar results were observed with 5-azacytidine, whose treatment also induced a significant increase in the population of cells with high expression of ALDH activity ((up to 18%). However, when we used bicalutamide, no changes were observed in the percentage of ALDH positive cells before and after treatment. Since this drug is a testosterone analogue, this result was expected, as its action depends on the presence of androgen receptors, which are not expressed in the cell lines tested. Furthermore, the results were confirmed through clonogenic assays, in which after a long period of incubation with the drugs the cells remained for a while in a "sleep state," before recovering the ability to form colonies.

Taken together, our results lead us to hypothesize that treatment with cytostatic drugs promotes enrichment of subpopulations of ALDH positive cells. This might have a great biological and clinical significance and opens new windows for the management of this devastating disease.

Conclusion. In the near future, it will be crucial to identify the activated pathways involved in this chemoresistance. Consequently, it may result in the development of new therapeutic strategies to specifically target cancer stem cells and thus, prevent tumor recurrence.

No conflict of interest.

248 Phosphatidylcholine-specific phospholipase C inhibition as a new therapeutic approach to control triple-negative breast cancer cells proliferation

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Introduction. Different studies have shown that an altered phosphatidylcholine (PC) cycle, responsible for changes in the profile of choline-containing metabolites, can be considered as an emerging cancer hallmark and some enzymes of PC metabolism have been proposed as powerful targets for anticancer therapy (Podo F et al, 2011; Glunde K et al, 2011). An upmodulation of PC-specific phospholipase C (PC-PLC) in breast and ovarian cancer (Iorio E et al, 2010; Paris L et al, 2010; Abalsamo L et al, 2012) is part of this scenario. In the present study we investigated whether the pharmacological inhibition of PC-PLC could affect both EGFR signalling and cell cycle progression in triple-negative breast cancer (TNBC), thus representing a potential antitumor strategy against this malignancy.

Materials and Methods. EGFR receptor expression and its interaction with PC-PLC were investigated in two TNBC cell lines (MDA-MB-231 and MDA-MB-468) and compared with non-tumoral epithelial mammary cells (MCF10A) using flow cytometry, confocal laser scanning microscopy, and immunoprecipitation techniques. The effects of PC-PLC inhibition by tricyclodecan-9-yl-xanthogenate (D609) were evaluated on EGFR-mediated signalling (phosphorylation/activation status of EGFR and some key downhill components such as ERK1/2 and AKT) as well as on cell cycle progression by SDS-page, western blotting, and flow cytometry.

Results and Discussion. PC-PLC expression was significantly higher in the two TNBC cell lines compared with non-tumoral MCF10A, suggesting that this enzyme could represent a specific target for cancer cells. Moreover, PC-PLC inhibition substantially reduced the EGFR signaling in tumoral cells, thus leading to cell cycle arrest in the G0/G1 phase, without induction of apoptosis. According to these findings, upon treatment with D609 only TNBC cells showed significant changes in the expression of key molecules regulating cell cycle progression, such as downregulation of cyclin D1, cyclin E and the Rb protein, and upregulation of p21.

Conclusions. Altogether these results pointed to a role of PC-PLC in regulating the proliferation of TNBC cells, suggesting that PC-PLC inhibition could represent a new therapeutic approach to control TNBC malignant transformation and tumor progression.

No conflict of interest.

249 Anti-angiogenic property of monoolein against human cervical cancer cells: An animal model

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Introduction. Monoolein, a glycolipid biosurfactant extracted from the fungus, E. dermatitidis has been demonstrated the anti-proliferative activity against cervical cancer cells lines and leukemia cells lines in vitro. Therefore, we explore the antitumor properties of monoolein against cervical cancer xenograft tumor in nude mice.

Materials and Methods. HeLa cell line was used to implant subcutaneously into the dorsal of BALB/c-nude mice to form xenograft tumors. Mice were divided into 2 groups; treated with 200 mg/kg bw of MO (HeLa-MO) and untreated group (0.05% ethanol vehicle control; HeLa-vehicle). When the tumor size reached approximately

50-90 mm3, mice were administered with MO or vehicle once daily by peri-tumoral injections for 30 days. Tumor size was measured every two days and tumor volume was calculated. The tumor blood flow was analyzed using the Laser Doppler blood flow meter. The tumor microvasculature was observed under intravital fluorescence video-microscopic and microvascular density was analyzed using digital image processing software (Image-Pro® Plus 6. 2).

Result and Discussion. To evaluate the anti-angiogenic potential of MO, tumor volume, tumor blood flow and tumor microvascular density were investigated. Tumor implanted mice treated with monoolein showed significantly decreased in the tumor volume, tumor blood flow and tumor microvascular density compared with those of HeLa-vehicle group ($p<0.001$). Tumor inhibition rate was 59.20%. Several experimental and clinical studies indicated that the development of a solid tumor growth is more rapid at a vascular phase or angiogenesis phase. Therefore, our results imply one of the mechanisms by which monoolein treated tumor-mice have a potential to retard tumor growth might be mediated by its anti-angiogenic property.

Conclusion. The present study demonstrated that monoolein suppressed tumor growth and microvascular angiogenesis in HeLa-cells implanted mice. Therefore, monoolein has potential in treating human cervical cancer however its' mechanisms and usage will be investigated further.

No conflict of interest.

250 Anoikis of colon carcinoma cells triggered by beta-catenin loss can be enhanced by Tumor Necrosis Factor Receptor 1 antagonists

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Background. Detachment of non-malignant epithelial cells from the extracellular matrix (ECM) causes their apoptosis, a phenomenon called anoikis. By contrast, carcinoma cells are anoikis-resistant, and this resistance is thought to be critical for tumor progression. Many oncogenes induce not only anti- but also pro-apoptotic signals. The pro-apoptotic events represent an aspect of a phenomenon called oncogenic stress, which acts as a safeguard mechanism blocking tumor initiation. In cancer cells oncogene-induced anti-apoptotic signals outbalance the pro-apoptotic ones. It is now thought that treatments blocking the anti-apoptotic events but preserving the pro-apoptotic signals can be particularly effective in killing tumor cells. Whether or not oncogenes induce any pro-anoikis signals that can be used for enhancing the efficiency of approaches aimed at causing anoikis of cancer cells is not known. β-catenin is an oncoprotein that is often activated in colorectal cancer and promotes tumor growth via mechanisms that are understood only in part.

Materials and Methods. To understand how β-catenin controls anoikis of tumor cells we examined the expression of various regulators of apoptosis in human colon cancer cells before and after ablation of β-catenin by RNA interference and the role of these regulators in anoikis resistance of such cells.

Results and Discussion. We found that β-catenin triggers both anti- and pro-anoikis signals in colon cancer cells. We observed that the anti-anoikis signals prevail and the cells become anoikis-resistant. We established that one pro-anoikis signal in these cells is triggered by β-catenin-induced downregulation of an apoptosis inhibitor Tumor Necrosis Factor Receptor 1 (TNFR1) and inactivation of a transcription factor NF-κB, a mediator of TNFR1 signalling. We also found that the effect of β-catenin on TNFR1 requires the presence of transcription factor TCF1, a β-catenin effector. We observed that β-catenin ablation in colon cancer cells triggers their anoikis and that this anoikis is enhanced even further if low TNFR1 or NF-κB activity is artificially preserved in the β-catenin-deprived cells.

Conclusions. β-catenin triggers both anti- and pro-anoikis signals in colon cancer cells. The latter ones are driven by β-catenin-induced TNFR1 downregulation and NF-κB inactivation. Preservation of low TNFR1 or NF-κB activity in colon cancer cells enhances anoikis of these cells triggered by the blockade of β-catenin signalling.

No conflict of interest.

251 Evaluation of Wnt receptor targeted theranostic nanoparticles in models of drug-resistant triple negative breast cancer

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Introduction. Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer with a poor prognosis. Therapeutic options for TNBC patients are limited to chemotherapy and surgery. More than 50% of patients with TNBC will not respond to chemotherapy, are at a higher risk for recurrence, and have a low 5-year survival. It is imperative that effective therapies are developed to enhance the drug response in drug-resistant TNBCs. The enrichment of cancer stem-like cells (CSC) with a CD44+/CD24- phenotype is a proposed mechanism for drug-resistance in TNBCs. Activation of the Wnt pathway can drive CSC signaling in TNBC. Our lab has developed short peptides that mimic Dickkopf-1, an inhibitor of the Wnt pathway. These inhibitor of Wnt (iWnt) peptides bind to Wnt pathway receptor LRP. iWnt peptides were conjugated to iron oxide nanoparticles (IONPs) carrying the chemotherapeutic doxorubicin (Dox). In addition, uPAR peptides were conjugated to iWnt-IONPs to facilitate intratumoral delivery and targeting to CSCs. uPAR is highly expressed in aggressive TNBC tissues and tumor stromal cells. This novel dual-targeting theranostic nanoparticle is a promising therapy.

Material and Method. We generated iWnt-uPAR-IONP with or without Dox for in vitro and in vivo studies. Cell viability and cell binding assays were conducted in a MDA-MB-231 cell line. In vivo studies were conducted in a TNBC MDA-MB-231 cell line-derived tumor xenograft model and a patient derived xenograft (PDX) model established from drug-resistant residual tumors from TNBC patients.

Result and discussion. Our in vitro data showed higher uptake efficiency of iWnt-uPAR-targeted-IONP in a MDA-MD-231 cell line compared to non-targeted IONPs. iWnt-uPAR-targeted-IONP-Dox treatment decreased cell viability compared to free Dox and no treatment groups. Systemic delivery of iWnt-uPAR-targeted-IONP led to the accumulation of the IONPs in both TNBC cell-line derived and PDX models. In MDA-MB-231 xenograft model, iWnt-uPAR-targeted-IONP-Dox treatment inhibited tumor growth compared to mice treated with free Dox or no treatment control group.

Conclusion. Our findings show that iWnt-uPAR-targeted theranostic IONP can effectively bind to and inhibit cell growth in vitro in human TNBC cells. We also demonstrated targeting and therapeutic efficacy in in vivo TNBC models. Our results support further investigation of theranostic nanoparticles as a viable therapeutic approach to drug resistant TNBC.

No conflict of interest.

252 Cross talk between bioenergetic and phosphatidylcholine metabolism in human glioma cells

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Introduction. Malignant transformation is driven by genetic mutations that promote unchecked proliferation, abrogate cell death programs, and reprogram metabolism. Several studies have reported an altered glucose and phosphatidylcholine (PC) metabolism in patients and in experimental models of glioblastoma (GBM). These grounds stimulated our interest in filling the still existing gaps in the characterization of a possible cross-talk between PC-cycle and cellular bioenergetic abnormalities in human glioma cells.

Material and Method. Metabolomics analyses were performed using magnetic resonance spectroscopy (MRS) on polar and organic cell extracts of either Metformin-treated or untreated human U87MG glioma cells.

In particular, Metformin was selected to perturb the adenosine monophosphate-activated protein kinase (AMPK), a sensor of cellular energy; oligomycin A was used as an inhibitor of ATP synthase; and D609 as a competitive inhibitor of PC- specific phospholipase C (PC-plc).

Results and Discussion. Metformin induced a cell growth arrest in U87MG cells, without cell death. In MR spectra of metformin-treated compared with untreated cells we found a significant accumulation of the glutamate/glutamine pool, metabolites linked to mitochondrial activities and an increase in phosphocoline content, a metabolite deriving from PC-biosynthesis and degradation, by choline kinase and PC-plc respectively. On the other hand, when the U87 cells were exposed to D609 (in the presence of oligomycin), the basal bioenergetic status was altered, with a 30% decrease of glycolysis activity, suggesting a cross talk in both bidirectional ways.

Conclusion. We here provide the first direct evidence of a cross-talk between bioenergetics and phosphatidylcholine metabolism in human glioma cells. This evidence could provide the identification of a new multi-targeted approach against this malignancy.

No conflict of interest.

253 miR155 drives metabolic and motile reprogramming of ER positive breast cancer cells following long-term estrogen deprivation

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Introduction. The majority of breast tumors are positive for estrogen receptor (ER+) expression and aromatase inhibitors (AI) have become the first-line endocrine treatment choice for postmenopausal ER+ breast cancers women. However, AI resistance still limits their benefit for many patients. Since metabolic reprogramming is considered a hallmark of cancer, we investigated whether metabolic reprogramming is linked to AI resistance.

Material and method. Long-term estrogen deprived cells (MCF7-LTED and ZR75-LTED) and cells overexpressing aromatase (MCF7-2A) were used as in vitro models of AI resistance and sensitivity, respectively. Data mining of publicly available database on tumor samples was used to confirm the in vitro experiments.

Results and discussion. AI-sensitive MCF7-2A cells subjected to different concentration of the AI revealed a positive correlation between glycolysis and cell viability inhibition, suggesting a role for AI in impairing glycolysis. Accordingly, targeting glycolysis with 2-Deoxy-glucose (2-DG) or 3-Bromo-pyruvate in combination with AI showed a synergistic effect in reducing MCF7-2A cell viability. Metabolic comparison of LTED-MCF7 cells versus parental MCF7 cells showed an increased glycolysis dependency of the AI-resistant cells. Importantly, in silico analysis of AI-

treated breast cancer patients revealed an enhanced glycolytic phenotype in patients that did not respond to AI treatment. However, the intriguing idea of targeting glycolysis in AI-resistant cells failed; when both cell lines were exposed to either 2-DG or metformin, an inhibitor of mitochondrial respiratory chain complex 1, only the parental MCF7 cells showed a decrease in cell viability. MCF7-LTED cells were able to switch to either OXPHOS or glycolysis when 2-DG or metformin were used and only the combination of the two compounds was able to induce MCF7-LTED cell death, thus suggesting a high metabolic plasticity. This effect is ER-dependent and the ER negative ZR75-1-LTED cells did show no metabolic plasticity. Importantly, MCF7-LTED cells were also more motile than parental MCF7 and once MCF7-LTED cells were subjected to 2-DG treatment, they further increase their motile ability. We showed that this metabolic and motile adaptability of MCF7-LTED cells is related to the enhanced expression of the ER-dependent miR155. In fact, targeting miR155, i.e. reducing its expression, sensitized MCF7-LTED cells to single agent metformin treatment and impaired 2DG-induced motility.

Conclusion. Glycolytic metabolism is associated with enhanced breast cancer aggressiveness and tumor cell growth in the absence of estrogen. Targeting miR155 to impair the metabolic reprogramming could be a potential therapeutic tool if used in combination with current therapeutic regimes.

No conflict of interest.

254 Characterization of adrenocortical carcinoma proteomic profile

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Introduction. Adrenocortical carcinoma (ACC) is a rare and aggressive tumor characterized by poor prognosis when metastatic at diagnosis. The biology of the tumor is still mostly unclear, thus justifying the limited specificity and efficacy of the anti-cancer drugs currently available. The present study reports the first proteomic analysis of ACC by using the two-dimensional-difference-in-gel-electrophoresis (DIGE) technique to evaluate a differential protein expression profile between adrenocortical carcinomas and normal adrenals.

Material and methods. Mass spectrometry associated to DIGE analysis of carcinoma (n=10) and normal (n=8) adrenal specimens was performed to identify differentially expressed proteins between tumor and normal specimens. The obtained results were validated by Western Blot analysis on the tissue samples of the same cohort and by immunohistochemistry performed on paraffin-embedded ACC and normal adrenal specimens of the same patients.

Results and Discussion. In this study we identified 22 proteins differentially expressed (fold variation <-2 or >2, $P<0.05$) between pathology and normal condition. All proteins appear to be overexpressed in ACC, except one which was downregulated (thiosulfate sulfurtransferase). Among the overexpressed proteins, the differential expression obtained by DIGE analysis for ALDH6A1, Transferrin, Fascin-1, Lamin A/C, CAP1 and Adrenodoxin Reductase was validated by Western Blot analysis (fold increase±SE7.5±1.4, 3.6±1.2, 2.9±0.2, 2.6±2.1, 1.9±1.4, 1.6±0.8, $P<0.05$, respectively). Immunohistochemistry performed for ALDH6A1, Transferrin and Fascin-1 showed a marked positive signal in almost all cells in the ACC, while it was negative in normal adrenals. Our preliminary findings reveal a different proteomic profile in adrenocortical carcinoma compared to normal adrenals, identifying 6 proteins significantly overexpressed in the tumor.

Conclusion. In conclusion, the identified proteins could represent novel valid protein ACC biomarkers if further validated in a larger cohort of patients.

No conflict of interest.

255 EP3 receptor on platelets modulates Prostaglandin E2-mediated angiogenic factors release

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Introduction. Platelets are a natural source of growth factors, cytokines and chemokines that regulate inflammation, angiogenesis, and malignancy. Prostaglandin E2 (PGE2) enhances aggregation of platelets sub-maximally stimulated in vitro. This results from activation of EP3, one of the four PGE2 receptors, which decreases the threshold at which agonists activate platelets to aggregate. We studied whether PGE2 promotes the release of angiogenic factors from platelets and the contribution of EP3 on this PGE2 effect on in vitro platelets isolated from mice wild type (WT) or knockout (KO) for EP3.

Methods. Platelets collected from WT or KO EP3 mouse were studied using PGE2 at different concentrations, and selective agonists and/or antagonists of the EP receptor subtypes. Angiogenic factors release and function was assayed by western blot, EIA and matrigel assays.

Results. Incubation of platelets with PGE2 promoted VEGF, FGF2 and PDGF release. The concentration of growth factors was higher in WT than in EP3 KO platelet releasates. Similar results were obtained by using PGE2 agonist and/or antagonist. Indeed, the EP3 antagonist significantly inhibited VEGF release from WP platelets incubated with PGE2. Further, incubation of the releasate from WT platelets with endothelial cells promotes pseudocapillary formation, while the releasate from EP3 KO platelets failed to induce pseudocapillary formation.

Conclusion. Our findings provide evidence that EP3 modulates angiogenic factor release from platelets in response to PGE2. Because PGE2/EPs signaling pathways can influence the behavior of multiple cell types involved in angiogenesis and cancer, selective targeting of EP3-mediated PGE2 signaling might represent an attractive therapeutic strategy.

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No conflict of interest.

Poster Session: Cell and Tumour Biology II

256 Investigate the role of Sialyltransferase inhibitor on MDA-MB231 breast cancer cells in vitro and in vivo

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Introduction. Metastasis is the primary cause of death in cancer patients. To find inhibitors to block this process is urgently needed. Increased sialyltransferase (ST) activity promotes cancer cell metastasis, our previous study showed that ST inhibitor, AL10, suppresses lung metastasis of A549 in tail-vein–injected animal model. We developed a series ST inhibitor by chemical synthesis named FCW34 and FCW66 to investigate the antimetastatic and antiangiogenic effects and mechanisms.

Material and method. Human breast cancer cell, MDA-MB-231 cells, were used in the study. Cells treated with FCW34 and FCW66 were subjected to migration, invasion. After treatment, cellular proteins were harvest for western blot.

Athymic nude mice (BALB/cAnN.Cg-Foxn1nu/CrlNarl) were raised and subcutaneously injected with MDA-MB-231/ Luc cells into the abdominal mammary gland area. While tumor grew to about 70-100mm3 (Day 20), we divided into two groups randomly and mice received 10mg/kg of FCW34 on every other day. Vehicle (40% PEG400+60% Normal Saline) treatment was used as control. The growth and the spontaneous metastasis of the tumors were observed under IVIS50 in vivo imaging system (Xenogen) with Firefly D-Luciferin substrate (NanoLight) injection. The metastasized tumor tissues were dissected on day 56. The blood sample and tissue were harvested for further assessment.

Results and discussion. By using FCW34 and FCW66 revealed that ST inhibitors might inhibited breast cancer cells migration via modulating the Integrin/FAK-Paxillin/NFκB signaling pathway. We also demonstrated that FCW34 significantly decreased tumor progression and metastasis in breast cancer-bearing mice. In histological study, the data also revealed the FCW34 inhibited tumor cells proliferation and angiogenesis in vivo.

Conclusion. Here we provide a novel selectivity ST inhibitor for future therapeutic strategy for blocking tumor progression and metastasis in patients with breast carcinoma.

No conflict of interest.

257 L-2-hydroxy acid oxidase 2 (Hao2): A new oncosuppressor in hepatocellular carcinoma development?

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Introduction. L-2-hydroxy acid oxidases are flavin mononucleotide (FMN)-dependent peroxisomal enzymes, that catalyze the oxidation of several L-2-hydroxy acids to ketoacids at the expense of molecular oxygen, resulting in hydrogen peroxide formation. Since no information about Hao2 and cancer are available, we analyzed the expression of these enzymes in mouse, rat and human HCC.

Material and Method. Two distinct series of human HCCs were used to detect by qRT-PCR the levels of Hao2. qRT-PCR and western blot analysis were also performed in two different rodent models i) HCCs induced in mouse liver by the administration of TCPOBOP, a ligand of the nuclear receptor CAR ii) the Resistant-Hepatocyte rat model, R-H.

Results and Discussion. We found that mRNA levels of Hao2, a member of this family, is strongly down regulated in human HCC when compared to both normal and cirrhotic peri-tumoral liver. The levels of Hao2 were inversely correlated with time of recurrence or overall survival and metastasis. Notably, a similar down-regulation was also observed in HCCs induced in mouse liver by TCPOBOP. To determine whether Hao2 down regulation occurs early in the development of HCC, we analyze the expression of Hao2 in the R-H rat model of hepatocarcinogenesis, which allows differentiating phenotypically of very early preneoplastic lesions. Using this model, we found a mRNA down regulation of Hao2 in rat early preneoplastic lesions, especially in those positive for the putative progenitor cell marker cytokeratin-19 (KRT-19) considered to be the progenitors of HCC. Hao2 down regulation was confirmed both at mRNA and protein levels all throughout the process (early and advanced HCCs).

Conclusion. These results describe, for the first time, the dysregulation of a metabolic gene whose expression is severely impaired in HCC generated in three

different species and by different etiological agents. They also demonstrate that dysregulation of Hao2 is a very early event in the development of HCC.

No conflict of interest.

259 Effects of phosphatidylcholine-specific phospholipase C inhibition on tumour growth, metabolism and HER2 expression in preclinical models of HER-2 overexpressing ovarian cancer

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Introduction. Previous studies in our laboratory revealed the ability of a neutral active 66-KDa phosphatidylcholine (PC)-specific phospholipase C (PC-PLC) isoform, involved in the PC-cycle, to accumulate on the plasma membrane of tumor cells and mitogen-stimulated fibroblasts, whereby the PC-PLC activation/deactivation status can modulate the expression of membrane receptors and proteins crucial for specific cell functions (Podo et al, NMR Biomed 2011; Paris et al, Breast Cancer Res 2010). We focused our attention on biological and metabolic effects of in vivo passage on the human HER2-overexpressing SKOV3 cell line, which allowed selection of cells (SKOV3.ip) endowed with a more aggressive phenotype, enhanced HER2 expression and higher PC-PLC activity (Pisanu et al, Br J Cancer 2014). Purpose of this work was to investigate the role of PC-PLC on tumour growth, metabolism and HER2 expression in preclinical models of HER-2 overexpressing ovarian cancer.

Materials and Methods. In vivo MRI/MRS experiments were performed using a Varian Inova system at 4.7 T on xenografts of in vitro cultured human SKOV3.ip cells in SCID mice (Canese et al, NMR Biomed 2012). High resolution MRS analyses were performed on SKOV3.ip cell and tissue extracts at 16.4 or 9.4 T (Bruker AVANCE). The PC-PLC inhibitor tricyclodecan-9-yl-potassium xanthate (D609, 1 mg/mouse x 9 days) or saline (SAL) was given, starting from day 7 post injection. Histological analysis of xenograft sections was performed on biopsies.

Results and Discussion. Separation of lipid rafts by sucrose gradient and immune-precipitation experiments allowed detection of co-localization and physical association of PC-PLC with HER2 in non-raft domains of in vitro-cultured SKOV3.ip cells. Cell exposure to D609 induced a long-lasting block of cell proliferation and a strong reduction in the overall HER2 content measured in total cell lysates. In vivo experiments showed a significant decrease in the growth of D609- vs. SAL-treated SKOV3.ip xenografts. Histological analyses are ongoing on xenografts-derived samples to assess if metabolic alterations may also impact on tumour histology.

Conclusion. The here reported decreases in the in vitro cell proliferation and in vivo tumour growth following PC-PLC inhibition suggesting that this enzyme plays an important role in HER2-driven EOC cell signalling and tumorigenicity.

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No conflict of interest.

260 The interaction between CD157 and fibronectin modulates acute myeloid leukemia cell behavior

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Introduction. Acute myeloid leukemia (AML) is a heterogeneous group of tumors characterized by blockage of myeloid differentiation and subsequent accumulation of blasts in the bone marrow (BM). The crosstalk between blasts and BM microenvironment promotes tumor cell survival as well as drug resistance.

CD157/BST-1 protein is expressed on the surface of myeloid cells, BM stromal cells and selected epithelial cancer cells. The implication of CD157 in the escape of solid tumors from apoptosis and from platinum-mediated cytotoxicity, and its role in adhesion and migration of normal myeloid cells and tumor cell lines lead us to hypothesize that it could interact with extracellular matrix proteins thus playing an active role in the cross-talk between AML blasts and BM microenvironment.

Materials and Methods. CD157 expression on the surface of AML blasts was analyzed by flow cytometry using anti-CD157 monoclonal antibodies (mAb) in combination with anti-CD45, CD117, CD123 and HLA-DR mAbs. The binding of CD157 to fibronectin (FN) was studied through solid-phase binding assays and surface plasmon resonance analysis using purified proteins. The biological significance of CD157-FN interaction was investigated using both native and engineered U937 leukemic cells. The sensitivity to cytarabine and daunorubicin in the presence or absence of FN was studied by MTT assays.

Results and Discussion. We demonstrated that CD157 is heterogeneously expressed by AML blasts. Its expression was detected in >60% of patients at diagnosis and in BM stromal cells primary cultures derived from the same patients, although at variable levels. Moreover, we demonstrated that CD157 binds fibronectin with high affinity and this binding is interfered by anti-CD157 mAbs. The CD157-FN interaction occurring in living cells modulates specific cell functions, including AML cell adhesion and drug sensitivity. Indeed, CD157 silencing by means of specific shRNA or blocking by means of specific mAbs reduce U937 cells adhesion to FN; moreover, CD157-mediated U937 cell adhesion to FN reduces their sensitivity to cytarabine (AraC) and daunorubicine.

Conclusion. These results suggest that the interaction between CD157 and fibronectin occurring in the BM niche might play a role in the biology of AML, ultimately influencing the response to therapy.

No conflict of interest.

261 Fibroblast role in the acquisition and maintenance of anti-Her2 target therapies resistance in breast cancer

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Introduction. Mechanisms underlying tumor progression after chemotherapy are not well understood. Therapeutic treatments can favor the clonal selection of cells with unique properties and different fitness for a given microenvironment. Indeed, tumor cells can induce changes in the structure and composition of the microenvironment to support their growth and spread.

Our aim is to study if clonal selection induced by target therapies like Trastuzumab and Lapatinib favors the outgrowth of cells with different microenvironmental crosstalk capability, and in particular the role of fibroblast in the selection of these particular clones.

Materials and Methods. In order to investigate if clonal selection induced by target therapies like Trastuzumab and Lapatinib favors the outgrowth of cells with different capability of microenvironmental crosstalk, we developed different cell lines resistant to these drugs from the parental SKBR3, BT474 and MDA-MB-453 Her2+ cells lines. We determined their molecular profile and investigated the changes in the expression of selected genes codifying soluble factors known to induce stromal changes, like chemokines, cytokines, matrix remodeling-related enzymes, angiogenic and neurogenic factors.

To study the role of fibroblast in the selection of the resistant clones, and the crosstalk between these two populations, we developed fibroblast immortalized lines derived from breast cancer tumors and normal breast tissue, and study the effect of the fibroblasts in resistant induction, as well as the effect of resistant clones in the fibroblast activation.

Results and discusion. We observed that the major changes in the genes investigated were obtained in the Lapatinib resistant cell lines, suggesting that the acquisition of Lapatinib resistance can provide the tumor with a higher capability of stromal interaction. In vivo, the resistant cell lines showed an invasive growth pattern and higher angiogenesis compared to the parental cell lines. We observed a different pattern of fibroblast distribution in the tumors derived from the resistant cell lines, which display a more infiltrative distribution. The resistant tumors were also more fibroblast-enriched suggesting a higher microenvironment crosstalk capacity. In vitro, we found that the supernatant of breast cancer associated fibroblast was able to induce an increase in the resistant phenotype of parental lines, and that the resistant cell lines were able to induce a slightly activation of fibroblasts.

Conclusion. These results highlight the importance of microenvironment in supporting tumor progression after chemotherapy.

No conflict of interest.

262 A novel function of Bcl-2 protein: miR-211 regulation in melanoma cells

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Introduction. Melanoma is the most lethal form of skin cancers, whose incidence and mortality has risen rapidly in recent years. It is frequently accompanied by alterations in several tumor suppressors and oncogenes. Among these, the anti-apoptotic Bcl-2 oncogene plays an important role in melanoma progression. Its overexpression is reported in 90% of melanoma cases where it contributes to lack of chemosensitivity. Bcl-2 has also apoptosis-independent functions related toautophagy, proliferation, metastatization and angiogenesis. Also micro RNAs(miRNAs) are emerging as modulators of melanoma development and progression, regulating several molecular pathways, such as invasion and metastasis. Among these, miR-211, located within the melastatin-1 (TRPM1) gene, is prevalently expressed in the melanocyte lineage and acts as oncosuppressor in melanoma, inhibiting cellular migration and invasion. Since we previously showed the capability of Bcl-2 protein to increase tumor progression, we evaluated whether there was a correlation between Bcl-2 and miR-211 expression in melanoma cells.

Material and methods. Human melanoma M14, PLF2, and A375SM cell lines and their Bcl-2 overexpressing derivatives were used. Pri-miR-211, mature miR-211and its target genes,and TRPM1 expression were assessed by quantitative Real-time PCR. Chromatin immunoprecipitation assay was performed to analyze consensus binding elements of MITF transcription factor at the TRPM1 and MLANA promoters. Immunofluorescence (IF) experiments were performed to analyze Bcl-2 and MITF subcellular localization.

Results and discussion. We found that expression of miR-211 is lower in Bcl-2 overexpressing than in parental cells. The mRNA levels of the known mir-211 target genes TGFBR2, IGF2R and NUAK-1 were upregulated in Bcl-2 overexpressing cells, while no modulation of BRN-2 and KCNMA-1 was observed. Reduced expression of pri-miR-211 and TRPM1 levels in Bcl-2overexpressing cells compared to the control cells was also observed. Since miR-211 and TRPM1 expression are co-ordinately regulated by MITF, we analyzed MITF occupancy to TRPM1 promoter and demonstrated that MITF is mainly recruited at TRPM1 promoter in melanoma parental cells compared to the Bcl-2 overexpressing ones. Similar results were observed analyzing MITF occupancy to MLANA promoter, another MITF target gene. IF experiments revealed a minor percentage of cells with nuclear MITF in Bcl-2 overexpressing cells compared to control ones. Complessively, our data evidenced a regulation of miR-211 expression by Bcl-2 protein. Results demonstrating that Bcl-2 reduces MITF nuclear translocation and occupancy of the target promoters indicate that this regulation occurs at the transcriptional level.

Conclusion. Our study demonstrated a new role of Bcl-2 protein as regulator of miR-211 through a mechanism MITF-dependent.

No conflict of interest.

263 Metabolic and proteomic characterization define two different groups of glioblastoma stem-like cells

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Introduction. Glioblastoma multiforme (GBM) represents the most common and malignant brain tumor in adults characterized by a high degree of cellular and genetic heterogeneity. The extremely aggressive behaviour of GBM has been ascribed to a fraction of self-renewing, multipotent tumor-initiating cells, called GBM stem-like cells (GSCs), responsible for tumor progression, maintenance, and recurrence. GSCs can be isolated to generate cell lines which reflect both the genomic and the gene expression profiles of the original tumor.

Material and method. We have collected GSCs from more than 50 GBM patients. These cells have been expanded and validated for their stem cell properties in vitro and in vivo. Gene expression data from all the GSC lines were collected using the Affymetrix GeneChip1.0ST array. Metabolic profiles were analyzed by 1H NMR spectroscopy. The molecular analysis of the signaling pathways was performed by reverse-phase protein microarray technology (RPPA).

Results and discussion. Genomic profiling defined four subtypes of GBM, pro-neural, neural, classical and mesenchymal, based on the expression of signature genes. We have analyzed a collection of 17 patient-derived GBM GSCs by applying complementary molecular approaches. Gene expression profiling of the 1,000 most variable genes/transcripts revealed two distinct GSC clusters reminiscent of the separation into GSf (full stem) and GSr (restricted stem) phenotypes described in the literature. According to our gene expression data, the GSf-like cluster is characterized by a 'pro-neural' gene expression signature, while the GSr-like is characterized by a 'mesenchymal-like' phenotype. NMR metabolic profiling identified the GSf-like subtype as characterized by high N-acetylaspartate (NAA) and α-aminobutyric acid (GABA) and low lipids, while GSr-like subtype showed lack of NAA and GABA and high lipids. These metabolic profiles confirm the association of the GSf-like group with the neural GBM subtype and GSr-like group with the mesenchymal GBM subtype. RPPA revealed a more stem-like signature in the GSf-like group, while GSr-like lines showed increased levels of phosphorylated proteins associated with the EGFR and PI3K/MTOR pathways. We observed that some of the RPPA analytes overlapped between TCGA and our data, displayed significant correlations with the clinical outcome of GBM patients.

Conclusions. These findings revealed that the study of the molecular landscape of GBM might promote the finding of appropriate clinical targets to be exploited for drug discovery.

No conflict of interest.

264 Establishment and characterization of a human metastasic melanoma cell line under serum-low conditions

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Introduction. Skin melanoma is a prevalent disease in the world which causes the majority of deaths related to skin cancer.We have generated a cell line derived from metastatic melanoma, within the strategy derivation of cell lines from tumor biopsies Andalusian Biobank.

Thanks to the obtaining structure of tumor samples launched in the Andalusian Biobank,we can implement a systematic generation and characterization of tumor cell lines.

Material and methods.A 58 years-old woman biopsy, was captured within the strategy of derivation of cell lines in the Andalusian Biobank.

The nature of the sample was cutaneous and subcutaneous tissue.

This tumor cell line was generated under a low concentration of fetal bovine serum. The sample was processed by mechanical and enzymatic digestion and cultured at 37 C, 5% CO2/90% humidity for 12-15 days.Culture media with 2.5% SBF was changed 2 times per week, and subsequently subcultured(1:3).The cell line has been maintained for more than 18 months and passaged over 60 times,and showed a doubling time of 72 hours. Cells culture was subjected to the following analyses: kariotype analysis by G banding ,the cell line was injected in nude mice in different concentrations, the generated line was compared to the source tissue by genetic fingerprinting by a multiple PCR amplification,and inmunohistochemistry detection with primary antibodies against S-100,EMA,CKAE1-AE3,HMB-45,Melan-A and p53. We tested for mycoplasma contamination in this cell line by conventional PCR.Cell line was analyzed morphologically each week,taking a photograph by equipment assembled in the inverted microscope.

Results and discussion. Morphologically these cells are star-shaped and rounded, with numerous accumulations of protein in the cytoplasm and the prominent large nucleus and show growth in monolayer which occasionally appears in cells with numerous extensions.

Multiple cytogenetic alterations,including hipotriploid alterations,with a modal number next to 3n.

The cell line was able to form tumor in one month, reaching a large size,demonstrating the ability of the cell line to form tumors in vivo.

The result of the fingerprinting between cell line and origin tumor sample was coincident.For this purpose,we checked the presence of contamination by mycoplasma, a major problem in many cell lines. The result was negative for this microorganism in different stages of derivation process.

The inmunohistochemistry result was completely negative in CK AE1-AE3 in both, the source and tumor cell line.Cell line showed a pattern of inmunoreactivity similar to tumor origin with respect to Melan-A and S-100,indicating that the cells adapted to in vitro culture come from the neural crest, in an unexpected way,cell line was negative in HMB-45 staining while origin tumor was moderate positive. Contrary,the cell line showed intense positive for p53 antibody being much less positive in the tumor of origin.

Conclusion. This melanoma cells possess morphological features and immunohistochemical markers of a melanoma cell,exhibited malignant melanoma such as high replicative capacity in under serum-low conditions and a strong potential for tumorigenicity and chromosomal instability.

This cell line is useful to advance knowledge of this disease, moreover, the scarcity of such lines, which makes the correct characterization of this line for proper use by the scientific community is very interesting.

No conflict of interest.

265 A therapeutic approach for Cu(II) complex, [Cu2(μ-(C6H5)2CHCOO)3(bipy)2)](ClO4): The potential cytotoxic and apoptotic effects of developed SLNs formulation as drug delivery system

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Introduction. Metal complexes demonstrate anticancer effects through binding to DNA, which is the main strategy to develop new or more efficient antitumor drugs. Cu(II) complex has extensively studied for anti-cancer research because of having a significant binding affinity for DNA and this complex indicates antiproliferative and antiapoptotic properties through ligand exchange. However this complex can not be able to target cancer cells without damaging normal cells.

Drug delivery systems have been used in recent years to overcome limitations of chemotherapeutic drugs. Solid lipid nanoparticles (SLNs) have a great potential in cancer treatment due to unique properties including controlled drug release and reduced side effects. Therefore, the aim of this work was to investigate the coordination properties Cu (II) complex with SLNs formulation and the potential cytotoxic and apoptotic effects of this complex-SLNs formulation on MCF-7 breast cancer and HUVEC control cells.

Material and Methods. A novel complex of Cu(II) has been synthesized with the reaction of 2,2'-bipyridine (bipy) and diphenyl acetic acid, (C6H5)2CHCOOH) (dpaa) and characterized by X-ray crystallography and other spectroscopic techniques. After synthesis of metal complex, Cu(II) complex loaded SLN formulations were produced by hot homogenization **Methods.** Cu(II)-SLNs formulation was characterized by Nano Zeta Sizer. The potential cytotoxic activity of Cu(II)-SLNs formulation on MCF-7 and HUVEC (control) cells was determined by WST-1 test compared with Cu(II) complex at the certain concentration (2,5–50 μM/mL). Additionally, the apoptotic death rate was measured by MuseTM Cell Analyzer.

Results. The size of SLN formulations ranged from 190 nm to 350 nm. When MCF-7 cells exposed to the concentration 50μM/mL of Cu(II) complex, the cell viability of reduced 33.1% after treatment of 48 h whereas the cell viability of HUVEC cells treated by Cu(II)-SLNs (70.1%) much less than that treated by Cu(II) complex (30.5%) ($p<0.05$). Furthermore, early and late apoptosis rates were found 24.30±2.35% after exposure Cu (II) -SLNs treatment on MCF-7 cells for 48 h.

Conclusions. The obtained result showed that the Cu(II)-SLNs formulations were less effective as compared to the Cu(II) complex against MCF-7 cells however, they

cause less damage on HUVEC cells. Thus, Cu(II)-SLNs are promising delivery system to reduce side effects of Cu(II) complex.

Keywords: Metal complex, Cu(II) complex, apoptosis, MCF-7 cells.

No conflict of interest.

266 Establishment of a program of cytogenetic cell lines control derived and deposited in a biobank for potential clinical use and research

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Introduction. The Biobanks are gaining importance as tools in the field of biomedical research. In the biobank, the processes, biospecimen and data collection are integrated. Besides, the future use of biological samples of human origin requires a comprehensive control assurance with regard to quality control, safety and traceability. Thus, the introduction of a program in determining cytogenetic chromosomal stability is a key point in the establishment of quality controls for the use of samples from the biobank. Therefore, one of the checks we carried out to biobank samples for use in biomedical research, is the detection of genetic instability and chromosomal abnormalities that represent a potential weakness in basic studies and future therapeutic applications based on the use of these biological samples of different nature.

Material and method. In our cytogenetic unit, a total of more than 300 samples have been analyzed in the last years of different origin as pluripotent stem cells (iPSCs and hESCS), fibroblast cell lines, tumor cells lines and multipotent stem cells, specifically mesenchymal stem cells (hMSCs), being these last kinds derived in the biobank. After obtaining a cell culture with 80% confluent cells, we characterized the cell lines using, conventional cytogenetics, in some cases, in combination with spectral karyotiping (SKY), and in situ hybridization (FISH). The techniques were developed in the context of a quality system with standardized procedures duly documented by internal validations and pertinent literature.

Results and discussion. The results in the characterization of pluripotent stem cells (iPSCs and hESCs), are consistent with what has been described in the literature that one of the main issues of these cells is the appearance of chromosomal abnormalities after prolonged periods of culture. Our results on the characterization of pluripotent stem cells have been strong to determine chromosomal abnormalities in a very high percentage of the cells characterized. Regarding fibroblast cell lines and derived msesenchymal stem cells (hMSCs) in the Biobank, have also been characterized, and in this case has prevailed chromosomal stability, the frequency of cytogenetic growth test set for these cells is lower. We have found very different results, for cytogenetic characterization in samples derived from solid tumors of different origin, depending on the nature of the starting tumor, and evolution of the culture. The result has been compared with the classification of http://atlasgeneticsoncology.org/index.html, and http://cgap.nci.nih.gov/Chromosomes/Mitelman.

Conclusion. Genetic instability and chromosomal abnormalities represent a potential weakness in basic studies and future therapeutic applications based on these stem cell lines, and may explain, at least in part, their usual tumourigenic properties. So, the introduction of the cytogenetic programme in the determination of the chromosomal stability is a key point in the establishment of the stem cell lines and tumoral lines. However, many aspects of the cellular biosafety about cells lines remained unclear. For this reason, is important to elucidate their potential susceptibility to malignant transformation and to ascertain the safety of these cells for clinical use and research use, and we consider, in our biobank, the need to know the chromosomal status of the cells lines.

No conflict of interest.

267 Endothelin A receptor drives invadopodia function and cell motility through ß-arrestin/PDZ-RhoGEF pathway in ovarian carcinoma

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Introduction. The endothelin-1 (ET-1)/ET A receptor signalling pathway plays a critical role in epithelial ovarian cancer (EOC) progression by driving different tumor promoting processes. One key process promoted by ET-1 stimulation is tumor cell motility, which requires the scaffolding functions of β-arrestin-1 (β-arr1); however, the specific protein complexes, activated by ETAR, controlling actin dynamic remain poorly understood. Here, we tested the hypothesis that ETAR /β-arr1 might regulate the formation of invasive protrusions, called invadopodia, by acting through the Rho-GTPase signalling pathway, associated with actin remodelling and cell motility. Material and methods. In different EOC cell lines, the expression, localization, activation and protein interactions are evaluated by qRT-PCR, immunoblotting, immunoprecipitation, immunofluorescence (IF), and pull down assays. The invasive and migratory capabilities are evaluated by wound healing and chemoinvasion assays. Formation of invadopodia are analyzed by IF and fluorescent gelatin degradation assays. In vivo analysis is performed in murine orthotopic EOC xenografts. Results and discussion. We found that in EOC cells, expressing RhoA and RhoC, ET-1 is able to activate Rho GTPase signalling, an effect associated with enhanced cell invasion. This is accomplished by the interaction of β-arr1 with one member of Rho-guanine nucleotide exchange factor (Rho-GEF) family PDZ-RhoGEF. The ETAR-mediated invasive phenotype is related to the reorganization of actin cytoskeleton in dot-like structures (invadopodia), colocalization of F-actin/cortactin, activation of cofilin pathway and subsequent invadopodia-mediated matrix degradation. The ET-1-induced ROCK/LIMK/cofilin pathway through the control of RhoC GTPase and invadopodia function, are significantly impaired by depletion of PDZ-RhoGEF or β-arr1, as well as by the treatment with ET-1 receptor antagonist macitentan, confirming that β-arr1/PDZ-RhoGEF interaction mediates ETAR-driven cell invasion. In vivo, macitentan is able to inhibit tumor growth, metastatic dissemination and both Rho GTPase expression. Moreover we found, by screening EOC human tissues, that PDZ-RhoGEF, RhoA and RhoC are overexpressed in these tumors samples compared with normal tissues and that ETAR expression positively correlates with RhoC expression. Conclusion. Collectively, the data establish a novel role for β-arr1/PDZ-RhoGEF as regulators of ET-1-induced motility, invadopodia function and metastasis, confirming the role of ET-1 axis in invadopodia formation and associated invasive activity required for EOC tumor progression.

No conflict of interest.

269 Cellular activity of microRNAs dysregulated in breast cancer

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Introduction. Breast cancer (BC) is one of the major health problems worldwide and it is the second cause of cancer-related death in women. Patients often develop resistance to the current therapies. For this reason, the identification of new specific clinical molecular markers and pharmacologic targets in cancer research is an ongoing challenge. In previous studies, we have identified miRNAs associated with metastasis and outcome in breast cancer. (Volinia S. et al. Genome Res. 2010; Volinia S. and Croce CM. PNAS 2013). Additionally, the embryonic stem cell MIR302/MIR203 asymmetry was associated with stem cell markers, metastasis and shorter survival in BC. (Volinia S. et al. JNCI 2014).

Materials and Methods. We studied a number of human BC-derived cell lines (MCF-7, MDA-MB-231, MDA-MB-468, MDA-MB-361, SKBR3, T47D, BT474, ZR75.1, MDA-MB-453, HBL-100) and breast non-tumor cell lines (184A1, MCF10A). All cell lines were transfected with either one of 38 miRNAs (miR-21, 26b, 28-5p, 33b, 99a, 126, 126*, 130b, 138, 142-5p, 143, 181a, 202, 203, 206, 210, 218, 222, 145, 301a, 302a, 320c, 326, 484, let-7d*, 93, 103, 1307, 148, 328, 874, 151, 10a, 25, 30a, 615, 27a, 9), identified as important in BC in our previous studies.

Cell proliferation was determined by means of the xCELLigence RTCA System. The analysis of cell viability was carried out by MTS. The miRNAs were further investigated for their capacity to affect cell migration, cell invasion, and RNA profiles.

Results. The main outcome of our studies has been the identification from such wide list of a few miRNAs involved in the regulation of cell growth and invasion. In a first, not yet exhaustive screening, we have also identified some genes, which negatively correlated with those key miRNAs and represent possible candidate targets.

Conclusions. In this work, we investigated the possible causal role of microRNAs associated to breast cancer. We can conclude that we could dissect and prioritize in vitro the functional role of miRNAs in breast cancer.

No conflict of interest.

270 Autocrine activation of HGF/c-Met signaling mediates acquired resistance to sorafenib in HCC cells

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Introduction. Liver cancer is the sixth most common cancer worldwide and ranks third in cancer-related mortality. Hepatocellular carcinoma (HCC), the most common type of primary liver cancer has limitations in treatment due to poor prognosis and resistance against traditional radiotherapy and chemotherapies.Sorafenib, a multikinase inhibitor of VEGFR, PDGFR and Raf, is the only FDA approved drug that can prolong survival for up to three months. Furthermore, second-line treatment for patients who cannot tolerate or develop resistance to sorafenib is an urgent medical need. The better understanding of sorafenib resistance in HCC is needed in planning effective treatment strategies, however; the underlying mechanisms still remain unclear. In this study, we developed two sorafenib resistant HCC cell lines from Huh7 and Mahlavu (MV) cells, by long-term sorafenib exposure. Through the comparison of the molecular changes between wild type and resistant (Huh7R and MVR) cells, we observed that hepatocyte growth factor (HGF)/c-Met signaling pathway plays a significant role in mediating acquired resistance to sorafenib in HCC cells.

Materials and Methods. Huh7 and MV cells were treated with sorafenib in increasing doses over a period of 3 months and MTT assay was regularly performed to assess reduction in sorafenib sensitivity. The morphology of resulting sorafenib-resistant cells was evaluated using phalloidin staining. The expressions of c-Met, p-Met and HGF were determined by Western blotting and immunofluorescence microscopy while HGF transcription was determined by RT-PCR. Cell motility and invasion assays were done using transwell inserts. Apoptosis was evaluated by flow cytometry using Annexin V/PI staining. SU11274 was used to inhibit c-Met in HCC cells.

Results and Discussion. The resulting sorafenib resistant Huh7R and MVR cells exhibited a mesenchymal-like morphology that was evident under light microscopy and by F-actin organization. In contrast to low basal levels of both c-Met expression and activation in wild type cells, we determined increased levels of c-Met expression and the active phosphorylated form of c-Met (Tyr1234/1235) in

HuhR and MVR cells. Moreover, HGF transcription and protein levels were increased in resistant cells indicating autocrine activation of HGF/c-Met signaling. Compared to wild type cells, HuhR and MVR cells displayed increased invasion and migration ability which can be reversed by the addition of c-Met inhibitor, SU11274. Furthermore, the combination of sorafenib and SU11274 restored the sensitivity of resistant cells to sorafenib induced apoptosis.

Conclusion. These results present a new mechanistic view into the acquisition of sorafenib resistance and suggest that HGF/c-Met inhibitors comprise promising candidates in combinational therapies with sorafenib and/or as second-line treatments after acquisition of sorafenib resistance.

No conflict of interest.

271 Deep sequencing of glioblastoma tissues and peritumoral regions reveals mRNAs and microRNAs depicting molecular pathways shared by tumors and surrounding areas and highlights differences between short-term and long-term survivors

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Introduction. Glioblastoma multiforme (GBM) is the most common and deadliest primary brain tumor. Its prognosis is inexorably unfavorable, as these tumors drive affected patients to death usually within 15 months after diagnosis (short term survivors, ST), with the only exception of a small fraction of patients (long term survivors, LT) surviving longer than 36 months. Even after the frontline therapeutic approach, including surgical resection followed by chemo- and radiotherapy, the cause of death in most cases is tumor recurrence, which occurs in peritumoral tissues in about 95% of patients. Here, we provide a comprehensive molecular analysis of a set of ST and LT samples derived from frankly tumoral areas (C) and from the peritumoral regions (P) of the same patients.

Material and Method. We collected frankly tumoral areas (C) as well as peritumoral areas from 4 long term (LT) and 9 short term (ST) patients diagnosed with primary glioblastoma, and we submitted total RNAs extracted from these samples to SAGE profiling and microRNA deep sequencing.

Results and Discussion. By performing SAGE analysis and microRNA deep sequencing, we collected data showing that P areas differ from healthy white matter, but share with C samples, a number of mRNAs and microRNAs. These molecules are representative of extracellular matrix remodeling, TGFb signaling, and of the involvement of cell types different from tumor cells but contributing to tumor growth, such as microglia or reactive astrocytes. Moreover, we provide evidence about RNAs differentially expressed in ST vs LT samples, suggesting the contribution of TGF-b signaling in this distinction too. We also add a further piece to this complex molecular view, by showing that the edited form of miR-376c-3p is reduced in C samples vs P ones and in ST tumors compared to LT ones.

Conclusion. As a whole, our study, besides confirming previous data about GBM-specific molecular markers, provides new insights into the still puzzling distinction between ST and LT tumors. In addition, it sheds new light onto that 'grey' zone represented by the area surrounding the tumor, which we show to be characterized by the expression of several molecules shared with the proper tumor mass. This provides a molecular basis to the contribution of the peritumoral areas to glioblastoma growth.

No conflict of interest.

272 Anti-apoptotic effects of lentiviral vector transduction promote Rituximab Tolerance in cancerous B cells

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Introduction. Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin Lymphoma in adults. It is a clinical and molecular heterogeneous disease where the 5 year overall survival rate is 60% illustrating that some patients are either unresponsive to initial treatment or develop drug resistance. Therefore, identifying mechanisms and biological markers involved in treatment resistance are becoming increasingly important. Rituximab, an antibody targeting CD20 that is expressed on pre-B-cells and more differentiated B-cell stages, is used as a treatment strategy against DLBCL. In recent years, lentiviral vectors have emerged as powerful tools to investigate and map mechanisms of drug response in malignant B cells through genetic manipulation of specific target gene expression. In this regard, lentiviral vectors effectively deliver genes to cancerous B cells, which are often hard to transfect with nucleic acids. However, the effects of lentiviral transduction itself on the recipient malignant B-cells remain unexplored. In the present study, we investigated the impact of lentiviral vector transduction on mechanisms that contribute to Rituximab resistance in cancerous DLBCL cells.

Materials and Methods. We used human embryonic kidney (HEK293T) cells as producer cells for production of VSV-G-pseudotyped lentiviral vector and performed flow cytometry analysis to determine transduction levels in human DLBCL cell lines. Cell proliferation was measured 48 hours after Rituximab treatment using Trypan Blue staining and direct cell counting and was confirmed by BrdU assay and flow cytometry analyses. Also, the apoptosis rate was measured using an anti-cleaved-PARP antibody. Expression levels of a set of markers were measured by qPCR and flow cytometry, and a protein dot blot-based screening approach as well as Western blotting was applied to detect apoptosis-related protein variations.

Results and Discussions. We demonstrate robust lentiviral transduction of cancerous B-cell lines of DLBCL origin and show that the resistance of transduced cells to Rituximab, but not to doxorubicin, is markedly enhanced relative to untransduced controls. This indicates a strong anti-apoptotic effect that is specific for Germinal Center B-Cell like (GCB) cells and is independent from complement-directed cell lysis. Comparing protein expression levels in transduced versus non-transduced cells, we find that Clusterin is upregulated in transduced cells and may thus play a role in acquired tolerance after lentiviral vector transduction. Notably, we find that cancerous B-cells lose the CD43 expression in response to Rituximab and that this effect is smaller in cells with lentiviral vector-induced tolerance. Our results point to CD43 as a potential marker for the response to Rituximab and demonstrate that the biological impact of lentiviral transduction of B cells may directly influence the outcome of drug sensitivity studies.

No conflict of interest.

273 A N-truncated isoform of the orphan nuclear receptor COUP-TFII modulates pancreatic cancer progression

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Introduction. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer Worldwide. COUP-TFII is a orphan nuclear receptor (NR) involved in cancer and other human diseases. We recently demonstrated that COUP-TFII is a marker of poor prognosis and a good candidate as PDAC biomarker; the NR expression is associated to more advanced clinical stages and the presence of metastases and patients positive for the NR had a lower overall survival. Interestingly, one variant of COUP-TFII lacking the DNA binding domain (COUP-TFII_V2, whereas COUP-TFII_V1 is the full-lenght isoform) has been recently described with conflicting results. Consequently, the AIM of our study was to evaluate the presence and function of COUP-TFII variants in PDAC.

Materials and Methods. COUP-TFII variants expression in PDAC cells and primary tumors was evaluated by qPCR, Western Blot (WB), and immunofluorescence (IF); proteins localization was evaluated by IF, EGFP-tagged proteins, and WB on cell fractions; transcriptional activity was tested by luciferase assay. Cell motility was tested by time-lapse experiments. Resistance to oxidative stress was tested by H_2O_2 treatment.

Results and discussion. By qPCR we demonstrated that COUP-TFII isoforms are differentially expressed in PDAC compared to normal duct cells. Using a new specific COUP-TFII_V2 antibody, fusion proteins, and WB, we demonstrated that the V2 isoform is both nuclear and cytosolic, conversely to the V1 variant, and that the two proteins co-assemble. COUP-TFII_V2 overexpression leads to COUP-TFII_V1 translocation in the cytosol. Noteworthy, the V2 isoform increases the transcriptional activity of COUP-TFII_V1 promoter. Overexpression of both variants modifies cell response to oxidative stress. Given that COUP-TFII has been associated to cell motility and Epithelyal to Mesenchymal Transition (EMT) we analyzed the role of COUP-TFII variants by time-lapse demonstrating that COUP-TFII_V2 alters cell motility, specifically increasing cell speed. Interestingly, cellular distribution of COUP-TFII_V2 varies among cells being apparently associated to cytoskeleton modifications.

Conclusion. Intriguingly, our results suggest that the isoform COUP-TFII_V2, lacking the DBD, may possess a not genomic function being implicated in cytoskeleton organization and EMT and might regulate COUP-TFII_V1 localization and expression. Considering COUP-TFII association to PDAC the parallel exploitation of COUP-TFII isoforms might results in novel therapies for PDAC.

No conflict of interest.

274 All-trans retinoic acid modulates epithelial to mesenchymal transition and inhibits cell motility in ERBB2/RARA amplified breast cancer cells: Role of NOTCH1 and TGFß pathways

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Introduction. All-trans retinoic acid (ATRA) is a natural compound and an agent proposed for the treatment/chemo-prevention of breast cancer. Increasing evidence indicates that aberrant regulation of epithelial-to-mesenchymal transition (EMT) is a determinant of the cancer cell invasive and metastatic behavior. The effects of ATRA on EMT are still largely unknown.

Material and Methods. The action of ATRA on cell plasticity and EMT was evaluated in 2D and 3D cultures of HER2-positive (HER2+) breast cancer cell lines. The anti-migratory activity of the retinoid was studied in a model of growth factor-induced EMT with the use of Boyden chambers. The same cellular model (SKBR3) was used to perform molecular studies on the determinants of ATRA anti-migratory action.

Results and Discussion. In HER2-positive SKBR3 and UACC-812 cells, which show co-amplification of the ERBB2 and RARA genes, ATRA activates a RARα-dependent epithelial differentiation program. In SKBR3 cells, this causes the formation/

re-organisation of adherens and tight junctions. Epithelial differentiation and augmented cell-cell contacts underlay the anti-migratory action exerted by the retinoid in cells exposed to the EMT-inducing factors, EGF and Heregulin-β1 (Herg). Down-regulation of SNAIL and NOTCH1, two major players in EMT, are involved in the inhibition of motility by ATRA. Indeed, the retinoid blocks NOTCH1 and SNAIL up-regulation by EGF and/or Herg and translocation of the latter protein to the nucleus. Inhibition of γ-secretase and NOTCH1 processing by DAPT also abrogates SKBR3 cell migration. Stimulation of TGFβ contributes to the anti-migratory effect of ATRA. The retinoid switches TGFβ from an EMT-inducing and pro-migratory determinant to an anti-migratory mediator. Inhibition of the NOTCH1-pathway is relevant also for the anti-proliferative activity of the retinoid in HCC-1599 breast cancer cells, which are addicted to NOTCH1 for growth/viability. This effect is enhanced by the combination of ATRA and DAPT supporting the concept that the two compounds act at the transcriptional and post-translational levels along the NOTCH1-activation process.

Conclusions. In retinoid-sensitive ERBB2+/RARA+ cells, ATRA regulates breast cancer cell-plasticity altering cell-to-cell contacts and cell-migration through regulation of crucial EMT determinants. NOTCH1 is a new target/mediator of ATRA anti-migratory and anti-proliferative activities.

No conflict of interest.

275 The effect of metformin on the proliferation of H295R cell line

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Introduction. Adrenocortical carcinoma (ACC) is a rare, heterogeneous malignancy with a poor prognosis, mainly dependent on tumor stage at diagnosis. To date, radical surgery, possibly associated to mitotane adjuvant therapy, is considered the best option for ACC treatment. However, in the case of metastatic ACC, the mean 5-year survival rate diminishes dramatically and mitotane efficacy has not been proved. Moreover, chemo-resistance often develops. Thus, more specific and effective drugs for ACC treatment are urgently required. The antidiabetic drug metformin, used as first line therapy for type II diabetes treatment, has been proved to exert antineoplastic effects in many type of malignancies. Our study aimed to evaluate the potential anti-cancer effects of metformin in H295R adrenocortical tumor cell line, looking for a possible therapeutic option for ACC treatment.

Materials and methods. H295R cells were treated with increasing doses of metformin (0,5-250 mM) for 24h, 48h, 72h and 7 days, alone or in combination with mitotane. Cell viability and proliferation were evaluated by MTS and cell count assay, respectively. [3H]thymidine incorporation assay was also perfomed. Western blot analysis at 6- and 24- hour metformin treatment (20, 50, 100 mM), was used to investigate the molecular pathways involved in mediating the drug effect.

Results and discussion. In our cell model, metformin inhibits cell viability and proliferation in a dose- and time-dependent manner. Moreover, metformin/mitotane combined treatment reduces cell viability to a higher extent than metformin alone, suggesting the presence of a synergistic effect. Considering the molecular mechanisms by which metformin may function in inhibiting cell growth, we observed a dose-dependent decrease in ERK1/2 and m-TOR phosphorylation and an increased AMPK activation.

Conclusion. Our data indicate that metformin is able to interfere with the in vitro cancer cell proliferation, showing an effect which depends on the drug concentration and treatment duration. Furthermore, the synergistic effect observed in the presence of mitotane suggests the possible use of metformin in combination with the current therapy for ACC. Further in vivo studies are necessary to prove metformin efficacy in adrenocortical carcinoma.

No conflict of interest.

276 Identification of cisplatin-resistance related genes in ovarian clear cell carcinoma using 3D cell culture system

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Introduction. Ovarian clear cell carcinoma (OCCC) is well known for its higher prevalence in Asian women and poorer prognosis as compared with other histotypes of epithelial ovarian cancer (EOC) due to higher rate of resistance to platinum based chemotherapy. However, the underlying mechanism has not been fully understood. Three-dimensional (3D) cell culture system with extracellular matrix components mimics the in vivo microenvironment and provides more accurate drug responses than two-dimensional (2D) monolayer cell culture. In this study, we adopted 3D cell culture system to study drug responses and to identify cisplatin-resistance associated genes.

Material and method. 3D cell culture system utilizing Geltrex® Matrix was developed to test cisplatin responses in a panel of OCCC cell lines and advanced serous ovarian cancer cell lines and compare with conventional 2D system. To further delineate the cell signaling associated with this drug response,the gene profiling analysis (Human Transcriptome Array 2.0) of OCCC cell line TOV-21G cells cultured in 2D and 3D system has been adopted.

Results and discussion. Ovarian cancer cell lines underwent morphologic changes in 3D cell culture system and showed remarkable higher cell survival ability upon cisplatin treatment than 2D cell culture system. Western Blot analysis using cleaved PARP and cleaved Caspase3 as well as TUNEL assay demonstrated that ovarian cancer cells including both OCCC and advanced serous cancer cells exhibited lower apoptotic rates in 3D cell culture system than 2D system against cisplatin treatment. Gene expression analysis revealed 120 up-regulated (>2.0-fold) genes and 323 down-regulated (>2.0-fold) genes in OCCC cells cultured in 3D cell culture system. The involved signaling pathways including regulation of cell cycle, DNA damage response, cell metabolism, autophagy, ErbB signaling and G Protein-Coupled Receptors signaling were significantly altered.

Conclusion. These results demonstrated that 3D spheroids of OCCC and advanced serous cell lines exhibited higher cisplatin-resistance than 2D system through dysregulation of numerous signaling pathways concerning different aspects of cell survival. This dysregulation may be generated by intrinsic modification and interaction with extracellular matrix. To study the underlying molecular mechanisms of matrix-mediated cellular changes, further studies to analyze the putative targets of the related signaling are warranted.

No conflict of interest.

277 Role of miR-1247 in prostate cancer progression

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Introduction. The Cancer Associated Fibroblasts (CAFs) are predominant components of the tumor microenvironment and key determinants in the malignant progression of cancer cells. CAFs enhance the invasion and proliferation potential of the PCa cells, favoring cancer progression. Here, we investigated the involvement of microRNAs in the tumor-stroma interplay as potential candidate mediators of the prostate tumor progression.

Materials and Methods. miRNAs expression were profiled by Illumina Human v2 miRNA bead chip. Invasion assay was performed by Boyden chamber coated with matrigel. Angiogenesis tubule formation assay were performed coating HUVEC on reduced growth factor matrigel.

Results and discussion. We performed the miRNAs expression profile in PCa cells undergoing CAF-induced EMT and we found that miR-1247 was significantly down-modulated. Moreover, DIO3OS, the gene coding for miR-1247 is down regulated in tissue explants from metastatic tumor than in normal prostate tissue, highlighting miR-1247 possible role as a negative regulator in prostate cancer metastasis.

Successively, we demonstrate that the ectopic expression of miR-1247 impairs the CAF-mediated invasion of PCa cells through the re-expression of the epithelial marker E-cadherin and inhibits the mesenchymal markers ZEB1, SNAIL, and vimentin. Furthermore, thanks to in silico dataset analysis we discovered that one of the target of miR-1247 is neuropilin-1 (NRP-1), a co-receptor of VEGFR-2 and EGFR, involved in pro-angiogenic and pro-survival pathways.

Immunoblot analysis confirms that NRP-1 is a specific target of miR-1247 and the overexpression of this miRNA in PCa co-cultured with endothelial cells leads to an inhibition of capillary morphogenesis.

Finally, upon EGF treatment, NRP-1 down-regulation induced by ectopic expression of miR-1247 reduces AKT and MAPK signaling, thereby impairing cell survival.

Conclusions. These findings suggest that miR-1247 is key negative regulator of the CAF-induced aggressiveness in PCa cells and it could represent a potential biomarker of prostate cancer progression.

No conflict of interest.

279 Molecular differences between 1,25-dihydroxyvitamin D3-sensitive and -resistant acute myeloid leukemia cells

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Introduction. Acute Myeloid Leukemia (AML) is a group of malignant blood disorders characterized by an accumulation of immature myeloid cells. Differentiation-induced therapy seems to be a very attractive alternative of medical intervention. 1,25-dihydroxyvitamin D3 (1,25D) is capable of inducing monocyte/macrophage differentiation, but not in all subtypes of AML. Due to the diversity of genetic aberrations and heterogeneity of AML cells, responsiveness to 1,25D is variable. Since the development of drug resistance is one of the major problems of cancers treatment, studies of this phenomena may lead to improved therapy. Therefore, in order to develop a new model for such studies, we have exposed 1,25D-sensitive HL60 cells to increasing concentrations of 1,25D for a period of 2 years. We obtained the stepwise 1,25D-resistant cell line, named HL60-EG. We will discuss here the molecular changes in these cells and their potent clinical importance for appropriate AML patient's selection in 1,25D-targeted therapy.

Materials and Methods. Cell lines, chemicals, antibodies - HL60 cells were from the European Collection of Cell Cultures, KG1 cells from DSMZ GmbH and were grown in standard conditions. 1,25D was from Cayman Europe, ATRA from Sigma. Anti-CD11b-FITC, anti-CD14-PE, isotypic controls were from ImmunoTools; primary antibodies for western blotting were from Santa Cruz Biotech.; secondary from Jackson ImmunoResearch.

Flow cytometry - after 96 h incubation with 1,25D, cells were stained with appropriate antibody and analysed on FACS Calibur.

Real-Time PCR - tRNA was isolated using TriPure (Roche), then was transcribed into cDNA using High Capacity cDNA RT Kit (Applied). Real-Time PCR reaction was

performed using SensiFastTM SYBR Hi-ROX Kit (Bioline) and Eco Real-Time PCR System (Illumina). The sequences of primers were published before.

Fractionation - cells were fractionated into cytosolic and nuclei fractions as published before. Mitochondria were isolated using Pierce Mitochondria Isolation Kit (Thermo). Fractions were separated in SDS-PAGE and blotted. Proteins were detected with antibodies.

Results and discussion. HL60-EG cells do not express CD11b and CD14 monocyte/macrophage markers after 1,25D treatment. Their resistance cannot be overcome by ATRA. VDR mRNA and protein levels are very low, and do not change after 1,25D or ATRA treatment. VDR is transcriptionally inactive in HL60-EG cells and does not activate CYP24 gene or protein in response to 1,25D.

Conclusion. HL60-EG cell line offers a novel study tool for leukemia cells resistance to 1,25D compounds. Detailed studies may explain mechanisms of resistance and help to select AML patients in which targeted therapy with 1,25D compounds will be effective.

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No conflict of interest.

280 Sirtuins as metabolic sensors of tumor: Stroma crosstalk

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Introduction. Tumor cells exhibit metabolic reprogramming according to nutrients availability to meet their demands for energy, rapid proliferation and metastasis. Furthermore, a vicious metabolic synergy between stromal and cancer cells has been reported in different tumor models. In particular, cancer associated fibroblasts (CAFs) play a key role in prostate cancer aggressiveness, as well as in its metabolic reprogramming toward a stroma-derived lactate-based oxidative metabolism. We have recently demonstrated that Src kinase activity is mandatory for lactate-dependent respiratory phenotype of PCa cells, acting by functional regulation of pyruvate kinase (PKM2) (manuscript submitted). In this context, tumor:stroma metabolic crosstalk should involve different regulations including nutrients sensing pathways and, in particular, the NAD+-dependent deacetylases Sirtuins.

Material and methods. Prostate carcinoma cell lines (PCa) and prostate fibroblasts isolated from healthy and cancerous regions of PCa-bearing patients (Gleason 4+5) were used. TMRE dye was used for label mitochondria. Redox-insensitive Src mutants were used to disrupt Src redox regulation.

Results and Discussion. PCa cells show an high [NADH/NAD+] ratio – due to CAF-derived lactate import – upon 24h CAFs contact, while such pattern is inverted after 48h as OXPHOS is activated. Lactate upload, either exogenously added or supplied by neighbouring CAFs, leads to increase of SIRT1 and mitochondrial SIRT3 in PCa cells. We also observed that CAF-mediated SIRT1 upregulation significantly decreases the acetylation of its target PPARg coactivator 1 alpha (PGC1a), a regulator of mitochondrial biogenesis. Accordingly, in cancer cells both mitochondrial mass and potential are increased upon CAF contact. Finally, Src-mediated redox regulation of PKM2 is mandatory for CAF-induced NADH/NAD+ fluctuations, SIRT1 upregulation and PGC1a activation in PCa cells.

Conclusion. Our data suggest that CAFs affect sirtuins expression in cancer cells and, in particular, SIRT1/PGC1a axis seems to be involved in the regulatory machinery of prostate tumor:stroma metabolic reprogramming.

No conflict of interest.

281 A novel role of phosphatydilcholine-specific phospholipase C in modulating CXCR4 chemokine receptor in human glioblastoma cells

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Introduction. CXCR4/CXL12 molecular axis has recently been described to play a crucial role in tumors. In gliomas CXCR4 is involved in several mechanisms as metastasis, tumor proliferation and angiogenesis. Previous studies, in our laboratory on the aberrant phosphatidylcholine (PC) metabolism in cancer cells demonstrated that inhibition of PC-specific phospholipase C (PC-PLC) in tumor cells down-modulates the expression of growth factor membrane receptors, interfering with cell receptor-activated signal transduction pathways involved in tumor progression. These data suggested a relationship between the PC cycle and cell receptor status. The present study was aimed to investigate the crosstalk between CXCR4 receptor and PC-PLC enzyme in a human glioblastoma (GBM) cell line.

Material and Method. A human GBM cell line, U87MG, was treated with a specific noncompetitive antagonist of CXCR4, AMD3100 (Plerixafor®), and a selective PC-PLC inhibitor, the xantate D609. Confocal Laser Scanning analyses, Immunoprecipitation and Western Blot experiments were performed on U87MG cells to analyze both CXCR4 and PC-PLC expression. To investigate the effects of drugs on cell metabolism Magnetic Resonance (MRS) experiments were performed on U87MG ethanol extracts using a Bruker Avance 400 spectrometer (9.4 Tesla). Intracellular glucose levels and lactate dehydrogenase (LDH) activity were detected using a colorimetric enzymatic activity assay.

Results and Discussion. Our studies demonstrated for the first time that CXCR4 and PC-PLC co-localize and are associated at the level of U87MG cell membrane. Both inhibitors, AMD3100 and D609, induced a down-modulation of PC-PLC and CXCR4 expression on cell surface, but D609 only induced a reduction in the CXCR4 total content in U87MG cells. Preliminary experiments showed a reduction in the U87MG cells invasive capacity after treatment with both inhibitors, D609 having a more pronounced effect that could be associated with CXCR4 downregulation. Exposure to D609 of U87MG cells induced a significant and long-lasting anti-proliferative effect (44%), as well as a decrease in the AKT phosphorylation level; while AMD3100 treatment even affecting the amount of phosphorylated AKT did not induce changes in the U87MG proliferation rate. MRS analyses revealed alterations of U87MG metabolism, with an increase of choline-containing metabolites (Glycerophosphocholine, GPCho; Choline, Cho) (77%) that could be linked with activation of lipases and phospholipases. MRS highlighted a decrease in intracellular Lactate concentration (38%), together with a decrement of 43% in LDH activity, suggesting that inactivation of PC-PLC could impair the glycolysis pathway in U87MG.

Conclusion. Our results suggest that PC-PLC inhibition could modulate CXCL12/CXCR4 axis and could represent a new molecular strategy to target CXCR4-overexpressing glioma cells.

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No conflict of interest.

283 Feasibility of a workflow for the molecular characterization of single circulating tumor cells by next generation sequencing

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Introduction. Circulating Tumor Cells (CTCs) represent a 'liquid biopsy of the tumor' which might allow real-time monitoring of cancer biology and therapies in individual patients. CTCs are extremely rare in the blood stream and their analysis is technically challenging.

The purpose of the study was to explore the feasibility of a protocol for the molecular characterization of single CTCs.

Materials and Methods. CTCs were immunomagnetically enriched and enumerated by the CellSearch® system in blood samples collected from four metastatic breast cancer patients and subsequently isolated by a single-cell sorter (DEPArray™ system) to obtain single CTCs to be submitted to whole genome amplification. Samples (3-5 single CTCs per patient) were analyzed by NGS on the Ion Torrent™ system using the Ion AmpliSeq™ Cancer Hotspot Panel v2 able to investigate genomic 'hot spot' regions of 50 oncogenes and tumor suppressor genes.

Results and Discussion. We found 53 sequence variants in 26 genes: 36 variants with possible deleterious consequences and 17 supposed benign variants on the basis of the PolyPhen software. Twenty-two mutations were already reported in the COSMIC database.

The gene with the highest number of sequence variants is TP53 (with 10 variants) followed by PDGFRA (5 variants) and KIT (4 variants).

We observed inter- and intra-patient heterogeneity in the mutational status of CTCs.

In 3 patients we could compare the NGS results from CTCs with those from the primary tissue evidencing few mutations common to the two different compartments. The discordance between the mutational status of the primary tumor and CTCs suggests that CTCs in advanced stages may reflect the disease status better than the primary tumor.

Conclusion. This study demonstrates the feasibility of a non-invasive approach based on the liquid biopsy in metastatic breast cancer patients.

No conflict of interest.

284 Pro-inflammatory cytokines and chemokines analysis in HPV-positive cancer cells

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Introduction. Recent data have expanded the concept that inflammation is a critical component of tumor progression. Many cancers arise from sites of infection, chronic irritation and inflammation. It is becoming clear that the tumor microenvironment is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. Tumor cells co-opt some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. Virus-induced tumors, like cervical high risk Papillomavirus-induced Squamous Cell Carcinomas (SCC), represent a paradigmatic example of the tight interplay between inflammatory responses and malignant transformation as inflammation is an integral part of the innate antiviral response.

Material and method. To study the tumorigenic role of inflammatory mediators in cutaneous and mucosal HPV+ cells, we analyzed by real time RT-PCR the

expression of selected inflammatory cytokines, chemokines and related molecules in human foreskin keratinocytes transduced by E6 and E7 from mucosal (HPV-16) or cutaneous (HPV-38) genotypes comparing them to primary Human Foreskin Keratinocytes (HFK). We also performed silencing experiments by using E6/E7 siRNA in HPV+ SiHa carcinoma cells to verify the involvement of the viral proteins in cyto- and chemokines deregulation.

Result and discussion. Our results indicate that IL-1α and IL-1β mRNAs are downregulated in K16 and K38 cells, whereas the level of mRNA for IL-1R1 is comparable in all cell lines. Level of IL-6 mRNA is unchanged in K16 and downregulated in K38. Experiments performed by using E6/E7 siRNAs in SiHa, confirm the specificity of these effects. MIP-3α, CCL-17 and CCl-22 mRNA levels in K16 cells are also deregulated compared to HFK. Deregulation of MIP-3α mRNA appears to be related to miR-21 over-expression detected in both K16 and K38. The effect of the antiviral cytokine IFN-β on the levels of these pro-inflammatory mediators will be also discussed. Experiments on paraffin embedded tissue are in progerss to verify MIP-3α deregulation in vivo.

Conclusion. Our results suggest that HPV is able to modify the tumor microenvironment through the synthesis and release of specific pro-inflammatory cytokines and chemokines. These effects could interfere with the leucocytes trafficking and/or allow a better tumor growth and infiltration.

No conflict of interest.

285 MMP2 as a molecular biomarker of stromal priming in lung cancer

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Introduction. Initiation and progression of malignant tumors are deeply influenced by surrounding stroma reactivity and undergo a strict microenvironment control in which a central role is played by stromal fibroblasts. Since extracellular matrix (ECM) remodeling forms an integral part in this process by influencing the structural architecture of the tumor and generating physical changes that facilitate tumor cell signalling and behavior, we focused on the study of extracellular proteinases and in particular on the role of MMP2 in the cross-talk between fibroblasts and cancer cells in lung cancer.

Materials and Methods. Different lung cancer cell lines were exposed to medium conditioned by primary fibroblasts, seeded in close-proximity transwell assays or co-cultured and flow-sorted to evaluate the relevance of physical contact. To clarify the mediators of tumor-stroma interaction, the expression of integrins was evaluated by FACS while ECM-related genes expression in cancer cells was assessed by real time PCR. To evaluate MMP2 functional relevance the endogenous expression was suppressed by RNA interference. Silenced cells were co-cultured with fibroblasts, flow-sorted and injected in nude mice to evaluate tumor growth. MMP2 expression in xenografts was evaluated by immunofluorescence. To investigate stability of priming co-cultured cells were flow-sorted and cancer cells injected in mouse immediately and after 7 days.

Results and Discussion. Expression of ECM related genes was markedly increased in lung cancer cells after co-culture with fibroblasts while a lesser increase was observed when exposed to fibroblasts conditioned medium indicating that tumor stroma cross-talk may be regulated by direct physical interactions involving integrins implicated in cell adhesion and/or communication with extracellular matrix. In cell lines responsive to microenvironment stimuli, in vivo injection of lung cancer cells obtained after co-culture with fibroblast resulted in increased tumorigenicity associated with changes in expression of specific genes involved in ECM composition among which MMP2. Silencing of MMP2 on cancer cells was able to prevent the initial increase in MMP2 levels after co-culturing but not the increase in tumorigenic potential or higher MMP2 expression in tumors obtained by injection of sorted cells suggesting long term persistence of the effects of fibroblasts–cancer cells interaction. The increased tumorigenic potential elicited by fibroblasts was maintained by cancer cells for up to 7 days after separation.
Conclusion. These data demonstrate that cross-talk between stroma and cancer cells can dictate ECM composition and that fibroblasts induce their pro-tumorigenic effect at least in part by priming cancer cells through MMP2 upregulation. Microenvironment stimuli are additionally integrated within lung cancer cells that respond to stromal cues depending on their plasticity potential.

No conflict of interest.

286 Novel 1,2,3-triazolium salts obtained via click chemistry – potential anticancer agents

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Introduction. The copper-catalysed azide–alkyne cycloaddition (CuAAC) is one of the most widely employed 'Click' reactions for the synthesis of complex molecular scaffolds. Novel 'Click' derived 1-(2-Picolyl)-, 4-(2-picolyl)-, 1-(2-pyridyl)-, and 4-(2-pyridyl)-3-methyl-1,2,3-triazolium salts have been synthesized and were selected for the biological screening along with three parent triazoles.

Material and method. The synthesis of pyridine tethered 1,3,4-trisubstituted 1,2,3-triazolium salts was achieved through the initial 'Click' reaction. Cytotoxic effect of selected triazoles and triazolium salts was evaluated on human cervical carcinoma HeLa cells and determined by MTT assay. The antiproliferative activity of the most active compound was examined against human tumour cell lines: large-cell lung carcinoma H460, colorectal carcinoma HCT-116 cells, laryngeal carcinoma HEp-2 cells, carboplatin, cisplatin and curcumin resistant HEp-2 subline 7T cells and normal primary fibroblasts. DNA binding activity of AB144 was determined in thermal denaturation experiment. Its effect on the cell cycle and the induction of apoptosis was determined by flow cytometry.

Results and discussion. Within the library of triazolium salts, the analogues that are functionalized at the triazole ring with strongly electron donating substituent were more potent in comparison to those with the electron neutral or electron accepting groups. Compound AB144 (4-(4-methoxyphenyl)-3-methyl-1-(2-picolyl)-1H-1,2,3-triazolium hexafluorophosphate(V)) exhibited the highest cytotoxic activity among all examined compounds, inhibiting strongly the growth of all examined tumour cell lines. This effect depended on the cell type with the difference in sensitivity over 7-times. Human large-cell lung carcinoma H460 cells were the most sensitive toward AB144 and were selected for further studies. It is very important to note that AB144 was significantly more cytotoxic to tumour than to normal cells. Additionally, this compound was similarly cytotoxic against parent laryngeal carcinoma HEp-2 cells and their cisplatin and carboplatin resistant 7T subline. On the basis of the structural features of triazolium salts it was anticipated that DNA would be the primary target. However, AB144 did not bind to DNA. AB144 efficiently arrests tumour cells in the G1 phase of the cell cycle and triggers apoptotic death.

Conclusion. Selected compound AB144 was a) highly cytotoxic against different tumour cells, b) it was more cytotoxic against tumour cells in comparison to normal cells, and c) exhibited similar cytotoxic effect against parental and their drug resistant subline. The results suggest that the triazolium salts as a simple to make, low molecular mass and non-metallic compounds are worth of further investigation as anti-cancer agents.

No conflict of interest.

288 Early onset of the adaptive response to drugs in colon cancer: Novel players and potential biomarkers

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Introduction. In colon cancer, the acquisition of resistance is a major problem since patients tend to relapse after few years. The fast onset of resistance and its likely modulation suggest an adaptive response of colon cancer cells to attain a survival advantage in a hostile microenvironment. Previous reports have related this process to the selection of a resistant phenotype. However, it is currently unknown whether gene expression changes represent an adaptive response mechanism that precedes the clonal selection of colon cancer cells fostered by the treatment itself. We sought to identify novel biomarkers of such an early adaptive resistance response in colon cancer.

Materials and Methods. Cell lines: HT29, HCT116wt and HCT116null sensitive and oxaliplatin- and 5FU-resistant human colon cancer cell lines. Sublethal drug doses: Annexin-V-Fluos analyses were performed to establish sublethal drug doses. Gene expression arrays: Gene expression profiles of chemo sensitive lines, treated with 2 sublethal oxaliplatin and 5FU doses, were compared to those of resistant lines by using GeneChip® Human Transcriptome 2.0 arrays from Affymetrix. Array analyses: Through statistical analysis, a list with the most interesting and differentially expressed genes was obtained ($p<0.05$, $FC>2$) and validated by qPCR.

Results and Discussion. Several genes significantly up/down-regulated after sublethal doses treatment and whose pattern of expression alterations was also detected in the resistant cell lines, were selected. Some of them were already reported to be involved in the chemoresistance process as well. One of the most interesting genes encodes for a non-previously reported soluble protein related to a growth factor family. We are currently testing - by MTS viability assays and siRNA gene expression abrogation studies both in vitro and on in vivo colon cancer models - whether the inhibition of these genes could represent a new strategy to reverse chemoresistance or even avoid the adaptation and survival of tumor resistant cells after treatment.

Conclusions. Our preliminary data suggest that even small doses of chemotherapy are able to awake a subset of genes involved in the first step for chemoresistance acquisition. Further and detailed studies could lead to new strategies to overcome chemoresistant tumors and improve treatment outcome.

No conflict of interest.

289 Melanoma inhibitors and microrna

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Introduction. The primary goal of our work was to identify miRNA contributing to melanoma progression and to the clinical outcome of melanoma patients. Vemurafenib, dabrafenib, SCH772984 and trametinib are selective and potent small molecule inhibitor for the V600 mutant form of BRAF, ERK and MEK, respectively, used in the treatment of melanoma. However, the tumor cells can exhibit resistance to these drugs. Then we aim to identify whether miRNAs are involved in the process of drug resistance .

Material and methods. Eighty samples, including matched primary/metastatic tumors, normal epidermis, cultured melanocytes, cultured keratinocytes, and metastatic melanoma cell lines were analyzed with miRNA microarrays and RT-PCR. The skin cutaneous melanoma (SKCM) dataset from The Cancer Genome Atlas (TCGA) was used for validation. Melanoma cell lines were transfected with mimics and antagomiRs, and western blot analysis was performed on putative targets and key cancer genes. Cancer drugs were used in combination with mimics and cell growth inhibition assess by MTS.

Results. We first studied melanoma cell lines and cultured melanocytes, and its surrounding environment, epidermis and keratinocytes. Clustering analysis confirmed that miRNA profiles were characteristic of the different cell types. After having identified the miRNAs deregulated through melanoma establishment and progression, we investigated their association to clinical covariates. MiR-204 and miR-211 were strongly expressed in normal melanocytes, while miR-203 was highly expressed throughout all layers of epidermis and miR-205 mostly in the basal layer. All these four miRNAs are also expressed in the normal melanocytes and are lost through melanoma progression. We then validated these melanoma miRNAs in two different cohorts. Additionally, we identified the putative target proteins for the melanoma miRNAs. Western blot was used to validate the predicted targets upon mimics' transfection in melanoma cell lines.In silico and in vitro, we showed that they are associated to the repression of genes such as CCND1, E2F1, ZEB1, ZEB2 and BCL2. Finally, to assess the possible role of melanoma microRNAs in drug resistance, melanoma cell lines were transfected with combinations of melanoma microRNAs and drugs.

Conclusions. We showed that some miRNAs deregulated in melanoma lead to the repression of cancer genes such as CCND1, E2F1, and BCL2 in vitro.

No conflict of interest.

290 Inhibition of uPAR-TGFß cross-talk blocks MSC-dependent EMT in melanoma cells

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Introduction. The capacity of cancer cells to undergo epithelial-to-mesenchymal transition (EMT) is now considered a hallmark of tumor progression, and it is known that interactions between cancer cells and Mesenchymal Stem Cells (MSCs) of tumor microenvironment may promote this program.

Materials and Methods. To evaluate EMT markers we performed real time PCR, western blot analysis and confocal microscopy. Cell invasion was studied in Boyden chambers in which the upper and lower wells were separated by 8µm pore size polycarbonate filters coated with Matrigel. uPAR gene expression was inhibited using a 18-mer phosphorothioate aODN, and as a negative control we used a degenerated oligodeoxynucleotide (dODN), which is a mixture of all possible combinations of bases that compose the aODN. TGFβ gene silencing was performed using small interfering RNA. For the in vivo experiments ND1 immunodeficient mice (6–8 weeks old) were injected subcutaneously with 1x106 melanoma cells alone or mixed with 0.5x106 MSCs (four animals/group). Tumor development was monitored at regular intervals by measuring tumor volume determined by the following formula:(L x W 2)/2, where L and W are the length and width of tumor mass.

Results and discussion. We demonstrate that MSC-conditioned medium (MSC-CM) is a potent inducer of EMT in melanoma cells. The EMT profile acquired by MSC-CM-exposed melanoma cells is characterized by: i) enhanced level of mesenchymal markers, including TGFβ/TGFβ-receptors system and uPAR up-regulation; ii) increased cell invasion and in vivo tumor growth. Silencing TGFβ in MSC is found to abrogate ability of MSC to promote EMT characteristics in melanoma cells, together with uPAR expression, and this finding is strengthened using an antagonist peptide of TGFβRIII, the so called p17. Finally, we demonstrate that the uPAR antisense oligonucleotide (uPAR-aODN) may inhibit EMT of melanoma cells either stimulated by exogenous TGFβ or MSC-CM. Thus, uPAR up-regulation in melanoma cells exposed to MSC-medium drives TGFβ-mediated EMT.

Conclusion. These results show the interplay between uPAR and TGFβ as an emerging crucial factor in cancer progression and metastasis. This crosstalk could be an attractive target for cancer therapy. By inhibiting TGFβ and uPAR it is possible to abrogate the positive and dangerous interaction between cancer cells and MSCs of tumor environment. Such a combined therapy can open a new therapeutically way for melanoma-bearing patients.

No conflict of interest.

291 A KRAS and dual concomitant PI3KCA mutations in a metastatic colorectal cancer patient with aggressive and resistant disease

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Introduction. Colorectal cancer (CRC) is the third most common tumor and shows globally increasing incidence. New therapies have improved the treatment and patient survival, even in metastasis cases. Today, KRAS and NRAS genotype addresses medical treatment of metastatic colorectal cancer (MCRC) patients including anti-epidermal growth factor receptor (anti-EGFR) or anti-vascular endothelial growth factor (anti-VEGF) targeted agents. Other genes activating downstream RAS-RAF-MAPK and PI3K-AKT pathways, such as PIK3CA and BRAF, and their prognostic and predictive clinical implication are under investigation. Here, we describe a MCRC patient carrying a KRAS and two PIK3CA mutations on the same allele.

Material and method. Liver tumoral sample was collected at diagnosis from an early-onset right CRC patient with synchronous liver, lung and lymph nodes metastases, treated with first-line intensive triplet chemotherapy plus bevacizumab, according to FIr-B/FOx schedule. DNA was extracted and KRAS, NRAS (exons 2, 3, 4), BRAF (exon 15), PI3KCA (exons 9, 20) were analyzed by direct sequencing.

Results and discussion. A KRAS exon 2 mutation (c.34G>A, GGT>AGT, G12S) and two PIK3CA exon 9 mutations (c.1633G>C, GAG>CAG, E545Q; c.1645G>C, GAT>CAT, D549H) were detected by direct sequencing. Mono-allelic sequencing demonstrated that both PIK3CA exon 9 nucleotide substitutions belong to the same allele. As thePIKCA c.1633G>C mutation was already described in CRC, the second one c.1645G>C was never reported in CRC, but described in literature with very low frequency in hepatocellular and cervical carcinoma. Patient showed an aggressive and resistant disease with 7 months progression-free survival and 15 months overall survival.

Conclusion. The analysis highlighted the presence of a KRAS and two PI3KCA mutations in a MCRC patient with aggressive disease, resistant to more active medical treatment regimen. Interestingly, the PIK3CA D549H substitution was never reported in CRC and previously described in scientific literature just in two samples, from cervical and hepatocellular carcinoma respectively.

No conflict of interest.

292 ID4-driven cross-talk between breast cancer cells and tumor-associated macrophages

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Introduction. ID-4 is a member of ID family of proteins (Inhibitors of Differentiation, ID-1 to 4) that act as dominant negative regulators of bHLH transcription factors. Several evidences indicate that ID proteins are associated with loss of differentiation, unrestricted proliferation and neoangiogenesis in diverse human cancers. In the context of breast cancer (BC), ID4 has been shown to be highly expressed in triple-negative tumors, 70% of which belong to the basal-like molecular subtype. We previously reported that ID4 expression is transcriptionally induced by mutant p53 proteins in breast cancer; the increased ID4 protein results in post-transcriptional induction of proangiogenic cytokines and in enhanced angiogenic potential of breast cancer cells, as well as in significantly higher microvessel density in a human breast tumor series. Angiogenic switch and progression to malignancy are tightly controlled by Tumor Associated Macrophages (TAMs) in BC. The presence of intratumoral proliferating macrophages was significantly correlated with high grade, hormone receptor negative tumors, and a basal-like subtype of BC.

Materials and Methods. Estrogen receptor negative BC cells depleted or not of ID4 expression were co-cultured with monocytes/macrophages (Mϕ) and changes in gene/microRNA expression were evaluated. Migration of Mϕ was evaluated in vivo using matrigel plugs containing conditioned media (CM) from BC cells depleted or not of ID4 expression. Correlations between ID4 protein expression and Mϕ biomarkers was evaluated on 62 triple-negative BC from Italian National Cancer Institute Regina Elena.

Results and Discussion. ID4 expression in BC cells promotes migration of Mϕ in vitro and enhances Mϕ recruitment in vivo. Analysis by immunohistochemistry revealed a significant association between ID4 protein expression and the Mϕ marker CD68 in 62 triple-negative BC specimens (P=0,012). ID4 expression is induced in Mϕ co-cultured with BC cells and this induction depends on activation of endogenous ID4 promoter in Mϕ. Of note, ID4 induction in Mϕ was significantly attenuated when ID4-depleted breast cancer cells were used for the co-culture.

Expression profiling analyses evidenced that ID4 expression in BC cells determines changes in angiogenesis-related mRNAs and microRNAs in co-cultured Mϕ.

Conclusion. ID4 expression in BC cells promotes recruitment of macrophages and enhances Mϕ angiogenic potential.

No conflict of interest.

293 Understanding the role of Cyclophilin A during cytokinesis; A novel role for the NEK kinase family

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Introduction. Disrupting mitosis represents an important anti-cancer approach that is evident in the widespread clinical use of microtubule-targeting agents such as paclitaxel for the treatment of cancer. Anti-mitotic agents disrupt mitotic progression, which culminates in anaphase arrest and eventual cell death. However, the development of resistance is a major factor that limits their utility, and is largely attributed to the occurrence of mitotic slippage as a result of gradual cyclin B degradation, leading to premature mitotic exit, adaption and survival of unstable malignant cells. Recently, it was demonstrated that targeting mitotic exit can overcome mitotic slippage, highlighting the importance of this new approach to prevent resistance.

Cytokinesis is the final stage of mitosis where two daughter cells are separated. Although less well understood than some of the earlier phases of the cell cycle, recent reports have shed light on the mechanisms involved, including the role of centrosome proteins in midbody assembly and abscission. Cyclophilin A (CypA) is a member of a family of peptidyl-prolyl isomerases (PPIases) that catalyse the cis-trans isomerisation of proline peptide bonds and as such they are implicated in protein folding and in catalysing functionally relevant structural changes on protein substrates thereby regulating cell signalling activity. CypA is overexpressed in cancer cells and is associated with acquired drug resistance. We have previously shown that the CypA is a centrosome protein that undergoes cell-cycle dependent re-localisation to the midbody during cytokinesis. Furthermore, CypA isomerase activity is required for the timely completion of cytokinesis and loss of CypA expression prevents clonogenic survival in vitro (Bannon et al., 2012). Moreover, CypA loss reduced tumour growth by 83% and significantly reduced metastasis in a xenograft mouse model (unpublished data). Thus, understanding the regulation of CypA localisation and function during cytokinesis is important and may reveal strategies to target the process in anti-cancer therapy.

Materials and Methods. Using biochemical (SDS-PAGE and Western blotting), molecular (molecular cloning and site-directed mutagenesis) and bioinformatic tools (www.elm.eu.org) we investigated post-translational phosphorylation as a mechanism to regulate CypA function during cytokinesis.

Results. CypA undergoes transient phosphorylation, which was evident by a mobility shift on Western blots. Further investigation revealed three putative Nek (NIMA-related kinases) serine-threonine kinase phosphorylation sites within CypA located at S51, S77 and T93. Mutation of the conserved S51 and S77 sites to a non-phosphorylatable form disrupts CypA localisation to the centrosome, whereas T93 does not. Furthermore, mutation of S51 and S77 significantly reduced cell proliferation in vitro, when compared to WT cypA.

Discussion. This data suggests that the CypA is phosphorylated by members of Nek serine-threonine kinase family, which is important for its localisation to the centrosome and function during cytokinesis. Overall, this work provides important new insight into the molecular mechanism of cytokinesis and may reveal novel strategies to exploit the process in the prevention of cancer chemoresistance.

No conflict of interest.

294 Characterization of a mantle cell lymphoma cell line resistant to a Chk1 inhibitor

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Introduction. Mantle cell lymphoma (MCL) is an aggressive, incurable disease. We recently observed that Chk1 inhibitors are strongly effective as single agents at very low concentrations in MCL. To investigate the molecular mechanisms at the basis of the Chk1 inhibitor activity in MCL, a MCL cell line, JEKO-1, resistant to a Chk1 inhibitor was isolated and characterized.

Materials and Methods. We have selected the JEKO-1 cell line resistant to the Chk1 inhibitor PF-00477736 (JEKO-1-R) by treating the cells continuously with growing concentrations of the drug for one year. The resistant cell line maintains stably in culture its acquired resistance for at least five months without the addition of the drug. A pharmacological, cellular and molecular characterization was conducted. Analysis of the cell cycle by FACS and BrdUrd incorporation was performed and the expression of cell cycle markers was investigated by Real Time PCR and Western Blot Analysis. Over-expression of cyclin D1 in JEKO-1-R was achieved by lentiviral infection.

Results and discussion. The JEKO-1-R cell line is at least 7 times more resistant to PF-00477736 as compared to the parental cell line; it is also more resistant to another Chk1 inhibitor (AZD-7762) and to the Wee1 inhibitor MK-1776. Cytotoxic activity of Bendamustine and Bortezomib, drugs commonly used for the treatment of MCL, was comparable in the parental and resistant JEKO-1 cell lines. The JEKO-1-R cell line has a shorter doubling time than JEKO-1 (20,6 hours vs 26,1 hours respectively), likely due to a faster S phase (10,2 hours vs 14,4 hours). The markers of S phase cyclin A and Cdt1 were indeed down-regulated in the JEKO-1-R as compared to the parental cell line. Interestingly cyclin D1 expression levels, which in MCL is constitutively expressed due to the presence of the t(11;14), were decreased in the resistant cell line. CyclinD1 over-expression by lentiviral infection, partly restored cyclin D1 protein level and partially re-established PF-00477736 sensitivity.

Conclusions. These data suggest that the cyclin D1 expression level is inversely correlated to PF-00477736 resistance even though this is not the only molecular mechanism.

No conflict of interest.

295 XMD8-92, an ERK5 inhibitor, in combination with imatinib, as a useful approach for CML treatment and prevention of relapse

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Introduction. Evidences obtained in our laboratory indicated that the Leukaemia Stem Cell (LSC) potential of Chronic Myeloid Leukaemia (CML) cell populations is resistant to, and selected in, severe hypoxia. The Extracellular signal-Regulated Kinase 5 (ERK5) is a Mitogen-Activated Protein Kinase involved in the control of cell survival and proliferation, as well as in the pathogenesis of a number of cancers, including CML. The main target of this study was to address the effects of ERK5 inhibition on the maintenance of hypoxia-selected LSC of CML.

Materials and Methods. The human stabilized CML cell lines K562 and KCL22, where ERK5 is constitutively activated, were incubated in normoxic (routine) or hypoxic (0.1% O2) primary cultures (LC1) in the absence or the presence of the ERK5-specific inhibitor XMD8-92 (10 mM). Cell survival, cycling, proliferation and apoptosis were assessed by counting Trypan blue-negative cells or by flow cytometry using propidium iodide (PI) and anti-annexin-V antibodies. Day-7 LC1 cells were transferred to drug-free, non-selective normoxic secondary cultures (LC2), to measure LC2 repopulation as a read-out of progenitor/stem cell recovery from LC1.

Results. XMD8-92 treatment, at concentrations used in the literature (i.e 10 μM), did not affect the growth kinetics of CML cells in normoxia (IC50 at 72 hours: K562, 20 μM; KCL22, 22.5 μM). Moreover, XMD8-92 did not affect the growth kinetics of CML cells incubated in hypoxia. Pharmacological ERK5 inhibition, on the other hand, resulted in a significant reduction of hypoxia-induced apoptosis. XMD8-92 also blocked cell progression to the S phase of mitotic cycle and the increase of p27Kip expression. These results point to a cytostatic, rather than cytotoxic, effect of XMD8-92 on the bulk of CML cell population. On the other hand, the pharmacological inhibition of ERK5 or its genetic knockdown impaired LC2 repopulation by hypoxia-selected LC1 cells. In CML cell lines as well as primary cells explanted from 5 CML patients, imatinib-mesylate (IM) was unable to suppress LC2 repopulation but very active on LC1 cell bulk. XMD8-92 maintained the capacity to suppress LC2 repopulation when administered in combination with IM.

Conclusions. XMD8-92 was inactive on the bulk of CML cell population, but capable to suppress completely hypoxia-selected LSC; thus, its combination with IM may be a useful approach to try, at one time, to induce remission and prevent late relapses of CML via the eradication of IM-resistant LSC responsible for minimal residual disease.

No conflict of interest.

296 Exploiting the hERG1/ß1/PI3K complex to overcome anti-angiogenesis therapy resistance in gastrointestinal cancers

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Introduction. The development of more efficacious chemotherapeutic drugs and of specific compounds (either small molecules or biologics) for a targeted therapeutic approach has greatly improved the prognosis of most types of cancer. However, systemic toxicity and chemoresistance are nowadays the main shortcomings of standard chemotherapy, while targeted therapies still suffer the lack of appropriate predictive markers and the occurrence of resistance (Wang B et al. See comment in PubMed Commons belowExpert Opin Drug Deliv. 2010). In the last years, the management of metastatic CRC has been relevantly changed by the availability of two classes of biological drugs: the anti-vascular endothelial growth factor (VEGF), Bevacizumab (BV) and the anti-epidermal growth factor receptor (EGFR), cetuximab and panitumumab (Stein A and Bokemeyer C. World J Gastroenterol. 2014).While KRAS mutations are consistently associated with increased treatment failure rates among patients treated with anti-EGFR antibodies (Dahabreh IJ et al. Ann Intern Med. 2011), no genetic and molecular markers for BV treatment response have been found.We identified (Crociani O. et al. Sci Rep. 2013; Crociani O. et al. Clin Cancer Res. 2014) a novel signaling pathway that sustains angiogenesis and progression in CRC and gastric cancer (GC). This pathway is triggered by β1 integrin-mediated adhesion and leads to VEGF-A secretion. The effect is modulated by the hERG1 K+ channel, which can recruit and activate PI3K and Akt. This in turn increases the Hypoxia Inducible Factor (HIF)-dependent transcription of VEGF-A and other tumor progression genes.

Material and Methods. We evaluated and characterized the protein-protein interactions of the hERG1/β1/PI3K-based intracellular pathway in wild type, p53-/-, BV resistant CRC cells by standard procedures and blocking/activating each single

component of the signaling cascade with specific pharmacological and biomolecular inhibitors.

Result and Discussion. We characterized a new signaling pathway with novel features in that the integrin- and hERG1-dependent activation of HIF (i) is triggered in normoxia, (ii) involves NF-kB and (iii) is counteracted by an active p53. Blocking the hERG1/β1/PI3K complex, we are able to inhibit tumor growth, angiogenesis and metastatic spread. Moreover, the combined treatment with hERG1 blockers and BV has an additive anti-tumoral effect.

Conclusions. The hERG1/β1/PI3K complex could be an alternative therapeutic target for anti-angiogenesis therapy, in particular to overcome anti-angiogenesis therapy resistance, in gastrointestinal cancers.

No conflict of interest.

297 Epithelial ovarian cancer: Affecting tumor aggressiveness and drug sensitivity by targeting aberrant metabolism

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Introduction. EOC possess a 'cholinic phenotype', characterized by increased intracellular phosphocholine content due to sustained expression/activity of choline kinase-alpha (ChoKα/CHKA). This metabolic reprogramming is involved in chemoresistance but the underlying mechanisms are yet unknown. Aim of the study is the evaluation of the biological relevance of increased ChoK expression and activity focusing the attention on its possible druggability in EOC.

Materials and Methods. Human EOC cell lines, non-tumoral immortalized ovarian cells and mouse models with human EOC xenografts were used. By in-vitro and in-vivo studies we evaluated the effects of stable CHKA silencing on EOC growth, global metabolic profiling and drug sensitivity. Independent validation was obtained by transient CHKA silencing (siCHKA).

Results. CHKA knockdown affects growth capability of two different EOC cell lines both in in-vitro and in-vivo models. It also altered antioxidant cellular defenses, decreasing glutathione and cysteine content while increasing intracellular levels of reactive oxygen species, overall sensitizing EOC cells to current chemotherapeutic drugs. Natural recovering of ChoKα expression after its transient silencing rescued the wild-type phenotype, restoring intracellular glutathione content and drug resistance. Rescue and phenocopy of siCHKA-related effects were also obtained by artificial modulation of glutathione levels. The direct relationship among CHKA expression, glutathione intracellular content and drug sensitivity was further demonstrated in four different EOC cell lines but notably, siCHKA did not affect growth capability, glutathione metabolism and/or drug sensitivity of non-tumoral immortalized ovarian cells. Alteration of expression levels of enzymes involved in glutathione metabolism by qRT-PCR and correlation of PCho levels and GSH content in xenograft models is currently ongoing. In order to investigate the putative role of CHKA in EOC pathogenesis, in silico analysis on available public data sets of gene expression microarrays will be performed.

Conclusion. We propose that a metabolic approach to EOC treatment might have the potential to address many of the issues that contribute to the high EOC lethality and open the way for the validation of ChoKα as a new therapeutic target to be used alone or in combination with conventional drugs for EOC treatment.

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300 Proteomic analysis of SHH Medulloblastoma stem-like cells

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Introduction. Medulloblastoma (MB) is a malignant brain tumor that comprises four distinct molecular subgroups, including Sonic Hedgehog (SHH)-MB group. In normal development, Hedgehog drives proliferation of granule neural precursors (GNPs) in the external granule layer. Its abnormal activation in GNPs leads to the development of one of the four MB subtypes and drives not only SHH-MB but also its cancer stem-like cells (SLCs), a fraction of the tumor cell population that maintain cancer growth. Since a thorough understanding of the molecular pathways sustaining MB SLCs is mandatory to design novel specific therapeutic strategies, we investigated proteome features of SHH-MB SLCs and their differentiated counterparts.

Materials & Methods. Human MB samples were collected and processed to obtain a single cell suspension cultured as neurospheres in selective medium. For differentiation studies, neurospheres were plated in differentiation medium for 48h. For proteomic analysis, cells were lysed and extracted proteins were digested and analyzed by nanoLC-MS. Proteins data set were filtered to identify significant up- or down-regulation and protein network analysis was performed by QIAGEN's Ingenuity Pathway Analysis (IPA).

Results and Discussion. 68 proteins were significantly modulated between SLCs and their differentiated counterparts. Heat Shock Protein 70 (Hsp70) was one of the protein that characterized the protein profile of SLCs. By means of IPA, Genomatix analysis and extending the network obtained using the differentially expressed proteins we found a correlation between Hsp70 and the NF-?B complex.

By western blot analysis, we confirmed the results of the proteomic analysis and also highlighted P-p65/NF-?B activatory complex highly expressed in SLCs.

Conclusion. Taken together, the presented proteomics study define the human SHH-MB SLCs proteins and signalling networks suggesting new key biological players involved in the process of malignancy transmutation.

No conflict of interest.

301 The receptor for urokinase-plasminogen activator (uPAR) controls plasticity of cancer cell movement in mesenchymal and amoeboid migration style

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Introduction. The receptor for the urokinase plasminogen activator (uPAR) is up-regulated in malignant tumors. Historically the function of uPAR in cancer cell invasion is strictly related to its property to promote uPA-dependent proteolysis of extracellular matrix and to open a path to malignant cells. These features are typical of mesenchymal motility.

Materials and Methods. To evaluate the mesenchymal ameboid transition we performed RhoA and Rac1 activation assay, immunofluorescence analysis of protein involved in cytoskeleton organization, and collagen degradation assay. For a clear visualization of collagen fiber breakdown in the process of proteolytic migration we used reconstruction by time-lapse video microscopy. Cell invasion was studied in Boyden chambers using filters coated with Matrigel. uPAR gene expression was inhibited using a 18-mer phosphorothioate aODN, and as a negative control we used a degenerated oligodeoxynucleotide, which is a mixture of all possible combinations of bases that compose the aODN.

Results and discussion. Here we show that the full-length form of uPAR is required when prostate and melanoma cancer cells convert their migration style from the 'path generating' mesenchymal to the 'path finding' amoeboid one, thus conferring a plasticity to tumor cell invasiveness across three-dimensional matrices. Indeed, in response to a protease inhibitors-rich milieu, prostate and melanoma cells activated an amoeboid invasion program connoted by retraction of cell protrusions, RhoA-mediated rounding of the cell body, formation of a cortical ring of actin and a reduction of Rac-1 activation. While the mesenchymal movement was reduced upon silencing of uPAR expression, the amoeboid one was almost completely abolished, in parallel with a deregulation of small Rho-GTPases activity. In melanoma and prostate cancer cells we have shown uPAR colocalization with β1/β3 integrins and actin cytoskeleton, as well integrins-actin co-localization under both mesenchymal and amoeboid conditions. Such co-localizations were lost upon treatment of cells with a peptide that inhibits uPAR-integrin interactions. Similarly to uPAR silencing, the peptide reduced mesenchymal invasion and almost abolished the amoeboid one.

Conclusion. These results indicate that full-length uPAR bridges the mesenchymal and amoeboid style of movement by an inward-oriented activity based on its property to promote integrin-actin interactions and the following cytoskeleton assembly.

No conflict of interest.

302 PDGF-D maintains tumor cell heterogeneity in an experimental model of pancreatic neuroendocrine tumors

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Introduction. Cancer progression is the consequence of dynamic, and yet poorly understood, cell-cell interactions driven by frequently deregulated signaling pathways. Further complexity arises from the notion that tumors are composed of phenotypically and functionally distinct subsets of malignant and stromal cells. An improved understanding of the different signaling pathways during tumor growth and how they contribute to genetic and phenotypic variation within the tumor stroma is therefore highly warranted. In the past decade, members of the platelet-derived growth factor (PDGF) family, PDGF-A, -B, -C and -D and their receptors (PDGFRα and PDGFRβ) have been extensively investigated and shown to be critical for numerous cellular processes such as proliferation, survival and motility during tumor growth and invasion. PDGF-D is the latest identified member of the family and, similarly to PDGF-B, exerts its functions by binding to and activating PDGFRβ expressed mainly by mesenchymal cells. However, unlike PDGF-B, the role of PDGF-D in tumor development is poorly understood.

Material and methods. Making use of a recently developed Pdgfd knockout mouse, we explored the role of PDGF-D in tumor growth by monitoring tumorigenesis in a mouse model of pancreatic neuroendocrine tumors (PNET), the RIP1-TAg2 mice, upon disrupted PDGF-D signaling. Pancreatic neuroendocrine tumor cells were analysed and sorted by flow cytometry and tumor tissue was stained by different immunohistochemistry techniques for evaluation of properties such as cell growth, metastatic dissemination and stromal cell recruitment upon PDGF-D inhibition.

Results and discussion. Genetic ablation of PDGF-D significantly impaired tumor growth in RIP1-TAg2 mice and improved survival. However, we did not observe any gross effect on the tumor vasculature or on recruitment of pericytes and immune cells. A likely explanation is that PDGF-B, the other described ligand for PDGFRβ, was upregulated in tumors of RIP1-Tag2;Pdgfd-/- mice compared to the wild-type littermates suggesting a partial compensatory effect. Furthermore, we identified both in vivo and in vitro a subpopulation of malignant cells in tumors from RIP1-TAg2 mice expressing PDGFRβ with accompanying responsiveness to PDGF-D and a preserved capacity to develop tumors when transplanted into NOD-SCID mice.

Conclusion. Our current understanding is that PDGF-D acts as an important growth factor in tumor development and in the modulation of PDGFRβ+ tumor cells in PNET. Our data has important implications for future studies exploiting the use of PDGF-D as a therapeutic target in PNET.

No conflict of interest.

303 Selective agonists for M2 muscarinic receptors inhibit cell proliferation and survival in human glioblastoma cells: Possible implications in drug resistance

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Introduction. The involvement of muscarinic receptors in cancer has been largely documented. Recently, we have demonstrated that the activation of M2 muscarinic receptors, by the agonist Arecaidine Propargyl Ester (APE), arrests cell proliferation and induces apoptosis in glioblastoma (GB) cell lines. In the present work, we investigated the effects mediated by M2 receptors in glioblastoma cancer stem cells (GSC), an undifferentiated GB subpopulation characterized by high chemioresistance.

Material and Methods. GB7 and GB8 cell lines obtained from human biopsies were cultured in Euromed-N supplemented with N2, B27, EGF and FGF. MTT assay and trypan blue staining were used to evaluate cell viability and cell death, respectively. By means of M2 silencing (by siRNA) and pharmacological competition we confirmed the ability of M2 agonists to selectively bind this receptor subtype. Transcript levels for muscarinic receptors and multidrug efflux pumps (e.g. ATP binding cassette, ABC) were analyzed by RT-PCR analysis.

Results. Our experiments were performed with the M2 agonist APE, the muscarinic orthosteric superagonist Iperoxo and its related dualsteric agonists P-6-Iper and N-8-Iper. In GB7, treatment with the M2 agonist APE (100 μM) decreased cell proliferation in a time and dose dependent manner. In GB8 APE induced cell death. Cell proliferation and survival of U251 and U87 cell lines and GSC cells were unaffected by treatment with Iperoxo and P-6-Iper. Conversely, N-8-Iper decreased cell proliferation in a time and dose dependent manner. Also in GB7 cells, N-8-Iper inhibited cell growth and survival also at lower concentration (12.5 μM). The co-treatment of GB cells with M2 agonists (APE or N-8-iper) and different muscarinic antagonists confirmed that the decreased agonist-induced cell proliferation and survival were dependent on selective activation of M2 receptor. Similarly, the silencing of M2 receptor abolished the M2-mediated agonists effects. Moreover, APE and N-8-Iper decreased, particularly in GB7 cells, the mRNA levels for the ABC drug efflux pumps (C1 and G2).

Conclusions. Our data suggest that M2 receptor agonists represent a new relevant tools to investigate glioblastoma-related mechanisms. Furthermore, the ability of M2 agonists to decrease the drug efflux pumps expression, in particular in GSC cells, suggests that they may have a role in reducing the GSC chemoresistance, and make them more responsive to conventional drugs (e.g. temozolomide).

No conflict of interest.

304 Significance of CDKN1B driver mutations in the growth and response to therapy of Luminal BC

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Introduction. Previous studies have shown that p27Kip1 is not only a cell cycle inhibitor, but that it also exerts additional functions when located in the cytoplasm, mainly through its C-terminal region. Increased p27 expression levels and p27 mislocalization have prognostic potential and therapeutic implications in various types of human cancers. Recent deep sequencing studies have shown that the gene CDKN1B, coding for p27, is frequently mutated in its C-terminal region in breast cancer (BC) of the luminal type, where it represents a 'driver mutation'.

Material and method. Given the complexity and relevance of p27 in BC, we exploited the Zinc Finger Nucleases (ZFNs) technology to generate p27 Knock-Out (KO) and mutant p27 Knock-In clones in the luminal-type breast cancer cell line MCF-7 (ER+/PgR+). We generated and characterized 3 p27KO cell clones, and compared them with the biological behavior of p27 KI cell lines, that we obtained by targeted integration of GFP-p27 WT, GFP-p27 K134fs (a frameshift mutation) and GFP-p27 170stop (a stop mutation at the 170 residue) in the AAVS1 safe harbor locus. Both these mutations have been identified in luminal BC patients. Three GFP-p27 WT, three GFP-p27 K134fs and two GFP-p27 170stop clones were generated and characterized.

Result and Discussion. Our functional analyses revealed that lack of p27 induced a significantly increase in both number and size of colonies grown in soft agar. Interestingly, mammosphere assays performed to evaluate the stem-like properties of MCF7 cells indicated that p27KO resulted in increased growth and self-renewal ability. In both experimental conditions, KI clones expressing GFP-p27 WT showed a rescue of the phenotype, while the GFP-p27 K134fs mutants displayed an intermediate behavior. Since radiation following by antiestrogen therapy represents the standard treatment for luminal BC patients, we tested the response of our MCF7 clones to gamma-irradiation, showing that p27 increased the radio-resistance of MCF7 cells. The response to different antiestrogen therapies (i.e. tamoxifen and aromates inhibitors) of p27KO and KI cell clones is in progress.

Conclusion. We have generated valuable tools to study the role of p27 in BC, which are also amenable for the study of specific mutations and/or domains involved in specific p27 functions. Our functional analyses reveal interesting features of p27 that could be exploitable for designing better BC diagnostic, prognostic and also therapeutic approaches.

No conflict of interest.

305 Therapeutic potential of metformin and phenformin in targeting the stem cell compartment in malignant melanoma

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Introduction. Malignant melanoma, the deadliest form of skin cancer, is highly resistant to conventional therapies. While inducing some degree of tumor regression, recently discovered drugs are often accompanied by disease recurrence. A possible mechanism explaining this phenomenon is the persistence of stem-like tumor cells, namely cancer stem cells (CSC), in treated patients. Therapies targeting the CSC compartment are warranted, however to date no currently available drug kills exclusively or preferentially melanoma stem cells. Chemoprevention is the use of natural or synthetic chemical agents suppressing or preventing carcinogenesis. Several chemopreventive agents have shown to be able to kill melanoma cells, however, little is known on the cellular targets of these drugs in melanoma.

Materials and Methods. We evaluated the effect of the biguanides Metformin (Metf) and Phenformin (Phen) on melanoma cells (cell lines and patient-derived primary cells) using monolayer and 3D spheroid cultures (in presence of serum, to mimic in vivo tumor cell growth). In melanoma, cells expressing high levels of aldehyde dehydrogenase (ALDH) are CSC. We therefore isolated ALDHhigh and ALDHlow melanoma cells by flow cytometric cell sorting and functionally analyzed these cells in presence or absence of 10mM Metf or 1mM Phen.

Results and Discussion. Metf and Phen abrogate melanoma cell viability in monolayer cell cultures and reduce melanosphere size and the number of viable cells/sphere at day10. Once sorted, ALDHhigh melanoma cells express stem cell markers including SOX2 and CD271 and generate slightly bigger and less necrotic spheres as compared to ALDHlow cells. Cell viability is higher in ALDHhigh derived spheres as compared to ALDHlow derived ones. Treatment of ALDHhigh and ALDHlow derived spheroids with Metf and Phen show a similar decrease in size. However, the number of viable cells/sphere is markedly and significantly decreased in ALDHhigh derived spheres treated with Metf/Phen, but not in ALDHlow ones. Melanospheres derived from total melanoma cells contain both ALDHhigh and ALDHlow cells. Interestingly, the treatment of melanospheres with Phen decreases ALDH1A3 and MITF expression and the number of ALDHhigh cells by FACS analysis.

Conclusions. Preliminary results suggest that both Metf and Phen decrease melanoma cell viability, with Phen preferentially targeting ALDHhigh melanoma cells. Further studies will be aimed at analysing the molecular mechanism (including AMPK involvement) underlying these events. Overall these data indicate a possible therapeutic approach to target melanoma stem cells with biguanides.

No conflict of interest.

306 PI3KC2a, a new spindle associated protein involved in genomic instability and tumorigenesis

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Introduction. PI3K signaling axis is one of the most frequently deregulated pathways in human cancer. Emerging evidences highlight the importance of class II enzymes in cell proliferation and survival.

Material and methods. Pik3c2a +/- mice were intercrossed with a transgenic strain expressing the activated HER-2/Neu oncogene in the mammary gland. Mice were weekly followed for survival, tumor appearance and growth. Primary Murine Mammary Epithelial Tumor cells were derived from early (E-MMET) and late (L-MMET) stage tumors. Effects of heterozygous loss of Pik3c2a were evaluated by cell proliferation, immunofluorescence, karyotype and CGH analysis. Truncating PI3KC2α mutants (N-terminal domain) were generated and interaction with TACC3 was tested by immunoprecipitation. Efficacy of anti-mitotic cancer drugs were examined.

Results and discussion. To evaluate the oncogenic role of PI3KC2α in cancer, we targeted its expression in a breast cancer mouse model (neuT). Heterozygous loss of PI3KC2α resulted in an initially delayed tumor onset followed by a faster growth rate in Pik3c2a+/-/neuT mice. In agreement with the delayed onset, Pik3c2a+/-/neuT E-MMETs displayed a reduced proliferative capacity and a delay in the progress from prophase to anaphase, accompanied by increased apoptosis. We found that Pik3c2a loss causes an aberrant microtubule (MT) spindle organization that, in turn,

promotes genomic instability. In line with this, PI3KC2α is specifically enriched at the metaphase spindle and stabilize K-fibers during mitosis, acting as a scaffold protein for clathrin and transforming acidic coiled-coil protein 3 (TACC3). Truncated constructs of PI3KC2α were generated to identify the region of interaction with TACC3. Despite the aberrant MT organization, we demonstrate that tumors bypass the requirement of PI3KC2α through a common mechanism of progression. The ability of tumors with low PI3KC2α to grow faster appeared to correlate with increased sensitivity to anti-microtubule agents like Paclitaxel.

Conclusion. We demonstrated that PI3KC2α is a new spindle associated protein interacting with TACC3 and clathrin to stabilize MT. The loss of PI3KC2α plays a crucial role in promoting genomic instability, altering chromosome congression/segregation during cell division. These findings will eventually validate PI3KC2α as a prognostic tool thus allowing the development of a new therapeutic option for breast cancer patients.

No conflict of interest.

307 p140Cap, a Chromosome 17q12-q21 scaffold protein, is a new prognostic marker in ERBB2 breast cancer

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Introduction. The HER2 breast cancer subtype defines ~20% of breast cancers. There is increasing evidence that amplification of additional genes in the genomic region surrounding HER2 gene (chr17q12-q21) may contribute to HER2 tumor heterogeneity in progression and treatment efficacy. p140Cap encoded by the SRCIN1 gene at Chr17q12, is a docking protein that behaves as a negative regulator of breast tumor growth and metastasis formation. Here we study the impact of SRCIN1 amplification and p140Cap expression in HER2 breast cancer patients.

Material and method. aCGH was performed on 200 HER2+ breast cancers together with matching gene expression profiles from 50 cases. FISH was performed with a mix of the home made probe for the SRCIN1 locus, and the commercial CEP17 of chromosome 17. Immunohistochemical analysis of p140Cap expression was performed on PFA-embedded breast TMA breast tumor sections using an anti p140Cap mAb. Immunocomplexes were visualized by the EnVision™+ HRP.

Results and discussion. aCGH analysis of the 17q12-q21 amplification pattern in 200 HER2 tumors from a Swedish cohort revealed that the SRCIN1 gene copy number is altered in 70% of the 200 cases, with 123 cases showing a gain and 18 cases displaying a loss. Kaplan-Meier survival curves stratified by SRCIN1 gene status, showed that the amplification of the SRCIN1 gene correlated with significantly improved survival. mRNA expression and SRCIN1 gene copy number were significantly correlated, giving a Pearson correlation of 0.77. FISH analysis on 34 HER2 consecutive breast cancer patients at diagnosis in Torino, showed that SRCIN1 is amplified in 56% of the specimens, and not amplified in 44%.

Analysis of p140Cap expression was performed on a Consecutive Cohort of Invasive Breast Cancer (N = 622) arrayed on TMA. Data for p140Cap expression was available for 515 out of 622 samples. Cancer patients with high expression of p140Cap have a lower risk of developing distant metastases (Hazard Ratio: 0.52, P= 0.012). In particular in patients with HER2 amplification, p140Cap overexpression correlated with a significantly less probability of develop a distant event (Hazard Ratio: 0.36, P=0.05). p140Cap overexpression correlated with a better survival in the whole cohort of patients (Hazard Ratio: 0.51, P=0.016) and in patients with HER2 amplification (Hazard Ratio: 0.32, P=0.021).

Conclusion. Together, we show that the SRCIN1 gene is amplified in >50% of ERBB2 amplified breast cancer cases and that p140Cap expression correlates with a lower risk of developing distant metastases and improved patient survival.

No conflict of interest.

308 Infantile myofibromatosis PDGFRB mutants are constitutively activated and sensitive to tyrosine kinase inhibitors

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Introduction. Infantile myofibromatosis is the most prevalent tumor of soft tissue of childhood. It is characterized by the presence of nodules in the skin, the subcutaneous soft tissues, the bones or the viscera. Recently, germline and somatic heterozygous PDGFRB mutations have been associated with familial infantile myofibromatosis (FIM) but have not been functionally characterized. PDGFRB mutations were also described in a particular case of overgrowth syndrome and in Fahr disease. Platelet-derived growth factor receptors are receptors-tyrosine kinases that stimulate cell growth and motility. So far, activating point mutation in PDGFRB has not been reported in human cancer.

Methods. In order to characterize these new PDGFRB mutations, we transiently expressed PDGFRB mutants in HT-1080 cells, a fibrosarcoma-derived human cell line and in Ba/F3 cells, a common model to test oncogene-induced proliferation.

Results and discussion. Three mutants induced constitutive signaling in the absence of ligand. The detailed analysis of the signaling pathways downstream these mutants showed that, in the absence of ligand, the somatic N666K mutant strongly activated mitogen-activated protein kinases (ERK), phospholipase Cγ and signal transducer and activator of transcription 3 (STAT3). The germline R561C mutant was less active than the N666K mutant whereas the P660T mutant showed no difference with the wild-type receptor. Moreover, in foci formation assays with NIH3T3 cells, we observed that the N666K mutant and, to a lesser extent, the R561C mutant were able to transform cells, unlike the P660T mutant. Interestingly, activated mutants were sensitive to imatinib, nilotinib and ponatinib.

Conclusion. Altogether, our results indicate that three of the reported FIM-associated mutations activate PDGFRB, supporting the hypothesis that these mutations cause the disease. Moreover, tyrosine kinase inhibitors seem to be promising treatments for patients with visceral tumors carrying these mutations. To our knowledge, these are the first confirmed gain-of-function point mutations of PDGFRB in human cancer.

No conflict of interest.

309 Role of miRNA-214 in melanoma progression

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Introduction. Malignant melanoma is one of the most aggressive human cancers with increasing incidence and poor prognosis in its metastatic stage. Although the mechanisms regulating metastatic progression are not yet completely understood, much progress has been made in unraveling the underlying molecular mechanisms. In the past years a substantial number of works have demonstrated the role of miRNAs in melanoma pathogenesis. Our laboratory has shown that miRNA-214 is highly expressed in invasive human melanomas and it contributes to melanoma spread by controlling tumor cell migration an invasion in vitro and, more importantly, tumor cell extravasation and metastasis formation in mice. Mechanistically, we showed that this occurs via the regulation of specific direct and indirect target genes in tumor cells, for instance TFAP2, ITGA3, ALCAM and miR-148b. A role for miR-214 in Cancer Associated Fibroblasts (CAFs) has been shown for ovarian cancers. We have evidences indicating that various stroma cells express high levels of miR-214 and that stroma cells can influence melanoma cell migration when co-culture are analyzed. We generated or obtained mice overexpressing or depleted for miR-214 and we are now investigating the role of stroma cells in tumor progression in these mice.

Materials and Methods. We injected B16.F10 melanoma cells (syngeneic) subcutaneously into the right flank of our miR-214 modified mice and evaluated tumor volumes and micro or macro metastases. In parallel we evaluated the number of circulating tumor cells by taking blood samples from these mice and expanding the cell content in culture. The same mice were also injected in the tail vein and the number of pulmonary nodules were counted.

Mouse embryo fibroblasts (MEFs) were derived from E13.5 miR-214 knock-out or over-expressing mice and characterized for their biological properties, such as growth, motility, transformation.

Results and discussion. Our results underline a role for miR-214 in stroma cells in the coordination of tumor dissemination. Detailed experiments will be presented at the meeting.

Conclusion. By using miR-214 modified mice we aim at the identification of miR-214 function in tumor and tumor-associated stroma cells.

No conflict of interest.

310 Monocytes/macrophages in cancer, from tumor aggressors to vascular components - a new insight for anti-angiogenic therapy

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The formation of new blood vessels in tumors is a crucial step for disease progression. Over the past decades we have witnessed the development and testing of drugs to treat cancer with anti-angiogenic activity. The failure of this therapeutic strategy tells us that we do not fully know the mechanisms underlying the formation of new blood vessels in cancer.

The role of monocytes as endothelial progenitor cells is not a new subject in cardiovascular diseases and inflammatory systemic disease, though in cancer monocytes have been underestimated as relevant cells to sustain vascular growth. Hence, anti-angiogenic therapy in cancer does not contemplate monocytes as targets.

Our experimental data shows that mononuclear CD11b+ cells (mainly monocytes) harvested from peripheral blood (PBMCs), when cultured with CXCL12 they increase the expression of CD11b whereas the incubation with VEGF induces the expression of CD34 (endothelial marker). We have also seen that cancer patients have higher levels of double positive CD31+/CD14+ cells in peripheral blood comparing to normal individuals. Moreover, cells cultured in VEGF proliferate more and presented a spindle cell like morphology.

In addition, a child having a brain cavernoma exihibited higher levels of double positive CD14+/CD31+ cells, before therapy with propranolol then normal individuals. During follow up a decrease in CD14+/CD31+ levels in peripheral blood was observed, coming close to normal profiles. Concomitantly, the levels of VEGF in peripheral blood also decreased to normal levels.

We believe this is a hot subject that must be brought into debate, since a new view on neovascularization mechanisms, different from the canonical one (angiogenesis), might contribute to a change in the paradigm of vascularization targeting cancer therapy. It is our belief that monocytes/macrophages, as stable non-malignant tumor 'helper' cells, represent powerful therapeutic targets, either as tumor-associated macrophages and/or as vasculature structure components, arising, herein, as an eventual new approach for anti-vascular therapy, whose common approach is directed to 'bona-fide' endothelium.

No conflict of interest.

311 BM-MSC loaded with PTX home to brain tumor and induce cytotoxic damage

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The key goal in cancer chemotherapy still remains to localize the drug effect specifically in the tumor microenvironment minimizing collateral toxicity. Mesenchymal stromal cells (MSCs) have recently gained great interest as therapeutic tool, because of their unique biological features. MSCs exert their therapeutic effects by several mechanisms, including the ability to home to pathological tissues. In previous studies, we demonstrated that MSC without any genetic manipulation, uptake and release the chemotherapeutic drug Paclitaxel (PTX) in an amount enough to impair tumor growth in subcutaneous mice models (1). Here we wanted to assess if PTX-loaded MSCs have a tropism in orthotopic glioblastoma multiforme (GBM) brain xenografts. Moreover, we characterized cytotoxic effect of PTX-loaded MSCs on tumor cells.

Materials and Methods. We stereotactically implanted the red fluorescent GBM cell line, U87MG Cherry, and the murine GFP-labeled SR4987 BM-MSCs loaded with PTX (SR4987-GFP-PTX) in the brain of immunosuppressed rats. The two cell lines were engrafted in adjacent but different brain sites. Serial brain sections were analyzed by confocal microscopy to, a) address tumor homing of MSC-PTX cells and b) to characterize the cytotoxic damage induced by PTX released in the tumor microenvironment by loaded PTX-MSCs.

Results and discussion. SR4987-GFP-PTX migrated from the injection site showing tropism toward the tumor. Notably, a significant number of cells either penetrated into or located around the tumor. Immunohistochemical detection of mitotic spindles and centrosomes revealed a remarkable increase of abnormal spindles as well as of centrosomes in the tumor exposed to SR4987-GFP-PTX. PTX released in the tumor microenvironment significantly increased the percentage of abnormal mitoses predominantly because of multispindles divisions, resulting in a dramatic increase of multinucleated tumor cells. These data demonstrate that dividing tumor cells proceeded through mitosis with abnormal spindles, resulting in chromosome mis-segregation, eventually leading cells to die.

Conclusions. The use of MSCs for local drug delivery has a therapeutic potentiality since a PTX-specific cytotoxic damage of brain tumors can be achieved avoiding the side effects of systemic delivery.

1. Pessina A et al, PlosOne, 2011.

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No conflict of interest.

312 Pharmacological characterisation of nicotinic receptors in gliomas and glioblastoma cells

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Introduction. Gliomas and glioblastomas (GBM) are a family of brain malignant tumours that originate from different glial elements and display high levels of proliferative, migratory and invasion activities. The therapeutic strategy consists of surgical resection and radiotherapy combined with chemotherapy, but more than 70% of patients die within 2 years of diagnosis.

Cigarette smoke is a major environmental risk factor for initiation and development of human malignancy, and cigarette smokers are prominently represented among individuals diagnosed with gliomas. Nicotine is an important active ingredient in tobacco, and promotes tumor progression by binding and activating cell-surface neuronal nicotinic acetylcholine receptors (nAChRs) an heterogeneous family of ligand-gated ion channels. However very little is known about the nAChRs expressed by glial cells and their signalling mechanisms in physiological and pathological conditions.

The aim of this study is to identify nAChRs in gliomas and glioblastomas cells, to investigate the molecular mechanisms by which nAChRs regulate cell proliferation and intracellular signalling and if this signalling is modulated by nicotine.

Methods. Analysis of mRNAs subunit expression was evaluated by qRT-PCR in U87MG glioma cell line and primary glioblastoma cultures derived from patients. Cell proliferation was tested using the MTS assay and cell counting after serum starvation. Wound-healing assay was used to evaluate cells invasive properties. Immunoblot analyses was performed to detect phospho-ERK and phospho-AKT levels.

Results. Nicotine, in a dose dependent manner, significantly increases migration and cell proliferation. These effects are blocked by incubating the cells with the specific nAChR antagonists αBungarotoxin, methyllycaconitine and mecamylamine. We also tested in cell lines and primary tumors three stylbene-derived α7 antagonists that reduces the viability of glioma cells in a dose-dependent manner with no effect on neuroblastoma and hepatocyte cell lines.

Preliminary results indicate also that nicotine affects the phosphorylation level of Erk and Akt pahways in a time dependent manner.

Conclusions. Nicotine activates signaling pathways that promote the proliferation and the invasive property of gliomas and glioblastomas cells. In particular, α-bungarotoxin sensitive receptors mediate this nicotine-induced proliferation and may represent a possible target for new therapeutic strategies.

No conflict of interest.

313 CXCR4 deregulation in Notch3-induced acute T cell lymphoblastic leukemia

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Introduction. One of the major cause of acute T cell lymphoblastic-leukemia (T-ALL) in humans and mice is the constitutive activation of Notch signaling. We previously demonstrated the oncogenic function of Notch3, since enforced expression of the intracellular Notch3 active form (N3-IC) in immature thymocytes (N3-ICtg) induces an acute form of leukemia (T-ALL). Deregulated proliferation and maturation at the preT/T transition phase and constitutive activation of preTCR were observed in N3-ICtg mice .

Cooperative signaling among the preTCR, CXCR4 and Notch are required at β selection for the continued differentiation from Double Negative (DN) to Double Positive (DP) T cells. The stromal cell derived factor SDF-1(CXCL12) and its receptor CXCR4 promote survival of DN thymocytes, regulate the migration during the DN/DP transition and has been suggested to play a role in the pathogenesis of T-ALL. Our aim is to define the possible crosstalk between the pathway of Notch3 and CXCR4.

Material and methods. We used as in vivo model, six- to twelve-week-old, N3-ICtg mice over-expressing the N3-IC under the control of the lck proximal promoter. N3 and CXCR4 expression analyzed in freshly isolated cells from thymus, spleen, and blood. CD4-CD8- (DN) T cells were purified from thymus of N3-ICtg and wt mice, by ' FACS-assisted cell sorting', to obtain mRNA further processed for Real-Time RT-PCR and miRNA analysis.

Results. DN-gated thymocytes display reduced percentages of CXCR4 positive cells in Notch3-ICtg with respect to wt mice, not attributable to any reduction of DN cell numbers. Furthermore, mRNA from selected DN thymocytes of N3-ICtg mice evidenced a reduced transcription of CXCR4 gene. miRNA regulatory network is essential in CXCR4 modulation, and some of them are regulated by Notch suggesting a possible interplay between the two pathways in our model. Our data may suggest that Notch3 disrupts early event in preT-cell progressive maturation by altering migration through the thymus and reducing EpCAM expression, a homotypic adhesion molecule. On the other hand, T/DP cells were anomalously represented in spleen, blood and lymph nodes of N3-ICtg mice. All characterized by an increased and combined Notch3/CXCR4 surface expression.

Conclusions. Our data are suggestive of a possible scenario in which Notch3 deregulating CXCR4 may lead to aberrant positioning of immature DN thymocytes and may modulate DP cells egress from thymus, in early steps of T-ALL development.

No conflict of interest.

314 Metabolomic profile of biological fluids of ovarian cancer patients

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Introduction. Clear Cell Carcinoma (CCC) has a very poor prognosis mainly due to late diagnosis and its low response to standard platinum-based chemotherapy. The 5 year survival of these patients is about 5%.

Cancer metabolism is a rediscovered, poorly known and tumour specific field. The impaired features of CCC among ovarian cancer, namely lipids and glycogen accumulation, indicate that CCC have a particular metabolic physiopathology. The identification/description of CCC metabolome will point out some genes and metabolic pathways that will be over active or de novo active in CCC. Those findings will allow the design of more specific and effective therapeutic strategies that can

contribute for a longer and higher quality life of CCC patients, which is our main goal. Besides that, the identified compounds can constitute early diagnosis, follow-up and recurrence markers as well.

Our main objective is to define peripheral blood serum and ascitic fluid metabolic profile in the CCC patients in order to find genes/proteins that can constitute specific therapeutic targets,

Methodology. Serum (n=77) and ascitic fluid (n=20) samples were analysed by 1H-NMR in a Bruker 600 Avance and 800 Avance, respectively. Compounds identification was performed by resorting to Chenomx NMR Suite program and Human Metabolome database. Classification of the different types of samples was be performed by multivariate analysis (PCA and OPLS-DA) implement in R and SIMCA.

Results and discussion. The 1H-NMR serum samples analyse allow the discrimination between the patients with malign ovarian tumour and benign tumour patients and healthy controls. The discrimination is based on increased levels of acetoacetate, acetone, 3-hidroxybutyrate, glutamine and lactate, while the levels of choline, alanine and valine are decreased on the malign tumour patients. In ascitic fluid, the levels of acetone and acetoacetate are higher than in serum, indicating an enhancement of the ketones bodies metabolism.

Conclusions. These preliminary results indicate that the presence of malignant ovary tumours lead to metabolic alterations that could be detect in the blood. These alterations are correlated with an increase of the ketone bodies.

No conflict of interest.

315 BMPR2 promotes doxorubicin-resistance in breast cancer cells

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Introduction. Chemoresistance is still a major obstacle to breast cancer treatment. Bone morphogenetic protein receptor type II (BMPR2) is a member of TGF-ß family, and can regulate bone, teeth, and vascular formation. On the one hand, TGF-ß is a well-known regulator in chemoresistance development by inducing epithelial to mesenchymal transition (EMT). However, the roles of BMPR2 in breast cancer chemoresistance remain elusive.

Material and methods. In this study we mimicked the clinical application of doxorubicin using in vitro and in vivo models. Gene expressions were analyzed by qPCR and western blots. Further physiological assays like cell viability assay, clonogenic assay, cell cycle analysis, comet assay and γH2AX staining revealed insights into the BMPR2-mediated chemoresistance.

Results and discussion. We demonstrate that BMPR2 promotes doxorubicin chemoresistance in breast cancer cells via regulating cell proliferation and cell cycle, DNA repair, and apoptosis. Moreover, in a xenograft mouse model we confirm an increased BMPR2 expression in doxorubicin-resistant breast cancer cells.

Conclusion. Our findings show novel roles of BMPR2 in breast cancer chemoresistance. Antagonizing BMPR2 action might improve clinical outcome of breast cancer patients by sensitizing resistant tumor cells to doxorubicin.

Keywords : BMPR2, chemoresistance, breast cancer, doxorubicin

No conflict of interest.

316 Identification and characterization of a novel ARID1a interaction with CAD (carbamoyl-phophate synthetase 2, aspartate transcarbamylase, and dihydroorotase)

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Introduction. The tumor suppressor ARID1a is mutated in ~50% of ovarian clear cell carcinomas and ~30% of ovarian/endometrial endometrioid carcinomas. We used a proteomic approach to identify a novel protein-protein interaction that for the first time links ARID1a to direct regulation of CAD, the rate controlling enzyme in pyrimidine biosynthesis.

Materials and Methods. Mass spectrometry analysis of the immunoaffinity purified ARID1a complex was done to identify interacting proteins. Validation was done by co-immunoprecipitation followed by immunoblotting, as well as pulldown assays using recombinant proteins. In ovarian and endometrial carcinoma cell lines, the effect of ARID1a knockdown (using targeting shRNA vs control shRNA) on CAD expression, phosphorylation, and proliferation, was evaluated by immunoblotting using total and phospho-specific antibodies, and by measuring BrdU incorporation, respectively. The effect of ARID1A overexpression (by transfection of HA-tagged full-length wildtype ARID1a vs empty vector) was evaluated using the same **Methods.** The ability of CAD knockdown (using targeting shRNA vs control shRNA) to rescue the phenotype of ARID1a depletion was assessed similarly.

Results and Discussion. The identification of the individual protein bands of the ARID1a complex was determined by mass spectrometry, with a 243 kDa band determined to be CAD. Co-immunoprecipation followed by immunoblotting validated the interaction of the endogenous proteins. Pulldown assays using recombinant tagged proteins showed confirmatory results. ARID1a depletion (in ARID1a wildtype cells) vs control shRNA increased CAD phosphorylation at the key regulatory site serine 1859, and increased cellular proliferation. This was reversed by CAD shRNA (vs control shRNA). ARID1a overexpression (in ARID1a mutant cell lines) reduced CAD phosphorylation and reduced cellular proliferation.

Conclusion. A novel function of the important tumor suppressor ARID1a has been identified. ARID1a directly interacts with CAD, the rate-limiting enzyme of pyrimidine biosynthesis. ARID1a loss promotes CAD phosphorylation at a key regulatory site and increases cancer cell proliferation in a CAD-dependent manner.

No conflict of interest.

317 Differential uPAR recruitment in caveolar-lipid rafts by gm1 and gm3 gangliosides regulates endothelial progenitor cells angiogenesis

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Introduction. Gangliosides and the urokinase plasminogen activator receptor (uPAR) tipically partition in specialized membrane microdomains called lipid-rafts. uPAR becomes functionally important in fostering angiogenesis in endothelial progenitor cells (EPCs) upon recruitment in caveolar-lipid rafts. Moreover, cell membrane enrichment with exogenous GM1 ganglioside is pro-angiogenic and opposite to the activity of GM3 ganglioside.

Materials and Methods. On these basis, we first checked the interaction of uPAR with membrane models enriched with GM1 or GM3, relying on the adoption of solid-supported mobile bilayer lipid membranes with raft-like composition formed onto solid hydrophilic surfaces, and evaluated by surface plasmon resonance (SPR) the extent of uPAR recruitment. We estimated the apparent dissociation constants of uPAR-GM1/GM3 complexes. These preliminary observations, indicating that uPAR binds preferentially to GM1-enriched biomimetic membranes, were validated by identifying a pro-angiogenic activity of GM1-enriched EPCs, based on GM1-dependent uPAR recruitment in caveolar rafts.

Results and discussion. We have observed that addition of GM1 to EPCs culture medium promotes matrigel invasion and capillary morphogenesis, as opposed to the anti-angiogenesis activity of GM3. Moreover, GM1 also stimulates MAPKinases signaling pathways, typically associated with an angiogenesis program. Caveolar-raft isolation and Western blotting of uPAR showed that GM1 promotes caveolar-raft partitioning of uPAR, as opposed to control and GM3-challenged EPCs. By confocal microscopy, we have shown that in EPCs uPAR is present on the surface in at least three compartments, respectively, associated to GM1, GM3 and caveolar rafts. Following GM1 exogenous addition, the GM3 compartment is depleted of uPAR which is recruited within caveolar rafts thereby triggering angiogenesis.

Conclusion. On the basis of these data and of our observations, we suggest that GM1-dependent localization of uPAR in caveolar-LRs accounts for GM1-dependent angiogenesis.

No conflict of interest.

318 Loss of Class II PI3K-C2A promotes aneuploidy in breast cancer and sensitivity to anti-mitotic agents

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Introduction. PI3K signaling axis is one of the most frequently deregulated pathways in human cancer impacting on cell growth, survival and metabolism. Whereas the majority of efforts have so far focused on class I PI3K, increasing evidence is pointing to the importance of class II enzymes in cell proliferation and survival.

Material and methods. We generated a mouse strain lacking PI3KC2A expression and found that the mutation is embryonic lethal. Pik3c2a+/- mice were intercrossed with a transgenic strain that specifically expresses the activated HER-2/Neu oncogene in the mammary gland. Mice were weekly followed for survival, tumor appearance and growth. We derived mouse embryonic fibroblast (MEF) and Primary Murine Mammary Epithelial Tumor cells (MMET). Effects of heterozygous loss of Pik3c2a were evaluated by cell proliferation, immunofluorescence, karyotype and CGH analysis. Efficacy of anti-mitotic cancer drugs were examined in MMET cells and in mouse models.

Results and discussion. We have generated MEFs from Pik3c2a+/+, Pik3c2a+/- and Pik3c2a-/- embryos, and their ability to proliferate was assessed. Karyotype analysis revealed that high levels of aneuploidy in Pik3c2a-/- MEFs compared to wt controls. Heterozygous MEFs also displayed haploinsufficiency and gene dosage dependency. To evaluate the oncogenic role of PI3KC2A in cancer, we targeted its expression in a breast cancer mouse model (NeuT). The heterozygous loss of Pik3c2a initially delays tumor onset and in the long run, leads to a faster growth rate compared to wt. We

found that Pikkc2a loss causes an aberrant microtubule (MT) spindle organization that, in turn, promotes genomic instability. In line with this, Pi3kc2a is specifically enriched at the metaphase spindle, interacting with transforming acidic coiled-coil protein 3 (TACC3)/colonic, hepatic tumor overexpressed gene (ch-TOG)/clathrin complex to stabilize K-fibers during mitosis. Despite the aberrant MT organization, we demonstrate that tumors bypass the requirement of Pi3kc2a through a common mechanism of progression. Multiple genes involved in the spindle-associated checkpoint (SAC), such as Bub1, Bub3 and APC/C genes, resulted either amplified or lost in fast growing compared to slow growing tumors. In addition, tumors with low Pi3kc2a and aberrant spindle organization showed increased sensitivity to anti-MT agents (Paclitaxel) both in vitro and in vivo. Expression profiles of breast cancer patients showed that reduced levels of PIK3C2A correlated with aggressive tumors, indicating that reduction in PIK3C2A expression provides a growth advantage in mice as well as in patients.

Conclusions. We demonstrated that loss of PI3KC2A plays a crucial role in promoting genomic instability and sensitivity to anti-mitotic agents. These findings will eventually validate PI3KC2A as a new diagnostic/prognostic tool that can be exploited to tailor more effective therapies for breast cancers.

No conflict of interest.

319 Concomitant intracellular retention of SPARC and CATHEPSIN B by SCD5-induced oleic acid production reduces melanoma malignancy

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Introduction. The increased demand of fatty acids (FA) to assemble the plasma membranes of continuously dividing cancer cells might unbalance their ratio and critically affect tumor outgrowth. Saturated FAs, as palmitic and stearic, are desaturated by Δ9 stearoyl-coA-desaturase enzymes (SCD1 and SCD5) to produce the monounsaturated palmitoleic and oleic FAs. The effect of desaturase activity in cancer cells is partially known for SCD1, while virtually unknown for SCD5. We have investigated the expression and function of SCD5 in melanoma.

Materials and Methods. Expression studies were performed by western blot, qReal time PCR, immunohistochemistry and immunofluorescence according to standard procedures. Quantification of FAs was evaluated by gas chromatography/mass spectrometry analysis. For in vivo studies, xenografted nude mice were utilized to evaluate SCD5 functional role on tumor growth and metastatic potential. The effects of different pH culture conditions were evaluate.

Results and Discussion. The close correlation between the expression pattern of SCD5 and the amount of oleic acid (OA), both downmodulated during melanoma progression, suggests their role against the aggressiveness of advanced melanoma. Accordingly, SCD5 enforced expression in A375M metastatic melanoma, besides inducing a significant increase of OA, blocks SPARC release leading to its significant intracellular accumulation paralleled by cathepsin B and collagen IV retention. The same results on the malignant parameters of melanoma were obtained by the exogenous supplementation of OA. More important, in vivo models of induced human melanoma metastases or murine spontaneous metastases confirmed a significant SCD5-dependent impairment through a process that modifies the ECM. Finally, results indicated that the SCD5- or OA-dependent reduction of the intracellular pH is associated with a more physiological condition (pHext > pHint) according to the notion that an acidic microenvironment enhances SPARC activity and ECM remodeling toward dissemination.

Conclusion. Our data support a protective role of SCD5 and its enzymatic product oleic acid against malignancy, a finding offering explanation for the beneficial Mediterranean diet. Furthermore, SCD5 appears to functionally connect tumor cells and surrounding stroma toward modification of tumor microenvironment with consequences on tumor spread and resistance to treatments.

No conflict of interest.

320 Tumor-associated stromal cells increase malignancy of human colorectal cancer inducing epithelial-to-mesenchymal transition

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Introduction. During tumor formation, normal tissue microenvironment is transformed in an 'altered' niche, composed of non-malignant supporting cells, which influence the homeostasis of cancer cells via paracrine regulators. Tumor-associated stromal cells (TASC) are the prominent stromal elements in most types of human carcinomas including colorectal cancer (CRC). The differentiation of TASC from other cell types, such as resident stromal cells or bone marrow-derived mesenchymal stem cells (BM-MSC) is mainly mediated by factors produced during the crosstalk with tumor. TASC produce various extracellular matrix proteins, chemokines, and other promoting factors which affect vascularization, tumor cell proliferation and invasiveness, and they also play a critical role in determining response to therapy. TASC-derived factors may contribute to the development of a protective milieu by influencing cell-cell/cell-matrix interactions, cell survival, and suppression of anti-tumor immune responses. Moreover, physical contact between TASC and malignant cells supports cell survival via activation of anti-apoptotic pathways or inducing epithelial-to-mesenchymal transition (EMT). In this scenario our purpose is to address phenotypic and functional characterization of TASC in vitro and analyzed TASC-mediated effects on CRC development and progression in vivo.

Material and Method. TASC were characterized for phenotype and differentiation capacity. Human CRC cells were cultured in the presence of TASC in 2D and in 3D perfused bioreactor, sorted by flow cytometry and evaluated for the expression of EMT-related genes by Real Time PCR and for in vitro invasiveness by chemoinvasion assay. Furthermore, their tumorigenicity was assessed upon injection in NOD/SCID mice and developing tumors were analyzed.

Results. Our results indicate that TASC freshly isolated from CRC samples comprise a multipotent subpopulation that is able to differentiate into adipogenic and osteogenic lineage. After coculture with CRC cells they express membrane-bound TGF-beta, through which they are capable to trigger EMT. Moreover CRC cells cocultured with TASC acquire an elongated shape and a more invasive phenotype. Upon subcutaneous injection in NOD/SCID mice, tumor cells cocultured with TASC show a significantly faster growth kinetic and develop significantly larger tumor masses with an higher vessel density as compared to tumor cells alone. Interestingly tumors developed from tumor cells cocultured with TASC display the presence of LGR5 positive cells.

Conclusion. Thus our data show that the stromal component of CRC comprises a multipotent subpopulation and increases the tumor cells malignancy triggering EMT induction through membrane-bound TGF-beta.

No conflict of interest.

321 Nrf2, but not ß-catenin, mutation represents an early event in rat hepatocarcinogenesis

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Introduction. Hepatocellular carcinoma (HCC) develops through a multistage process but the nature of the molecular changes associated to the different steps, the very early ones in particular, is largely unknown. Recently, dysregulation of the NRF2/KEAP1 pathway and mutations of these genes have been observed in experimental and human tumors, suggesting their possible role in cancer development.

Material and method. To assess whether Nrf2/Keap1 mutations are early or late events in HCC development, we investigated their frequency in the Resistant-Hepatocyte (R-H) model, a chemically-induced rat model of hepatocarcinogenesis, analyzing preneoplastic and neoplastic liver lesions by Sanger sequencing.

Results and discussion. We found that Nrf2/Keap1 mutations were present in 71% of early preneoplastic lesions and in 78.6% and 59.3% of early (eHCC) and advanced HCC (aHCC), respectively. Nrf2 mutations were more frequent, missense and involved the Nrf2-Keap1 binding region. Mutations of Keap1 occurred at a much lower frequency in both preneoplastic lesions and HCCs and were mutually exclusive with those of Nrf2. Unlike Nrf2, mutations of Ctnnb1, which are frequent in human HCC, were a later event, as they appeared only in fully advanced HCCs (18.5%). Functional in vitro experiments show that Nrf2/Keap1 mutations lead to pathway activation, as demonstrated by the strong upregulation of NRF2 target genes (Nqo1, Gclc, Gsta4) in mutated preneoplastic lesions compared to control liver or to non-mutated preneoplastic nodules, suggesting that these are, actually, activating mutations. We found a strong upregulation of Nqo1 in eHCCs and aHCCs compared to normal liver, as well. Interestingly, unlike preneoplastic lesions, no significant difference in Nqo1 expression was found in mutated vs. non mutated HCCs, suggesting that, at late stages, the Nrf2-Keap1 pathway no longer depends on the presence of activating mutations only, but it can be activated by other mechanisms. In vivo studies showed that Nrf2 silencing inhibited the ability of tumorigenic rat cells to grow in soft agar and to form tumors, if subcutaneously injected in syngeneic rats.

Conclusion. Our results demonstrate that in the R-H model of hepatocarcinogenesis, the onset of Nrf2 mutations is a very early event, likely essential for the clonal expansion of preneoplastic hepatocytes to HCC, while Ctnnb1 mutations occur only at very late stages. Moreover, functional experiments demonstrate that Nrf2 is an oncogene, critical for HCC progression and development.

No conflict of interest.

322 Inhibition of CXCR4 receptor by a novel peptide antagonist modulates microglia reactivity and angiogenesis in a human glioblastoma model

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Background. The chemokine receptor CXCR4 is widely expressed in cancer. Its activation by the chemokine CXCL12 has been shown to sustain metastasis and angiogenesis and regulate the crosstalk between tumor and microenvironment. In

gliomas, cancer cells attracts surrounding microglia/macrophages into the tumor mass polarizing infiltrating cells into an anti-inflammatory, immunosuppressive, proangiogenic phenotype (M2) that promote cancer growth. In this context CXCL12/CXCR4 axis is implicated in macrophages/microglia recruitment into tumor bulk and promote M2 phenotype polarization. Aim of our study was to evaluate the effects mediated by a novel CXCR4 antagonist, Peptide R (Portella L., Plos One 2013) in a glioblastoma multiforme (GBM) experimental model.

Material and Method. Studies were performed using intracranial xenografts of U87MG, a human GBM cell line. Cells were injected in CD1 nude mice, administered for 15-20 days since the day of cells implantation, with Peptide R, a newly synthetized CXCR4 antagonist, or AMD3100 (Plerixafor ®), a well-known CXCR4 antagonist. Tumor volume was assessed by magnetic resonance imaging (MRI) analyses (4.7 T) at different time intervals during the treatment. Immunofluorescence (IF) and Confocal Laser Scanning Microscopy (CLSM) analyses on mouse brain sections were conducted to analyze the expression of microglia/macrophages markers (CD11b, CD68) and markers associated to pro-inflammatory microglial phenotype (M1) or immunosuppressive phenotype (M2), such as inducible nitric oxide synthase (iNOS) and Arginase-1 (Arg-1) respectively. Moreover, we examined the expression of vascular endothelial growth factor (VEGF), and the endothelial marker CD31.

Results and Discussion. IF and CLSM analyses of samples revealed that Peptide R reduced the number of macrophage/microglial cells (CD11b+/CD68+) recruited at the tumor edge and increased the percentage of CD11b+/iNOS+ cells, suggesting that Peptide R could favor the acquisition of pro-inflammatory phenotypic features by CD11b+ cells. Peptide R reduced Arg-1expression associated with intratumoral endothelial structures as well as that of VEGF and CD31 in tumor core. **Conclusion.** Our results suggest that the disruption of CXCL12/CXCR4 axis by Peptide R modulates tumor microenvironment creating a niche less favorable for tumor expansion. In view of the anti-angiogenic and M1 polarizing effects observed, our data suggest that Peptide R could represent a promising tool to modulate local inflammatory responses mediated by CXCR4/CXCL12 signaling axis.

No conflict of interest.

323 Ovarian cancer peritoneal dissemination is characterized by a loss of miR-506 expression associated to acquisition of mesenchimal phenotype and increased resistance to platinum treatment

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Introduction. Epithelial ovarian cancer (EOC) has a peculiar dissemination process: tumor cells slough-off from primary tumors and spread throughout the peritoneal cavity. This process is frequently associated with acquisition of mesenchymal phenotype with a cell reprogramming also involving development of resistance to platinum-based chemotherapy. However, a clinically useful definition of molecules driving these behaviours is still lacking.

Materials and Methods. Forty-four couples (formalin-fixed paraffin embedded tissues) of chemo naïf primary tumors and synchronous secondary peritoneal localizations, obtained at primary surgery from MITO2 clinical trial, have been profiled for microRNA (miRNA) expression on an Agilent Platform. Direct targeting of selected predicted genes was confirmed and functional biological assays performed in appropriated cellular models. Relevant miRNAs/genes modulation has been validated on an independent set of samples with similar characteristics.

Results and discussion. By class comparison analysis, imposing a false discovery rate <10%, 45 miRNAs were identified as differentially expressed: 32 down-modulated and 13 up-modulated in secondary localizations compared to primary tumors. Among the miRNAs down-modulated in the secondary localizations we detected most of the miRNA belonging to the Xq27.3 cluster, whose low expression we previously described to be associated with EOC early relapse. One of the miRs belonging to the cluster, hsa-miR-506, is a key regulator of epithelial/mesenchimal transition (EMT) and is associated to platinum resistance; its ectopic expression in EOC cell lines increased their sensitivity to the drug. Besides targeting the E-cad transcriptional repressor, SNAI2, we show that miR-506 simultaneously suppresses vimentin and N-cadherin thus representing a new class of miRNA that regulates master players of EMT and metastatization process. On the other hand, among the miR-506 predicted target, we validated RAD17 involved in DNA damage repair pathways; it actively guides BRCA1/2 to DNA and stabilizes strand break DNA-repair active signaling.

Conclusion. Loss of miR-506 expression in EOC peritoneal dissemination is associated to acquisition of mesenchimal characteristics and resistance to chemotherapeutic treatment.

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No conflict of interest.

324 Phospholipid scramblase 1: At the cross-road between autophagy and apoptosis in mantle cell lymphoma

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Introduction. Phospholipid scramblase 1 (PLSCR1) belongs to a family of proteins which bi-directionally flip lipids across membranes and it is responsible for cell surface exposure of phosphatidylserine during apoptosis. Recently, new functions have been described about PLSCR1 involvement also in cell proliferation and differentiation. Herein we suggest a new role of PLSCR1 in autophagy control.

Autophagy and apoptosis are two processes tightly associated and, commonly, the first one is a resistance mechanism to avoid drug-induced cell death. Currently different clinical trials evaluate the possibility to combine autophagy inhibitors with pro-apoptotic agents to enhance their antitumor activity. Recent studies demonstrated that is possible to overcome resistance to mTOR inhibitor (everolimus®) in mantle cell lymphoma (MCL) by blocking autophagic flux.

Material and method. Autophagy and apoptosis were studied by conventional and multispectral imaging flow cytometry and confirmed by immunoblotting analysis of specific molecular markers. Mino cell line expressing ectopic PLSCR1 (+/- LC3B-GFP) or silenced for the same protein allowed us to analyze PLSCR1 contribution to drug-induced autophagy/apoptosis. PLSCR1 expression in tumor biopsies was evaluated by immunohistochemistry.

Results and Discussion. Our results showed that the combination of retinoic acid (RA) with Interferon(IFN)-a induced anti-apoptotic autophagy in MCL cells. Microarray-based expression profiling allowed the identification of PLSCR1 as one of genes significantly up-regulated by this treatment. Interestingly, immunohistochemical analysis of 28 tumor biopsies revealed heterogeneous expression of PLSCR1 in this setting and stimulated an in-depth analysis. In particular, the expression of PLSCR1 was induced by RA/IFN-α at both transcriptional and protein levels and a further increase was obtained, in association with a higher apoptosis extent, by blocking the autophagic flux with chloroquine. Interestingly, comparable results were observed when doxorubicin or bortezomib were added to cell culture after RA/IFN-α pre-treatment, likely as consequence of an autophagy blocking as shown by sequestosome1/p62 accumulation. Ectopic PLSCR1 expression reduced cell susceptibility to starvation-induced autophagy, while PLSCR1 silencing increased MCL cells resistance to drug-dependent apoptosis.

Conclusion. In summary, our data suggested that the presence of PLSCR1 could improve MCL response to anticancer pro-apoptotic treatments counteracting protective autophagic process.

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No conflict of interest.

325 miR-199a-3p in vivo study in papillary thyroid carcinoma mouse model

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Introduction. We recently reported that miR-199a-3p under-expressed in papillary thyroid carcinoma (PTC) down-regulates MET protein and displays tumor suppressor function in PTC cell lines. Moreover, we demonstrated for the first time that miR-199a-3p induces in PTC cells a form of non-apoptotic cell death, named methuosis, caused by the cytoplasmic accumulation of macropinosomes. To date, methuosis has been showed in several cancer cells mainly in vitro and its occurrence in vivo remains to be demonstrated. In this study, to investigate if miR-199a-3p may exert tumor suppressor function and trigger methuosis in vivo, we used a PTC xenograft mouse model and tested a recently reported protocol for the analysis of macropinocytosis in vivo.

Material and methods. CD-1 nude mice were injected subcutaneously with the PTC-derived K1 cell line. When tumors reached a volume of 100-150 mm3, miR-199a-3p mimic or negative control (NC) were intratumorally injected. A treatment schedule of an injection every 4 days for a total of 4 injections was followed. 2 days after the last injection, tumors were collected and analyzed for tumor volume, MET protein expression by IHC and histopathological features by HE staining. To assess macropinocytosis in vivo, a preliminary experiment was performed in miR-199a-3p treated mice by the intratumoral injection of 2mg FITC-dextran 1 hour before the tumor collection. Dextran uptake was analyzed by IF.

Results and Discussion. miR-199a-3p tumors compared to NC displayed on average reduced MET protein level and tumor volume, suggesting that also in vivo miR-199a-3p may efficiently regulate its target and exert tumor suppression function. Nevertheless, histopathological analysis revealed the presence of necrotic areas surrounding the intratumoral injection site both in miR-199a-3p and NC tumors, suggesting that in our experimental set technical problems are associated to this procedure. This issue makes difficult the assessment of methuosis-like features, underling the need of an alternative methodological approach. The preliminary tests on macropinocytosis in

vivo showed that miR-199a-3p xenografts displayed FITC-positive areas indicative of dextran uptake, indicating that this protocol is applicable in our experimental set.

Conclusions. Further methodological set up is required to assess if methuosis could occur in vivo. As alternative approach, we are planning to develop xenograft model from K1 cells expressing miR-199a-3p.

No conflict of interest.

326 Identification of chromosomal alterations in different breast cancer subpopulations reveals distinctive molecular profiles and therapeutic responses

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Introduction. A small subpopulation of stem/progenitor cells can give rise to the diversity of differentiated cells that comprise the bulk of the tumor. Many of the current modalities for breast cancer treatment are based on different pharmacological compounds which, although have been successfully used in clinical settings, show variability in the efficacy of treatment. Patients displaying exactly the same clinical-pathological features may present different clinical outcomes and may frequently relapse. The establishment of long-term cultures of stem/progenitor cells may help for understanding how the molecular features of subtypes of breast cancer may be targeted in breast cancer patients.

Materials and Methods. From breast cancer tissue, we obtained cultures of CD44+/CD24- cells with stem/progenitor cells properties, which were able to form tumors when injected into SCID mice. Semi-quantitative RT-PCR and Western blot analyses have been performed to evaluate gene and protein expression of biomarkers representative for breast cancer (ER/PgR, HER2) and stem (Hedgehog) cell phenotypes. Conventional (Caryotype) and molecular cytogenetic analyses (Multi-Fish Assay) have been carry out on the aforementioned cells. Conventional drugs for breast cancer (tamoxifen, aromatase inhibitors, trastuzumab, lapatinib) were administered in vitro to breast cancer cells and in xenograft- derived tumor cells to investigate their effect on growth (MTT assay).

Results and Discussion. The cells analyzed have revealed different genetic profiles in vitro. Interestingly, in cells derived from in vivo selection of triple negative breast cancer cells, we found a gain in estrogen receptor expression, coupled with a reduction in canonical GLI1 expression, as measured by WB analysis. Lapatinib displays a higher cytotoxic effect on xenograft- derived tumor cells, where numerous and different numerical and structural chromosomal rearrangements have been observed.

Conclusion. The knowledge of molecular mechanisms by which during tumorigenesis the genome evolves towards catastrophic chromosomal rearrangements (chromoplexy or chromotripsis) may provide important information for clinical intervention, a 'customized' picture of patient outcome and identify new targets treatment.

No conflict of interest.

327 The effects of nicotine in non small cell lung cancers by binding neuronal nicotinic acetylcholine receptors

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Introduction. Lung cancer is the leading cause of cancer death worldwide and smoking accounts for approximately 70% of non-small cell lung cancer (NSCLC) and 90% of small cell lung cancer (SCLC) cases, although there is a subset of patients who develop lung cancer without a history of smoking.

Tobacco smoke contains multiple classes of carcinogens, and nicotine, the most active component of tobacco smoke, by binding to cell-surface neuronal nicotinic acetylcholine receptors (nAChRs), promotes tumour growth and metastasis by inducing cell-cycle progression, migration and invasion.

In this work we have analysed the nAChR expression in lung cancer cell lines and lung cancer tissues. We have investigated the effects of nicotine in regulating cell proliferation and cell migration and studied the intracellular signalling of nicotine.

Material and method. We used two human NSCLC cell lines (A549 and H-1975) and lung cancer tissues obtained from patients.

Analysis of mRNAs subunit expression was evaluated by qRT-PCR in both NSCLC cell lines and tissues. Cell viability assay (MTS) and cell counting experiments have been performed in cells quiescent by serum starvation for 48 hours and stimulated with nicotine or nAChR antagonists. After the pharmacological treatments the cells were lysed and used for immunoblot analyses to detect phospho-ERK and phospho-AKT levels.

Boyden Chamber assay and wound healing assay measure A549 invasion property.

Result and discussion. qRT-PCR shows the presence of $\alpha 7$–containing receptors in the A549 but not in H1975 cells, while tumour samples show an increase in the level of CHRNA5 subunit mRNA with no significative change of CHRNA7 mRNA.

Nicotine, in a dose-dependent manner, significantly increases the A549 cell viability and proliferation but has no effect on H1975 cells.

To determine whether the $\alpha 7$ nAChR might mediate the nicotine-induced proliferation we treated A549 cells with α-bungarotoxin (α-bgtx), an $\alpha 7$ selective antagonist and we found that α-bgtx blocks the nicotine-induced proliferation in A549 cells .

Nicotine enhances the migration of A549 cells and induces a selective time-dependent activation of phospho-ERK and phospho--AKT pathways in A549 cells.

Conclusion. Collectively our data suggests that nicotine promotes proliferation, invasion and migration of NSCLC A549 by activating the AKT/ERK pathway. We would therefore investigate the role of drugs targeting nAChRs for new therapeutic strategy.

No conflict of interest.

328 Use of a perfusion-based bioreactor to maintain in vitro the tumor microenvironment of primary human colorectal cancer specimens

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Introduction. Two-dimensional (2D) in vitro culture systems and in vivo animal models are the primary tools used to test cancer cell responses to drugs. However, drug sensitivity data obtained via 2D culture systems can be misrepresentative, while, in the opposite end, patient derived xenografts (PDTX) are time consuming, expensive and the non-transformed components of tumor microenvironment are lost.

The use of three-dimensional (3D) systems based on human tissue could be an innovative and efficient tool able to bridge the gap between 2D cultures and animal model for the development of new chemotherapeutic strategies.

Material and methods. Fresh surgically colorectal cancer (CRC) specimens were cut into fragments, inserted into a collagen scaffold sandwich and then cultured for 10 days using a perfusion-based bioreactor system. We assessed tissue in vitro survival, proliferation and the maintenance of the microenvironment cell components by histo-morphological analysis, immunofluorescence and gene expression for Ki67, EpCAM, vimentin, CD90, CD45, CD4, and CD8.

Results and discussion. After 10 days of culture the tissue partially maintained its original architecture with typical neoplastic disorganization. Phenotypic analysis confirmed that expanded tissues included epithelial and stromal cells. Tumor cell proliferation, as provide by Ki67 staining, was assessed. Furthermore, CD4+ and CD8+ T lymphocytes were detected within cultured tumor fragments.

Our results indicate that culture of primary tumor fragments within perfused bioreactors allows the preservation of the diverse CRC cellular components, thus representing a relevant tool for the evaluation of anti-tumor treatments.

Conflict of interest

Other substantive relationships: Cellec Biotek AG, Basel, Switzerland

329 The role of Nek 6 gene expression in multidrug resistance and apoptosis in MCF-7 and K-562 cell lines

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Introduction. Chemotherapy is the most efficient and commonly used treatment strategy for cancer therapy. The simultaneous resistance of cancer cells to a number of structurally or functionally unrelated chemotherapeutic agents is called as multidrug resistance (MDR), a major complication for successful chemotherapy. Chemoresistance to apoptosis induction is one of the mechanisms for development of acquired resistance. Never in mitosis gene A-related kinase 6 (Nek6), is newly identified member of Nek gene family which is important in cell cycle progression. Nek6 transcript is highly upregulated and its kinase activity increased in many cancers. Studies show that the overexpression of Nek6 increases the anchorage-independent growth of cancer cells and that the knockdown of Nek6 induce apoptosis and results in the reduction of tumors in the nude mouse xenograft model. However, the biological functions and mechanisms of Nek6 activity in carcinogenesis and MDR has not been clarified yet.

In current study, the role of Nek6 on multidrug resistance and apoptosis were investigated in drug-sensitive and drug-resistant MCF-7 and K-562 cell lines.

Materials & Methods. Resistant cell lines were developed from parental MCF-7 and K-562 cell lines by stepwise selection in drug increments. Total RNA isolated from all cell lines and cDNA was synthesized from 1 ug total RNA by using Random Hexamer. Gene expressions of Nek6 and apoptotic genes; Bax, survivin, PUMA, NOXA, Bcl-2 and c-FLIP; were examined by qRT-PCR. Nek6 gene expression was knocked down by Nek6 specific siRNA delivery. Resistance level of cells were examined after Nek6 gene

silencing by XTT cell proliferation assay. Changes in the expression levels of apoptotic genes were investigated by qRT-PCR. The data were represented mean±SEM and analyzed by one-way ANOVA and post-hoc Tukey's test. The results were significant when $p<0.05$.

Results. The results showed that Nek6 gene expression shows no significant change between MCF-7 sensitive and resistant subtypes. On the other hand, Nek6 gene expression is downregulated in resistant K-562 cell lines compared to sensitive one. After Nek6 siRNA delivery, the expression level of some apoptotic genes were changed in K-562 cell line.

Conclusion. It was shown that Nek6 expression is important for the MDR development in K-562 cell line but not in MCF-7 cells. On the other hand, Nek6 knockdown affects the expression levels of several apoptotic genes. However, further analysis should be performed to reveal the function of Nek6 in MDR.

No conflict of interest.

330 Modulation of Acute Myeloide Leukaemia (AML) metabolism by the Vascular Endothelial Growth Factor (VEGF) - in vitro study

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Several studies have highlighted cancer metabolism as a suitable target to develop new therapies. This thesis aimed at determining the role of lactate metabolism in AML, both VEGF dependent and independent, by developing an in vitro study (pro-myelocytic-HL60 and erithroblastic-HEL).

We observed that HL60 and HEL have different metabolic profiles when exposed to lactate and/or VEGF. NMR analysis showed that HL60 uses lactate to synthetize acetate, amino acids and malate and upon VEGF stimulation nucleotides are detected though not originated from lactate or glucose. Concordantly, VEGF exposure increases the expression of MCT1 in HL60 cells. HEL cells' metabolic profile is not altered by VEGF stimulation and lactate is mainly converted into malate and proline. However, VEGF decreases MCT1 and MCT4 levels in HEL. In both cell lines, LDHA and LDHB expression levels are equivalent between conditions. Concerning VEGF receptors, in HL60 VEGF increases and lactate decreases the levels of KDR, expressing more FLT1. HEL cells barely express KDR and all the stimulations tested increase the expression of FLT1.

Cell cycle analysis showed that VEGF and lactate increase proliferation of respectively HL60 and HEL cells. The percentage of dead cells in the same conditions was higher, assenting the concomitant media consumption.

In order to validate our results, we verified that the majority of BM samples express higher levels of MCT1 than MCT4. By immunohistochemistry, MCT1 is expressed in aberrant and large cells whereas MCT4 is expressed in normal mature and progenitor cells. MCT1 positive cells are preferentially localised in paratrabecular regions, the VEGF rich niches.

Our study showed VEGF regulates lactate metabolism and proliferation in monocytic AML cells. In human samples, higher levels of MCT1 are expressed at diagnosis and relapse, being MCT1 expressed in cells morphologically aberrant. Taking together out results indicate MCT1 as a suitable therapeutic target in AML.

No conflict of interest.

332 Role of WNT/ß-Catenin pathway in endocannabinoid-mediated antitumor effects in human CRC

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Introduction. Colorectal cancer (CRC) arises through a multistep process involving a series of pathological alteration. Increasing evidence showed that the endocannabinoids control tumor growth and progression, both in vitro and in vivo, acting as antiproliferative, antiangiogenetic and antimetastatic compounds.

In a high percentage (≥ 85%) of both sporadic and familial adenomatous polyposis forms of CRC, the inactivation of the APC tumor suppressor gene initiates tumor formation and modulate the WNT/ β-catenin transduction pathways involved in the control of cell proliferation, adhesion and metastasis.

In this study we evaluated the potential direct effect of SR141716, a Cannabinoid Receptor 1 (CB1) antagonist/reverse agonist, on the WNT/β-catenin pathway in HCT116, a human CRC cell line.

Matherials and **Methods.** In HCT116 (deleted at Ser45 β-catenin allele and wild-type for both APC and p53), treated with SR141716 (10μM) we studied the expression of protein belonging to the WNT/β-catenin pathway by western blot and the activation of TCF/LEF- and AP1-regulated target genes by Luciferase assay.

Results and Discussion. In HCT-116 cell line, SR141716 reduced the expression of WNT3 and increased both β-catenin phosphorylation and APC protein. Moreover, SR141716 significantly reduced luciferase expression controlled by a TCF/LEF responsive element.

On the other hand, SR141716 was able to induce WNT5 and to activate CaMKII. Moreover, in HCT116 cells, SR141716 significantly reduced the luciferase activity controlled by the transcriptional response element for AP1, and reduced the phosphorylated form of JNK, one of the upstream regulators of AP-1 activity.

Conclusion. Obtained data strongly suggested a direct effect of SR141716 in the regulation of both canonical and non-canonical β-catenin signaling in human CRC cellular models.

No conflict of interest.

333 Antiproliferative effect of N(6)-isopentenyladenosine in human colorectal cancer cell lines: New potential role in epigenetic regulation

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Introduction. N(6)-isopentenyladenosine (iPA) is a naturally occurring modified nucleoside, characterized by an isopentenyl chain derived by dimethylallyl pyrophosphate (DMAPP), an intermediate of the metabolic pathway of mevalonate.

iPA is present in mammalian cells as a free mononucleoside in the cytoplasm, or in a tRNA-bound form, displaying well established pleiotropic biological effects, including a direct anti-tumor activity against several cancers. However, the precise mechanism of action of iPA in inhibiting cancer cell proliferation remains to be clarified.

Here, we investigated the molecular mechanisms of the antitumor effects triggered by iPA in colorectal cancer (CRC) cell lines.

Materials and Methods. Human CRC cell lines DLD1, SW620 and HCT116, were treated with iPA for increasing concentrations. Cell proliferation was evaluated in vitro by BrdU incorporation assay. FACS analysis was performed to assess apoptosis and cell cycle progression. Identification and validation of iPA-protein interactors have been performed through a proteomic approach and protein levels were analyzed trough western blot analysis.

Results and Discussion. Our results demonstrate that iPA significantly inhibits the proliferation of CRC cell lines, in a dose- and time- dependent manner. FACS analysis of propidium iodide (PI)-stained DLD1 and SW620 cells revealed a strong accumulation of CRC population in a pre-G1 phase, after treatment with iPA. Moreover, flow cytometry assay using Annexin V/PI double staining, confirmed that iPA induces apoptosis in DLD1 and SW620 cells starting from 24h of treatment.

Through fishing for partners gel free approach, in both cytosolic and nuclear extract of DLD1, we identified, among others, histone H2B as a iPA-direct protein partner.

Since histones undergo a wide variety of reversible covalent modifications, we evaluated the iPA-mediated effect on post-translational histone H2B modifications. Western Blot analysis after iPA exposure, showed a clear modulation of mono-ubiquitinated Lys120-H2B (H2BK120ub). In agreement with the role of H2BK120ub, iPA also regulates Lys-4-H3 and Lys-79-H3 methylation, revealing the ability of iPA to control 'H2B-H3 histone cross-talk' involved in the transcriptional regulation.

Conclusion. The preliminary results suggested that anti-proliferative effect of iPA could arise from epigenetic regulation of genes involved in cell survival.

No conflict of interest.

334 SGK2: A new modulator of platinum drug resistance in epithelial ovarian cancer

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Introduction. Epithelial Ovarian Cancers (EOC) represent the most lethal gynaecological malignancies due to the advanced stage at diagnosis and the development of drug-resistant recurrences after a initial response to platinum drugs. Therefore the identification of novel genes involved in chemoresistance is mandatory for the design of new therapeutic strategies. Alterations of different cellular pathways such as DNA repair, apoptosis and p53, have been involved in chemoresistance mechanisms. Our aim is to identify new modulators inside these pathways, which could help to overcome drug-resistance in EOC.

Material and Method. We performed a functional genomic screening evaluating cell survival arising from the combination of gene silencing (of 685 genes related to the mentioned pathways) and platinum treatment in different EOC cell lines. Bioinformatics analysis and validation screening identified 8 genes as targets involved in chemo-resistance. One of these genes is SGK2 (serum and glucocorticoid inducible kinase 2). The expression levels of SGK family members in different EOC cell lines were evaluated. Cell viability tests validated the screening results. Commercially available SGK inhibitors were tested. Role of SGK2 over-expression in EOC cells' growth was also evaluated in vitro and in vivo in mouse models.

Results and Discussion. SGK2 is a member of a family of serine/threonine kinases consisting of three distinct but highly homologous genes: SGK1, SGK2 and SGK3. In a series of primary EOC SGK2 is expressed in about 50% of the cases. Our results confirmed that SGK2 silencing (but not SGK1 and SGK3)increased cell sensitivity to both carboplatin (CBDCA) and cisplatin (CDDP) in different EOC cell lines. Pharmacological inhibition of SGK activity using specific inhibitors, mimicked the effect of SGK2 is downregulation by shRNAs on platinum sensitivity. SGK inhibitors sensitized EOC cells to platinum only when SGK2 is expressed, confirming that it represents the only member of the family that have an impact on drug sensitivity. **Conclusion.** SGK2 inhibition sensitizes EOC cells to platinum treatment and the

combination of SGK2 inhibition and platinum treatment may represent a promising strategy to improve the management of EOC patients. Mechanism by which SGK2 protects cancer cells from platinum induced death is currently under investigation, in particular downstream partners and how SGK2 silencing/inhibition induced cell death under platinum treatment.

No conflict of interest.

335 Characterization of a murine orthotopic xenograft model of glioblastoma multiforme: The glioma stem cell gamble

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Background. Glioblastoma multiforme (GBM) is a grade IV astrocytoma and the most common malignant brain tumor. Current therapies provide a median survival of ~14 months after diagnosis. The failure of current therapies may be due to the presence within tumor of cells with stem-like properties, called glioma stem cells (GSCs). Moreover, the GSC subpopulation is directly involved in GBM relapse because of its sharp chemo and radioresistance.

The development of in vivo models that faithfully mirror human diseases is essential for the validation of new therapeutic approaches. In particular, xenografts of GSCs appear to be promising tools for recreating a human brain tumor in an immunocompromised host. The generation of GBM orthotopic xenograft models from GSCs could play a crucial role in the design of GSC-targeted treatments and in the comprehension of the resistance molecular mechanisms.

Material and Method. In this study, we develop a murine orthotopic xenograft model using a GSC line (G144) from GBM. Fifteen NOD/SCID (4-6 weeks old) male mice were intracranially injected with 2*105 cancer cells. After six months, they were sacrificed. Brains were fixed in paraformaldehyde and embedded in paraffin. Serial sections of brains were cut and several sections were stained with H&E to histologically detect brain lesions. Remaining sections were used to perform immunohistochemistry analysis (IHCs) using antibodies against several stem and differentiation markers. Furthermore, we set up a FISH using a human EGFR-specific probe to confirm the human origin of the proliferating cells observed in murine brains.

Results and Discussion. Brain lesions were observed in 4 out of 15 mice, with a 27% engraftment efficiency. Tumor cells showed the characteristic infiltrative behavior of this cancer: in fact, GSCs invaded healthy brain tissue and migrated in the controlater hemisphere. IHC evidenced immunoreactive cells for CD44 (++) and Nestin (+++), which are stem markers, and for GFAP (+++) and MAP2 (+), two differentiation markers, while we did not observe immunoreactive cells for Vimentin (-). FISH analysis confirmed the presence of human cancer cells. In the very next future, we will perform a genome-wide comparative analysis on engrafted cells by aCGH.

Conclusion. Our GBM in vivo model recapitulates the typical features of this cancer and could represent an interesting tool to develop novel GSC-targeted therapies, aimed at the eradication of the cancer stem compartment.

Keywords: Murine orthotopic xenograft, GBM, Glioma stem cells.

No conflict of interest.

336 Validation of let-7c cluster as breast carcinoma early biomarkers using a bioluminescent mouse model

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Introduction. Breast cancer is a heterogeneous disorder characterized by many tumor subtypes with different biological characteristics. Recently, microRNAs (miR) have been proposed as potential biomarkers in the development and progression of several tumor types, including breast. In fact, the level of expression of these small RNAs that act as tumor suppressors or oncogenes are finely modulated during tumor progression. Among these, let-7c cluster, that include miR-99a and -125b, is an intriguing miRs in human breast cancers with multiple functions as a regulator of cell proliferation, differentiation and apoptosis that strictly depends on the cellular context. Here, we evaluated by using a mouse models engineered to study the dynamics of cell proliferation, let-7c cluster as early biomarkers in breast carcinoma progression.

Material and method. We used the mouse model generated by crossing MMTV-neuT breast cancer mouse model and MITO-Luc reporter mice a recently generated model in which it is possible to follow proliferation events in every body district in alive animals. The expression of transgenes was assessed by PCR. Animals MITO+/neuT- and MITO+/neuT+ have been subjected to in vivo bioluminescent imaging every week from 7 to 18 weeks of life. The tumors of mice MITO+/neuT+ have undergone ex vivo imaging sessions. Liquid and solid biopsies were collected from mice MITO+/neuT- and MITO+/neuT+. Expression of let-7c, miR-99a and miR-125b was evaluated by stem-loop qRT-PCR in transformed and non-transformed breast tissues at the moments of the animal life with an increase of systemic proliferation index.

Results and Discussion. The mouse model described above allowed us to visualize the temporal evolution of breast cancer within the entire animal since the early stages of the disease. We found that cell proliferation at the level of the breast does not play a key role in the progression of breast cancer. In contrast, during the cancer development, are present in bone marrow cells, waves of proliferation detectable even in pre-neoplastic stages. We evaluated the expression levels of the let-7c cluster in breast tissues during the bone marrow waves of proliferation. Our ongoing experiments show that, the expression of the oncosuppressor let-7c, miR-99a and miR-125b is significantly down-regulated during breast cancer progression.
Conclusion. By using non-invasive bioluminescent molecular imaging, in genetically modified mouse models, we identified the members of let-7c cluster as potential early biomarkers during breast cancer progression.

No conflict of interest.

337 Chemopreventive activity of olive mill wastewaters

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Introduction. Angiogenesis is a crucial event for cancer progression. Several diet derived compounds have been reported to exert anti-oxidant, anti-proliferative, anti-angiogenic and pro-apoptotic effects in a variety of cancers. The consumption of extra virgin olive oil represents an important constituent of the mediterranean diet. The presence of several phenolic molecules, including hydroxytyrosol, is believed to prevent the occurrence of a variety of pathological processes, including cancer. While the strong antioxidant potential of these molecules is well characterized, their anti-angiogenic activities remain unknown. Here we assessed the anti-angiogenic and anti-tumor properties exerted by extracts from olive mill wastewaters (OMWWs), which represent a waste product from olive oil industry, in vitro and in vivo in endothelial and colorectal cancer (CRC) models.

Material and Methods. OMWWs ability to affect cell proliferation and survival were evaluated on HUVECs, six different tumor cell lines and murine CT26 CRC cells with MTT assay, while the induction of apoptosis and reactive oxygen species (ROS) were assessed by flow cytometry. Functional studies evaluated the capacity of OMWWs to interfere with endothelial cell tube formation, migration and invasion by morphogenesis and Boyden chamber assays, respectively. Finally, the inhibition of angiogenesis and tumor cell growth was evaluated in vivo, by the matrigel sponge assay and tumor xenograft.

Results and Discussion. OMWWs were able to inhibit both HUVECs and tumor cell growth in a dose dependent manner, exerting a stronger inhibitory effect comparing to purified hydroxytyrosol. This effect was directly associated with the induction of apoptosis and ROS on HUVECs. Moreover, OMWWs were able to inhibit HUVEC morphogenesis, migration and invasion in a dose dependent manner. Finally, OMWWs inhibited tumor angiogenesis and CT26 tumor cell growth in vivo.

Conclusions. Our results suggest that polyphenol enriched extracts from olive oil processing (OMWWs), show an anti-angiogenic and anti-tumor potential. In particular, our data demonstrate that a pool of specific polyphenols are characterized by stronger anti-angiogenic/anti-tumor properties compared to hydroxytyrosol alone, a well known polyphenol with anti-tumor activity. These data could be explained by the presence, in OMWWs, of different polyphenols that act synergistically, thus improving their single component effects.

No conflict of interest.

338 Roles and mechanisms of NANOG-mediated drug resistance in human colorectal cancer cells

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Introduction. Tumours form a disorganized hierarchy of heterogeneous cell populations, on which current chemotherapy regimens fail to exert discriminative cytotoxicity. A small sub-population of poorly differentiated cancer stem cells (CSCs), exhibit an embryonic stem cell gene expression signature1,2. CSCs evade conventional drugs, and significantly contribute to adverse survival rates3.

We and others have reported the expression of embryonic proteins, including carcino-embryonic antigen (CEA), alkaline-phosphatase, and NANOG in human colorectal cancer (CRC) and other cancers, which may contain undifferentiated self-renewal cells4-6. These suggest that NANOG may be an essential modulator of the cancer cell drug-resistance mechanisms of which preventing differentiation in CSCs. Dedifferentiation is an established hallmark of carcinogenesis, accompanied by key signaling pathways mediated in drug resistance such as DNA damage, mitogen-activated protein kinases (MAPKs) & GSK3-ß)/ß-catenin pathways via epithelial-mesenchymal transition (EMT).

Material and methods. a) Cytotoxicity and viability analysis of stable HCT116 cell lines; expressing GFP and GFP-NANOG (HCT116GFP vs. HCT116GFP-NANOG cells), treated with 5-FU, MEK/ERK & GSK3-b, and tested by Fluorometric, Colonosphere & stem-cell associated gene reporter assays.

b) Western blotting, qRT-PCR & immunofluorescent assays used to explore the specific cellular & molecular mechanism of action of NANOG-expressing cells.

Results and discussion.

- Markedly increased Nanog expression correlates with 5-FU resistance in CRC cells
- NANOG-expressing cells exhibited aberrant expression of phospho-ERK and phospho-GSK3-β levels.
- Overexpression of NANOG enhanced stemness and proliferation activity of CRC cells via ERK/GSK-3b mediated EMT activation in CRC cells.

Conclusion. NANOG could be a novel biomarker for CSCs in CRC, and that NANOG could play a crucial role in maintaining the self-renewal of CSCs through the ERK/GSK3-b mediated EMT activation in CRC cells.

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No conflict of interest.

339 Development of a novel tumor homing compound with antiproliferative and anti-metastatic profile for glioblastoma multiform

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Introduction. Glioblastoma Multiform (GBM), is one of the most fatal types of cancer with a median patient survival of 12 to 15 months. Current therapies include radiotherapy concurrent with temozolamide. Bevacizumab has been approved recently, as adjuvant therapy for patients with progressive GBM. Another therapeutic perspective involves tyrosine kinase inhibitors (TKIs), investigating single or multiple targeting concomitantly. Unfortunately, the majority of therapeutic approaches are so far disappointing, emerging crucially the need for novel, more effective treatment agents. In the current project we developed a tumor homing peptide able to selectively kill glioblastoma multiform cells without affecting normal cells.

Materials and Methods. Synthesis of the peptide was performed using classical Fmoc-a-aminoacid based synthesis. The novel agent was tested for its effect in both GBM cells (U87, LN18 and M059K) and non cancer cells (L-929). Cell number was estimated using MTT assay. Furthermore, we evaluated the novel compound's effect on 3D culture in soft agar. In addition, several types of cell death were determined including apoptosis (programmed cell death type I), autophagy (programmed cell death type II) and necrosis as well as cell cycle arrest. Annexin V and dead cell assay kit were used for apoptosis and necrosis, western blot with a-beclin-1 for autophagy and a cell cycle assay kit for the distribution of cell cycle phases. Wound healing and boyden chamber assay were used for evaluating the ability of cells to migrate.

Results and Discussion. The novel compound exerted an antiproliferative effect in all GBM cell lines in 2D and 3D cell cultures. However, it did not affect L929 cells, a normal fibroblast cell line that was used as control. The anti-proliferative effect was associated with a specific type of cell death. This effect was in accordance with reduced cell migration.

Conclusions. Herein, we have developed a novel tumor homing peptide that selectively targets glioblastoma cells and decreases both cell proliferation and migration. The novel compound is able to be used as a carrier of biological cargoes and/or cytotoxic agents, too. In addition, the novel compound could be used as a theranostic agent if combined with an appropriate fluorophore.

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No conflict of interest.

340 Vorinostat induces HNF1ß expression and latency in ovarian clear cell carcinoma (OCCC) and resistence in ovarian serous carcinoma (OSC)

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Background. Ovarian clear cell carcinoma (OCCC) is a rare type of ovarian cancer known to be highly chemoresistant and having a very poor prognosis comparing to most common histological type, ovarian serous carcinoma (OSC). Unique histology, de novo expression of HNF1β, molecular changes in tumor suppressor ARID1A gene, and overexpression of histone deacetylases (HDAC) are also associated with OCCC.

Aims: To evaluate the action of vorinostat, a HDAC inhibitor, in the regulation of HNF1β expression and cell survival.

Methods. We used an OCCC cell line (ES2) and OSC cell line (OVCAR3) cultured with and without vorinostat (5μM). Cell death was evaluated by flow cytometry, Annexin V-FITC and PI. HNF1β expression was evaluated by RQ-PCR and western blotting. HNF1β promoter activity was evaluated by luciferase reporter gene assay. ARID1A binding to HNF1β promoter was evaluated by chromatin immunoprecipitation.

Results. Vorinostat induces cell death in both cell lines, but in OVCAR3 induces resistance after long term exposure whereas latency is induced in ES2. In OCCC cell line vorinostat increased HNF1β promoter activity as well as HNF1β expression, consistent with the increased relative occupancy of ARID1A in HNF1β promoter.

Conclusions. Vorinostat may not be an appropriate therapy in ovarian cancer. However, exposure to vorinostat pointed out a regulation mechanism of HNF1β expression, mediated by ARID1A. This mechanism can be used to define more effective therapeutic strategies.

Key words: clear cell carcinoma (OCCC), vorinostat, HNF1β, ARID1A

No conflict of interest.

341 Diverse role of mesenchymal stromal cells in tumor biology

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Introduction. The discovery that mesenchymal stromal cells (MSC) are recruited to the tumor site has led to an increased interest in the function of MSC in tumors. Their preference for injured, inflamed and tumor tissue is exploited in regenerative medicine. MSC levels in the peripheral blood of cancer patients were described to increase in comparison to healthy person, where were exposed to chemotherapy and could alter the behavior of tumor cells on multiple levels. Understanding this crosstalk and also the network of cytokines and secreted growth factors may lead to important new therapeutic approaches in controlling the growth and metastasis of tumors.

Material and Methods. The tumor cells were cultivated in conditioned medium from adipose tissue-derived MSC (CM-MSC) or in chemotherapy pretreated CM-MSC. We analysed chemosensitivity, migratory potential and proliferation of breast cancer cells by fluorimetric, mammosphere culture assay and IncuCyte Zoom™ Kinetic Imaging System. The expression of specific genes was analysed by qPCR.

Results and discussion. We have shown that MSC were able to stimulate an epithelial-to-mesenchymal transition (EMT) in breast cancer cells associated also with changes in morphology and expression of EMT-related genes and genes responsible for stemness. EMT phenotype corelated as well as with increased migration of Sk-Br-3 and MCF-7 cells in the presence of MSC-secreted factors. We have analysed chemotherapy pretreated AT-MSC and their secreted factors protecting the tumor cells against apoptosis and increasing resistance of tumor cells to cisplatin and doxorubicin in 2D, 3D and in vivo conditions. We have analysed possible signalling pathways and cytokines involved in the MSC-mediated chemoresistance.

Conclusion. We have described complex role of MSC in the tumor microenvironment and have shown that they express a wide scale of cytokines, chemokines and growth factors, which were able to confer increased metastatic potential and resistance of breast cancer cells. There is a need to consider intrinsic properties of MSCs during their application in cancer-related diseases and potential interaction that might be important for the therapeutic efficiency.

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No conflict of interest.

342 Immodin and its effect on 4T1 derived mouse breast cancer in vivo when combined with Paclitaxel or Manumycin A

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Introduction. Nowadays, it is well known that surgical antitumor treatment as well as radiotherapy and chemotherapy improves the disease prognosis and increases the survival rate of cancer patients. Despite the positive aspects of chemotherapy and radiotherapy, various side effects on bone marrow and lymphocyte proliferation which can induce a patient immunodeficiency were demonstrated. Immodin is an ultrafiltered dialysate released from disintegrated blood leukocytes of healthy donors which possesses a number of non-specific activities, such as the ability to increase the number of immunocompetent cells, to stimulate phagocytosis and hemopoiesis and others.

Material and method. Mouse mammary adenocarcinoma 4T1 cell line was inoculated to BALB/c mice in the number of 100,000 cells/per mouse. Immodin (administered i.p. in a dose of 0.05 U/per mouse) was administered to mice 10 times (each day) alone or together with Paclitaxel (administered i.p. in a dose of 200μg/mouse, 5 times) or Manumycin A (administered p.o. in a dose of 100μg/mouse, 6 times) after the onset of palpable tumors. All immunological parameters were analyzed by flow cytometry 24 hours after the last dose of the treatment and for survival of animals the Kaplan-Meier curve was built.

Results and discussion. In our study, we have analyzed the effect of Immodin and its combination with conventional chemotherapeutic agent Paclitaxel or alternative therapy Manumycin A (natural compound isolated from Streptomyces parvulus) on the growth of 4T1 mouse breast cancer cells inoculated to BALB/c mice as well as survival of tumor-bearing mice. Our results showed that the combinations of Immodin with Paclitaxel or Manumycin A compared to the individual treatments were associated with longer overall survival and reduced tumor volume. Moreover, the combinations of Immodin with Paclitaxel or Manumycin A eliminated the negative impact of single therapeutic agents on immune cells in blood and immune organs

such as spleen. Indeed, Immodin increased the activity of NK cells in spleen tissue and positively modulated the number of T cells declined after Paclitaxel or Manumycin therapy.

Conclusion. The combinations of Immodin with Paclitaxel or Manumycin A revealed longer overall survival with reduced tumor volume and the elimination of the negative impact of single therapeutic agents on immune cells in blood and spleen of 4T1 derived tumor-bearing mice.

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343 Characterization of non-competitive inhibitors of androgen receptor identified from high throughput screening

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Introduction. There are limited therapeutics for Castration Resistant Prostate Cancer (CRPC). Studies have shown that Androgen Receptor (AR) reactivation through mutations, amplification, truncation, and other mechanisms contribute to the development of CRPC. Since AR mutations can turn a competitive inhibitor into an agonist, we screened for non-competitive AR inhibitors.

Material and Method. We constructed a cell line stably expressing an androgen-responsive promoter-driven firefly luciferase and a truncated AR without ligand binding domain, called AR-V4. The cells were also transfected with a CMV promoter-driven Renilla luciferase for the identification of non-specific inhibitors. High throughput screening (HTS) of about 2,400 compounds in LOPAC and Prestwick chemical libraries led to identification of three compounds that inhibited the AR-V4-induced firefly luciferase activity with little effect on Renilla luciferase activity within the assay period. These three compounds were further characterized in several prostate cancer cell lines for their ability in blocking AR activity and in inhibiting the growth of AR positive prostate cancer cells with MTT and soft agar assays.

Results and Discussion. The three compounds are disulfiram (DSF), nelfinavir (NFV) and thiostrepton (THS). DSF and NFV are both FDA-approved drug for treating chronic alcoholism and Human Immunodeficiency Virus (HIV) respectively. THS is an antibacterial agent and also a proteasome inhibitor. All three compounds were not able to inhibit estrogen-induced transcriptional activity in estrogen receptor (ER) positive cells suggesting that they target AR, but not ERs. All three compounds inhibited AR transcriptional activity in a dose-dependent manner, apparently by down-regulating AR expression. They also inhibited the growth of prostate cancer cells in a dose-dependent manner. The AR positive prostate cancer cells were in general more responsive to them than AR negative prostate cancer cells. The combination of DSF+NFV or DSF+THS showed even a greater growth inhibitory effect whereas the combination on NFV+THS showed no additional growth inhibitory activity. We have also shown that the compounds inhibited the anchorage-independent growth of AR positive prostate cancer cell lines in soft agar assay.

Conclusion. Our cell-based system was effective for high throughput screening of non-competitive inhibitors of AR. The three drugs we have identified may have potential to be repurposed for treating AR-driven CRPC.

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No conflict of interest.

344 M2 receptor activation inhibits cell growth and survival in human breast cancer

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Introduction. The involvement of muscarinic receptors in the modulation of tumor growth and progression has been largely demonstrated in several tumor types. Recently we demonstrated that the M2 agonist, Arecaidine Propargyl Ester (APE) arrests cell proliferation and induces apoptosis in glioblastoma and urothelial bladder cancer cells. In this work we investigated the effects mediated by M2 receptors in breast cancer cell lines and the combined effect of APE with paclitaxel, a conventional breast cancer drug.

Materials and Methods. MCF-7 and MDA-MB-231 cell lines were cultured in DMEM supplemented with 10% FBS. MTT assay and trypan blue staining were used to evaluate cell viability and cell death respectively. M2 silencing (by siRNA) was used to confirm the ability of M2 agonist to selectively bind this receptor. Transcript levels for muscarinic receptors, EGFR and multidrug efflux pumps (e.g. ATP binding cassette, ABC) were analyzed by RT-PCR. Results. We detected the expression of M2 receptor subtype by RT-PCR analysis and protein expression was confirmed by western blot analysis both in MCF-7 and MDA-MB-231 cell lines, being higher in MDA-MB-231 than in MCF-7 cells. The M2 agonist APE, added at micromolar concentration, decreased cell growth in a time and dose dependent manner in both cell lines. The effect appeared more evident in MDA-MB-231 than in MCF-7 cells. The silencing of M2 receptor abolished the M2 agonist effect confirming that APE selectively binds M2 receptors. Interestingly, low doses of APE plus paclitaxel administered to the cell cultures caused a significant decrease of cell growth. The combined effect of the two drugs presents a synergism of potentiation. Finally the co-treatment with both drugs negatively modulates the mRNA levels of ABC (G2 type) and EGFR.

Conclusions. The data obtained suggest that M2 receptor agonist APE may represent a new interesting therapeutic tool in breast cancer therapy as well as in other tumor types (glioblastoma, bladder cancer and neuroblastoma). Its ability to decrease ABC efflux pumps and EGFR expression also suggest that APE may reduce cell chemoresistance and make the tumor cells more responsive to conventional drugs (e.g. paclitaxel).

No conflict of interest.

345 The role of apoptosis regulator programmed cell death 10 (PDCD10) in multidrug resistance

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Introduction. Multidrug resistance (MDR) is the collection of different cellular mechanisms of cancer cells to avoid cytotoxic effects of chemotherapeutics. MDR is the primary reason of failure in cancer chemotherapy. Cancer cells can acquire multidrug resistance through the alterations in several mechanisms, one of which is the evasion of apoptosis. Apoptosis is a form of programmed cell death that is important in development and maintenance of homeostasis. Programmed cell death 10 (PDCD10) is an apoptosis regulator, involving in the modulation of cell survival and apoptosis. The role of PDCD10 in the development of MDR has not been clearly established yet, however, the microarray studies previously conducted in our laboratory showed that PDCD10 expression was significantly downregulated in drug-resistant MCF7 cell lines. In this study, we aimed to investigate the role of PDCD10 in the development of multidrug resistance.

Materials and Methods. Drug-sensitive and drug-resistant MCF7, K562 and HeLa cell lines were grown in RPMI 1640 medium supplemented with 10% FBS at 370C and 5% CO2. Total RNA content was isolated by TRI Reagent according to the manufacturer's instructions. cDNA was synthesized from 1 ug of total RNA and random hexamer primers. PDCD10 expression was examined by qRT-PCR. The change in resistance levels of cells were examined by XTT cell proliferation assay before and after silencing PDCD10 with specific siRNA. The changes in the expression of apoptosis-related genes, Bax, Bcl-2, survivin, PUMA and NOXA, were examined by qRT-PCR after PDCD10 silencing. The data were presented mean±SEM and analyzed by one-way ANOVA followed by Tukey's test. The results were significant when $p<0.05$.

Results and discussion. Gene expression analysis showed that PDCD10 was significantly downregulated in drug-resistant MCF7 and K562 cells and upregulated in drug-resistant HeLa cells. The expression of PDCD10 in drug-resistant cells could be tissue-specific. PDCD10 was successfully silenced in drug-sensitive MCF7 and K562 cell lines. Results showed that silencing PDCD10 altered the resistance level of drug-sensitive MCF7 and K562 cells. This indicated that PDCD10 could have important roles in MDR development. Further studies are required to determine the exact role of PDCD10 in multidrug resistance.

Conclusion. PDCD10, as an apoptosis regulator, is also important in the development of multidrug resistance. Our study showed that PDCD10 could be a suitable target for MDR reversal studies.

No conflict of interest.

346 Inhibition of PDGFRß signaling reduces glioblastoma progression due to BM-MSCs recruitment

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Introduction. Recently, bone marrow-derived MSCs (BM-MSCs) were shown to migrate towards sites of active tumorigenesis where they contribute to cancer progression by enhancing tumor growth and metastasis, or by inducing anticancer-drug resistance. Furthermore, emerging studies suggest that tumor cell-derived molecules and factors could specifically attract BM-MSCs to invasive glioblastoma. In vitro studies suggest that platelet derived growth factor (PDGF-BB) may mediate this tropism. Therefore, the aim of this study was to inhibit BM-MSC recruitment by glioblastoma cells through modulation of PDGFRβ signaling.

Material and Methods. Human glioblastoma cell lines, U87MG and U87MG/EGFRvIII, stably expressing the mutant oncogenic EGFRvIII, were grown in medium with 10% fetal bovine serum for 72 h to obtain conditioned medium (CM). PDGFRβ and p-PDGFRβ protein level was evaluated in BM-MSCs exposed to glioblastoma cells-CM. Proliferation of BM-MSCs cultured in presence of glioblastoma cell-CM and treated with a nuclease-resistant RNA-aptamer against PDGFRβ, was evaluated by MTT assay. BM-MSCs migration, in the presence or absence of the aptamer, was assayed in transwell Boyden chambers by using glioma cells-CM as chemoattractant. Furthermore, involvement of AKT and/or ERK signaling pathways in glioblastoma progression BM-MSCs-mediated was examined.

Results and Discussion. Our results showed that the treatment of BM-MSCs with CM from glioblastoma cells caused an increase of cell migration and in parallel an enhancement of p-PDGFRβ levels. The treatment of BM-MSCs with PDGFRβ-aptamer hampered the activation of AKT and ERK in response to glioblastoma cells-CM resulting in inhibition of BM-MSCs proliferation and migration. Furthermore, we observed that the enhancement of p-PDGFRβ levels in BM-MSCs treated with glioma-CM, compared to untreated BM-MSCs, correlated with a major sensibility of these cells to aptamer treatment. This result suggests the possibility to target specifically BM-

MSCs recruited by glioblastoma and not BM-MSCs involved in tissue homoeostasis. **Conclusion.**In the present study, we demonstrate that the inhibition of PDGFRβ signaling by a specific aptamer reduces glioblastoma progression due to the BM-MSCs recruitment suggesting the possibility to interfere with cross-talk between tumor cells and microenviroment.

No conflict of interest.

347 Bone marrow-derived mesenchymal stem cells drive epithelial-mesenchymal transition in osteosarcoma cells through CXCL12/CXCR4

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Bone marrow-derived mesenchymal stem cells drive epithelial-mesenchymal transition in osteosarcoma cells through CXCL12/CXCR4 Raffaela Fontanella,1,2 Alessandra Pelagalli 1,3, Stefania Scala 4, Luigi Auletta5, Rosanna Palumbo1 and Antonella Zannetti1

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Introduction. Recently, bone marrow-derived mesenchymal stem cells (BM-MSCs) were shown to participate in tumor progression by establishing a favorable tumor microenvironment that through a cytokine networks promote metastasis. However, the mechanism of homing and recruitment of BM-MSCs into tumors and their potential role in malignant tissue progression is poorly understood. The aim of this study was to elucidate the role of BM-MSCs to promote in osteosarcoma cells the epithelial-mesenchymal transition (EMT) epigenetic program, which ultimately promotes metastasis to secondary tumor sites. Therefore, we analyzed the role of chemokine receptor type 4 (CXCR4) in EMT of osteosarcoma.

Materials and Methods. BM-MSCs were grown in medium with 1% fetal bovine serum for 48 h to obtain conditioned medium (BM-MSCs-CM). Human osteosarcoma cell lines, U2OS and Saos-2, were grown in presence of BM-MSCs-CM for 72 h and CXCR4 expression was analyzed by western blot. Tumor migration was assayed in transwell Boyden chambers using BM-MSCs-CM as chemoattractant in the presence or absence of CXCR4 antagonist, AMD3100. For EMT evaluation, E-cadherin, N-cadherin and Vimentin protein level was analyzed in human osteosarcoma cells exposed to BM-MSCs-CM and with or without AMD3100 treatment.

Results. We found that osteosarcoma cells treated with BM-MSCs – CM caused an increase of cell migration and in parallel an enhancement of CXCR4 levels. Furthermore, we observed that BM-MSCs conditioned medium decreased E-cadherin level, whereas increased N-cadherin and Vimentin levels in osteosarcoma cells. When we treated tumor cells grown in BM-MSCs-CM with CXCR4 antagonist we inhibited U2OS and Saos-2 migration and EMT program.

Conclusions. In conclusion these findings suggest that BM-MSCs recruited by osteosarcoma are able to promote tumor migration and epithelial-mesenchymal transition through CXCR4. Thus suggesting that inhibition of this receptor could prevent the processes that lead to tumor metastasis.

No conflict of interest.

348 Angiogenic switch in prostate cancer: PGE2 and miRNA regulation

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Introduction. Prostaglandin E-2 (PGE-2) promotes tumor angiogenesis via secretion of pro-angiogenic growth factors (1, 2, 3). miRNAs have emerged as key regulators of several cellular processes including angiogenesis; however, whether miRNAs influence the PGE-2-mediated angiogenesis is unknown. In prostate cancer cells, the tumorigenic drive has been associated to the constitutive expression of microsomal prostaglandin E synthase-1 (mPGES-1). In this work, we investigated whether PGE-2 induces the angiogenic switch in prostate tumors modulating miRNA expression.

Methods and Results. By comparing tumor cells bearing the mPGES-1 enzyme (SC) with those in which the enzyme was knocked down (KD), we demonstrate that mPGES-1 signaling modulates Dicer expression and miRNAs biogenesis. miR-15a and miR-186, associated with mPGES-1 signaling, were down-regulated. As a consequence, SC compared to KD tumor cells expressed higher levels of proangiogenic markers, such as vascular endothelial growth factor (VEGF) and hypoxia inducible factor-1α (HIF-1α), induced angiogenesis in vitro, and promoted highly vascularized tumors. In human prostate cancer specimens, mPGES-1 was

expressed in tumors with high Gleason score and associated with elevated expression of VEGF and HIF-1α, microvessel density and decreased Dicer expression measured by immunohistochemical analysis.

Conclusion. These results present evidence for a new regulatory mechanism in which PGE-2, by modulating miRNA processing pathway, promotes angiogenesis in prostate tumors and provides a rationale for the development of aggressive traits in prostate cancer.

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No conflict of interest.

349 Exosome-mediated transfer of miR-222 promotes tumor progression of melanoma

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Introduction. Our previous results indicated that miR-221&222 are key factors for melanoma development and dissemination. Growing evidence is showing that miRNAs are not strictly cellular, but are secreted in extracellular compartments through the release of small vesicles of endocytic origin, called 'exosomes' (EXO). These vesicles can alter diverse biological functions including tumor cell invasion, cell-cell communication and antigen presentation, through transfer of proteins, mRNAs and microRNAs. In this study, we analyzed whether the release of miR-222 and its specific targets into EXOs is coupled to a metastatic behavior of melanoma cells.

Material and method.EXOs were isolated by UltraCentrifugation or Exoquick-TC® methods according to standards procedures or manufacturer's instruction. To evaluate the fidelity of our EXOs preparations, we performed a western blot analysis. Using Nanosight™ technology, we established the size of our melanoma-derived vesicles. The expression level of endogenous and exosomal miRNAs were examined by Real Time PCR. Furthermore, the functional significance of exosomal miR-222 was estimated by biological assays according to standard procedures.

Results and discussion. In this study, we demonstrated that miR-222 was carried by exosomes secreted from melanoma and that its expression shows a direct correlation with melanoma malignancy. Exosomes carrying miR-222 can be transferred among different cell lines through direct uptake. We performed a series of biological assays looking for the capability of EXO/miR-222 to convey the same effects obtained by direct overexpression of miR-222 in melanoma cells. In EXO/miR-222-fused melanoma cell line we observed a significant induction of the invasion and chemotactic capacities compared to EXO-control fused cells. In order to gain insight in the molecular mechanisms underlying this EXO-based increased malignancy, we observed that miR-222 transported by exosomes can suppress the protein level of its target genes, such as p27kip, and induces PI3K/AKT pathway indicating its functional significance in cancer progression. Furthermore we investigated by a TaqMan Array Plate those genes differentially expressed in EXO/miR-222 and EXO/control observing that some tumor promoting genes, like VEGF and FGF2, were upregulated in EXOs secreted by miR-222 overexpressing melanoma cell line.

Conclusion. These data indicate that EXOs secreted by miR-222-overexpressing melanoma cells can act on target cells by inducing a protumorigenic program.

No conflict of interest.

350 Study of mitochondrial energy metabolism in the sensitivity of breast cancer cells to anthracyclines and in their chemosensitization by n-3 polyunsaturated fatty acids

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Several studies indicate that changes in one or more of mitochondrial functions (related to the synthesis of energy, cell death or oxidative stress) are involved in chemoresistance of some cancers. Nevertheless, the mechanisms involved in breast cancer remain unknown. Moreover, it was observed that a diet enriched in n-3 PUFAs such as docosahexaenoic acid (DHA) is able to overcome some resistance mechanisms and sensitize tumors to chemotherapy. The chemosensitizer effect of DHA could be related to its integration with the side chains of cardiolipin which are specific phospholipids of mitochondria known to play an important role in various mitochondrial functions. Our first study was designed to compare the mitochondrial energy metabolism of sensitive breast cancer cells (MCF-7S) and resistant to doxorubicin (MCF-7doxR). Moreover, it was analyzed if the chemosensitizing effect of DHA involved changes of mitochondrial energy metabolism.

Our results show that basal mitochondrial oxygen consumption is less in MCF-7doxR compared with MCF-7S. At the same time, we see a better synthesis of energy (ATP) mitochondrial associated with a decrease in mitochondrial oxygen consumption is not dedicated to the synthesis of ATP in the MCF-7doxR. This results in improved efficiency of ATP synthesis in the MCF-7doxR compared to MCF-7S. Moreover, we found that accumulation of Dox is 2-fold lower in mitochondria from MCF-7dox-R than MCF-7-S. In condition where mitochondrial ATP synthesis is inhibited, mitochondrial accumulation is increased in MCF-7dox-R. Whatever the studied cell line, we do not observe a significant effect of DHA supplementation on basal mitochondrial oxygen

consumption. However, DHA specifically reduces the ATP / O ratio in the MCF-7doxR at the value of MCF-7S.

In conclusion, our results show that the mitochondria of MCF-7R have a better efficiency of ATP synthesis and accumulate less doxorubicin that mitochondria of MCF-7S. Of doxorubicin accumulation studies are underway to assess whether DHA, decreasing the synthesis of ATP, affects efflux pumps mitochondrial ATP-dependent and increases mitochondrial accumulation of doxorubicin.

No conflict of interest.

351 Interplay between p53 and miR-34a in T-cells transformed by HTLV-1

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Introduction. Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of an aggressive neoplasm of CD4+ T-cells termed adult T-cell leukemia/lymphoma (ATLL). HTLV-1-transformed T-cells exhibit defects in the p53 pathway due to functional inactivation of the protein or mutations in the TP53 gene. In an effort to find new treatment strategies for ATLL, our laboratory is studying the effects of drugs that activate p53 on HTLV-1-transformed T-cell lines.

Materials and Methods. HTLV-1-transformed cell lines C91PL and MT-2, both of which express wild-type p53, were treated with selected drugs. Expression of p53 and target genes was measured by quantitative RT-PCR and immunoblotting. The cell cycle and cell viability were assessed with standard assays.

Results and Discussion. In both cell lines, treatment with nutlin-3a led to an increase in the levels of p53 protein and a consequent increase in expression of key p53 transcriptional targets, including CDKN1A (p21), MDM2 and miR-34a. In turn, several miR-34a targets were downregulated, including the inhibitor of apoptosis Survivin, the transcription factor YY1 and the deacetylase SIRT1, all of which are highly expressed in HTLV-1-transformed cells. Nutlin-3a induced cell cycle arrest in G1 in both cell lines, but was more effective in killing MT-2 cells than C91PL cells. Combined treatment with nutlin-3a and the SIRT1/2 inhibitor salermide potentiated the death induced by either drug alone in MT-2 cells.

Conclusions. Combined treatment with drugs that activate p53/miR-34a and inhibit SIRT1 could be explored for treatment of ATLL and other malignancies with wild-type p53. Further investigation of the HTLV-1 model may aid in identifying markers to predict the anti-proliferative and pro-death effects of these drugs.

No conflict of interest.

352 Nck2 pathways promote aggressiveness and angiogenesis in ovarian cancer

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Introduction. We investigated the role of NCK2 correlated to ITGB1 and ITGB4 integrins in driving ovarian cancer aggressiveness and we evaluated as NCK2 and integrins may influence the prognosis in ovarian cancer patients.

Material and method. Nanofluidic technology was used to analyze the expression of NCK2, ITGB1 and ITGB4 in 220 ovarian cancer patients. To evaluate expression of NCK2, ITGB1, ITGB4 and VEGFA we performed Real Time PCR in Ovarian Cancer cell lines: A2780, OVCAR3, HEY, PEO1, OV2774 and COV318. Transient overexpression of NCK2 was obtained in Ovcar3 and Hey. Real Time PCR was performed to evaluate expression changes of VEGFA, β-CATENIN, VINCULIN, ITGB1 and ITGB4. Western blot was used to evaluate protein expression. Coimmunoprecipitation (Co-IP) method was performed in A2780 cells to found interaction between NCK2 and the proteins of interesting.

Results and Discussion. Patients with increased levels of NCK2 and ITGB1 in nanofluidic analysis exhibited a poor prognosis (RR>1). Patients with increased levels of ITGB4 exhibited a favorable outcome (RR<1). In ovarian cancer cell lines we observed a general increase of NCK2, ITGB1 and VEGFA expression levels, instead ITGB4 is only expressed in OV2774, COV318 and PEO1 cells. NCK2 overexpression correlates with higher levels of VEGFA mRNA (about 5 times in OVCAR3) and we observed no variation of ITGB1, ITGB4, β-CATENIN and VINCULIN expression. By western blotting analysis, we compared protein levels in NCK2 overexpression cells: only ITGB1 and VEGFA are overexpressed. Therefore, NCK2 may be related to angiogenesis as acts on the pathway linked to VEGFA. Co-IP showed that NCK2 can bind ITGB1, but not VEGFA. Moreover NCK2 may be involved in mediating cell-extracellular matrix interactions in ovarian cancer cells by influencing tumor aggressiveness.

Conclusion. The results obtained show that NCK2 could effectively influence tumor progression in ovarian cancer cell lines, including through the formation of molecular complexes at the level of the extracellular matrix. The link between NCK2 and ITGB1 observed at the molecular level by Co-IP could be attributed to NCK2 a functional role in the regulation of extracellular matrix, instead VEGFA upregulation by NCK2 overexpression indicates a possible correlation between their pathways in modulating angiogenesis. It may be interesting to consider how Nck2 modules anchorage independent growth in ovarian cancer cells.

No conflict of interest.

353 Identification and characterisation of pancreatic cancer stem cells

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Introduction. Pancreatic cancer has the highest mortality among the cancer types. Each year approximately 250,000 people have been diagnosed with pancreatic cancer and as many people die worldwide. There have been significant improvements in our understanding of the etiopathogenesis of pancreatic cancer over the past decade. One of the these important improvements is the demonstration of existence of stem cells in pancreatic cancer. These stem cells named CSCs are also identified as 'cancer initiating cells'. CSCs have the potential of excessive proliferation, differentiation, high tumor formation, metastasis and one of the reasons for the resistance to the treatments. Human pancreatic CSCs express high levels of CD44 and CD24.

Material and method. Three pancreatic cancer cell lines, BxPC-3, Capan-2 and AsPc-1 were analyzed for the expression of CD44 and CD24 by flow cytometry. Fluorescence-activated cell sorting (FACS) analysis was used to separate CD44/CD24 + and CD44/CD24 - cells. We used sphere cultivation method to enrich the CD44/CD24 + stem cell population. Pluripotency maintaining factors (Nanog, Oct-4 and BMI-1) were examined by immunofluorescence analysis in CD44/CD24 + CSCs.

Results and Discussion. Cell surface marker expressions were analyzed by FACS. 99 % of BxPC-3 cells expressed the cell surface marker CD44, 68 % expressed CD24. For Capan-2 and AsPC-1, few cells (2-5 %) were positive for CD24, 97 % was positive for CD44. Sphere formation assay was performed to evaluate the sphere forming abilities of BxPC-3, Capan-2 and AsPc-1 CD44/CD24+ CSCs. All of CD44/CD24+ CSCs formed spheres in vitro. Pluripotency maintaining factors were positive in CD44/CD24 + spheres.

Conclusion. We found that the expression patterns of CD44 and CD24 in pancreatic adenocarcinoma cell lines were different. In BxPC3, 68 % of cells expressed CD24, while few Capan-2 and AsPC-1 cells expressed CD24. All pancreatic cancer CSCs showed self-renewal capacity. As a next step, the epigenetic differences between cancer stem and non-stem cells will be identified by microarray analysis. Obtained data will have a high potential for targeting cancer stem cells for a better treatment of pancreatic cancers. This project supported by TUBITAK (Project number:113S941)

Poster Session: Experimental/Molecular Therapeutics, Pharmacogenesis I

359 MK2 inhibitor targeting microtubules: Novel drug candidate for glioblastoma therapy

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Introduction. Glioblastoma is among the most lethal and least successfully treated solid tumours. With solely one chemotherapeutic agent available in clinic, novel therapies for glioblastoma are urgently needed. A challenge to find a suitable agent for brain tumour treatment lies in its lack of neurotoxicity but also its ability to cross the blood-brain barrier. MAPK-activated protein kinase 2 (MK2) is a cell cycle checkpoint kinase involved in DNA damage response. MK2 inhibitors enhance efficacy of conventional chemotherapeutic agents, but their effectiveness as a single agent has not been investigated.

Material and method. The anti-cancer effectiveness of an MK2a substrate-selective p38 inhibitor CMPD1 (Boehringer-Ingelheim; Davidson et. al., Biochemistry 2004; 43(37):11658-71) was determined in a panel of glioblastoma cell lines and normal cells (primary human microglia, astrocytes and neurons). Cell viability was assessed by Alamar Blue, apoptosis by Annexin-V staining and cell cycle by flow cytometry. Immunofluorescence and tubulin polymerization assays were conducted to study the effect of CMPD1 on microtubules and tubulin polymerization respectively.

Results and discussion. The MK2 inhibitor CMPD1 demonstrated single agent anti-cancer efficacy with a submicro-molar IC50 in glioblastoma cells yet exhibited minimal toxicity on normal cells. Treatment of U87 cells with CMPD1 resulted in significant G2/M arrest and accumulation of a polyploid (>4n) population. However, these cell cycle alterations were less evident in primary astrocytes. CMPD1 potently induced apoptosis and affected the expression of anti-apoptotic proteins in glioblastoma cells but not in astrocytes. Interestingly, while reported to be MK2a substrate-selective p38 inhibitor, CMPD1 did not inhibit MK2 or its downstream target Hsp27 at doses that are cytotoxic in U87 cells. siRNA knockdown of MK2 did not alter the IC50 of CMDP1 suggesting that MK2 is not involved in cell death. Instead, we identified CMPD1 as a tubulin depolymerizing agent causing microtubule disruption and mitotic defects in glioblastoma cells. Furthermore, we discovered that CMPD1 reduces the expression of tubulin in U87 cells and inhibits the self-renewal capacity of glioblastoma cells.

Conclusion. Collectively, we have discovered a novel microtubule targeting drug candidate with selective toxicity for glioblastoma therapy. We are currently working on developing analogues with enhanced blood-brain barrier permeable properties.

No conflict of interest.

360 A novel mitocan compound selectively and efficiently suppresses resistant Her2high breast carcinomas

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Introduction. Cancer is one of the prevailing causes of death worldwide. The prognosis of this disease is poor and may be attributed by high rate of mutations leading to heterogeneous populations of cells. It has been suggested that an invariant target is needed to selectively eliminate malignant cells. Mitochondrial targeting anti-cancer agents, mitocans, are often selective for cancer cells and may induce apoptosis in resistant cancers, such as Her2high breast carcinomas. Tamoxifen is a frequently used drug for breast cancers; however it fails to suppress resistant Her2high breast cancers. Thus, our lab synthetised a novel mitocan, mitochondrially targeted tamoxifen, MitoTAM, postulating this compound to efficiently target resistant breast cancers.

Material and Method. We used MCF7 cells with varying levels of Her2. The effects of treatment were assessed using the MTT assay and western blots. Her2 localisation was evaluated by fractionation and immunofluorescence staining. Mitochondrial membrane potential and respiration were analysed using flow cytometry and oxygraph, respectively. Xenografted Balb/c nude mice and FVB/N c-neu transgenic mice were treated with mitochondrial targeting compounds intraperitoneally to assess tumour volume by ultrasound imaging. Tumour tissue was further analysed for apoptosis and respiration by TUNEL staining, western blotting and Oxygraph analysis.

Result and discussion. Her2high cells exhibited higher mitochondrial membrane potential making them a good candidate for mitocan treatment. We found that Her2high cells were more sensitive to MitoTAM treatment at a 10 times lower dose compared to tamoxifen. Her2high cells treated with MitoTAM triggered the apoptotic signalling pathway. Balb/c nude mice and FVB/N c-neu mice treated with MitoTAM showed a significant decrease in tumour volume compared to control and tamoxifen-treated mice. Interestingly, we observed Her2 to be highly expressed in the mitochondria of Her2high cells and tumours, probably altering their bioenergetics metabolism.

Conclusion. We established that MitoTAM is more effective in killing Her2high cells and induces apoptosis via the involvement of the mitochondria. A remarkable decrease in tumour growth was observed with MitoTAM, suggesting the agent to be of translational importance. We also show that Her2 is highly expressed in the mitochondria of Her2high cells and tumours, however; the mechanism of Her2 translocating to mitochondria is yet to be evaluated.

No conflict of interest.

361 Targeted inhibition of casein kinase II enhances IKZF1-mediated repression of PI3K pathway in primary acute lymphoblastic leukemia xenografts

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Introduction. Casein Kinase II (CK2) is a pro-oncogenic kinase that is essential for cellular proliferation. Increased CK2 activity is associated with malignant transformation and poor prognosis in leukemia. Our previous work revealed that in leukemia, CK2 directly phosphorylates and impairs the tumor suppressor function of IKZF1 (Ikaros) in acute lymphoblastic leukemia (ALL). We hypothesize that the targeted inhibition of CK2 will enhance IKZF1 activity as a transcriptional repressor and have a therapeutic effect on leukemia in vitro and in vivo – in a mouse preclinical model of human leukemia.

Material and Method. Patient-derived B-cell acute lymphoblastic leukemia (B-ALL) xenografts were treated with a specific CK2 inhibitor. In vivo DNA binding of IKZF1 to its target genes was evaluated by quantitative chromatin precipitation (qChIP). Gene expression was determined by quantitative real-time PCR (qRT-PCR). Cellular viability and proliferation was evaluated by trypan blue and MTT test.

Result and discussion. Treatment of primary B-ALL cells with CK2 inhibitors severely reduced cellular proliferation. CK2 inhibition resulted in increased binding of IKZF1 to promoters of several genes that promote PI3K pathway, including PIK3CD, PIK3C2B and PI3KFYVE. Increased binding of IKZF1 was associated with reduced transcription of these genes, as evidenced by qRT-PCR. Knock-down of IKZF1 with shRNA severely impaired the ability of CK2 inhibitors to repress expression of the genes that promote the PI3K pathway. This suggests that transcriptional repression of the PI3K pathway genes following CK2 inhibition is mediated via enhanced IKZF1 activity. The use of novel CK2 inhibitors in vivo, in primary B-ALL xenografts, resulted in a strong anti-leukemia effect with reduced numbers and percentages of leukemia cells in both bone marrow and spleen of mice treated with CK2 inhibitors. Expression analysis of the PI3K pathway genes showed reduced expression of these genes in treated mice as compared to controls. These results demonstrate the therapeutic efficacy of CK2 inhibitors in a preclinical model of B-cell leukemia.

Conclusion. Targeted inhibition of CK2 enhances IKZF1-mediated transcriptional repression of PI3K pathway-promoting genes and has an anti-leukemia effect both in primary cells and in patient-derived leukemia xenografts. Results suggest that CK2 inhibitors can be an efficient treatment for leukemia and reveal a possible mechanism of their therapeutic activity.

No conflict of interest.

362 Structural activity studies on L-sugar derived glycosylated antitumor ether lipids: Evaluation of antitumor effects on cancer cell lines and cancer stem cells.

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Introduction. Glycosylated Antitumor Ether Lipids (GAELs) are an emerging class of glycolipids with potent antitumor activity. Of special importance is their non-apoptotic mechanism of killing cancer cells. Non-apoptotic cell death induction in cancer is an attractive strategy to avoid cross resistance. The significant drawback to development of GAELs as clinically useful anticancer agent is their lack of in vivo anticancer activity, which is attributed to metabolic cleavage by glycosidases. The sugar moiety employed to date in the development of GAELS are glucose, glucosamine, galactosamine, galactose, mannose and mannosamine which occur naturally in humans and are prone to hydrolysis by glycosidases. To prevent hydrolysis in GAELs a variety of techniques have been employed including thioglycosides and N-acylated sugars. However, use of both thioglycoside- and N-acylated-based GAELs resulted in significantly reduced antitumor activity. Here we report on the use of L-sugars that are not naturally present in humans and are expected to resist glycosidase-catalyzed breakdown in human.

Material and method. Nine compounds were tested which include seven new glycolipids based on three L-sugars namely L-glucose, L-rhamnose and L-mannose against drug resistant and aggressively growing cancer cell lines derived from human breast, prostate and pancreatic cancers, using MTS assay. The most potent analog was also studied against cancer stem cells (CSCs) obtained from breast BT 474 cell line and prostrate DU 145 cell.

Results and Discussion. The anticancer activities of these L-sugar derived GAELs were compared to that of our lead structure Gln, a D-glucosamine derived GAEL, the most studied compound in this class and chlorambucil a known clinical anticancer agents. Our results indicate that positive charge, the position of the amino substituent and the nature of the sugar have significant effects on the anticancer activities of these compounds. The most active analog has CC50 values in the range of 4.8 to 11 μM and CC90 values in the range of 6.5 to 14.0 μM across all cell lines tested. This analog is more active than Gln and chlorambucil. This active analog also demonstrated significant cytotoxicity against CSCs with CC90 values of 9.0 and 5.0 μM against stem cells obtained from breast BT 474 and prostrate DU 145 cell lines respectively, and also disrupt preformed CSC spheroids. The ability of this compound to disrupt CSC spheroids and kill the cells is an indication that it shares a common mechanism of killing via an apoptosis-independent mechanism as our lead compound Gln.

Conclusion. L-sugar derived GAELs showed significant cytotoxicity, comparable or better than D-sugar derived GAELs, against both cancer cell lines and CSCs in single digit micromolar concentrations. This shows that changing of the sugar moieties of GAELs from D- series to L-series did not alter the mechanisms of action and cytotoxic activity of GAELS while stability against human glycosidases is expected.

No conflict of interest.

363 Proteomic study highlights the anticancer effects of thieno-triazolodiazepines. Role of kinoma inhibition

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Introduction. L'HCC is one of the most common malignancies worldwide. So far there is no effective chemotherapeutic treatment for HCC and the prognosis of advance stage remains poor. The thieno-triazolodiazepine are well known anti-inflammatory drugs that acts as PAF-receptor antagonists. Recently an antineoplastic pleiotropic effect of this molecules have been shown, where treatment with WEB2086 and WEB2170 induce differentiation, growth arrest and apoptosis in murine and human leukemia cells. The effects of WEBs on HCC remain untested. Aim of this study was to investigate the anti-tumor efficacy of the WEBs in vitro and in vivo models of HCC.

Material and Methods. The in vitro biological effects of WEBs was assessed by thymidine incorporation, growth in soft agar, cell migration in a Wound Healing assay and chemoinvasion on Matrigel of cancer cell lines (HepG2, Hep3B, HuH7 and Hepa 1-6). Cell cycle, apoptosis and authophagy was determined by MUSE cytometry and senescence with β-galactosidase stainig. The effects of WEBs in vivo study was valuated in three animal model, HBV transgenic mice model, orthotopic model and in HepG2 xenograft model. 2D-DIGE proteomic study was performed in WEB 2086 treated Hepa 1-6 cancer cell line.

Results and Discussion. WEBs were able to reduce proliferation of cancer cell lines (HepG2, Hep3B, HuH7 and Hepa 1-6) and eliminates the ability of these cells to form colonies in soft agar that was associated with cell cycle arrest, apoptosis, and senescence. In addition, WEBs impair hepatoma cell migration and chemoinvasion. WEBs administration in HBV transgenic mice and in orthotopic mouse model reduces the number and the dimension of tumor masses. WEB anticancer effects were also

confirmed in a HepG2 xenograft model of HCC. A 2D-DIGE proteome study highlights that the WEB2086 inhibits the binding function in particular inhibits protein binding of ATP and GTP that results in kinoma inhibition. In addition 2D-DIGE analysis showed that WEBs down-regulate chaperone proteins such as hsp90 and hsp70 that are involved in protein refolding, an important mechanism in tumor cell resistance. The down-regulation of Hsp90 could lead to failure of 'clients' protein refolding and mediate their degradation.

Conclusions. The WEBs are able to reduce HCC progression in human and murine HCC models blocking cancer cell proliferation and migration and inducing apoptosis and senescence probably whit a mechanism that involve kinome inhibition.

No conflict of interest.

364 Metformin inhibits proliferation but does not induce death in colorectal cancer cells

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Introduction. metformin, a widely used and well-tolerated antidiabetic drug, may reduce cancer risk and/or cancer mortality. In vitro and in vivo studies on several cancer models have evaluated the potential antitumorigenic effects of metformin and many clinical trials are looking to its use for suppressing tumor growth. However, the mechanism for its anti-cancer effect remains uncertain and needs to be clarified. The aim of this study is to investigate the anti-cancer activity of metformin on colorectal cancer (CRC) cell growth.

Materials and Methods. the human CRC cell lines HT29, HCT116 and HCT116P53-/- were treated with metformin at a dose of 5mM. Treatment-induced alterations in protein levels were evaluated by western blotting and the biological effects exerted by the drug were assessed in terms of apoptosis (TUNEL assay), autophagy (immunofluorescence assay for LC3B) and senescence (β-galactosidase staining). Analysis of cell cycle was performed by flow cytometry. The ability of a single cell to grow into a colony was determined through a clonogenic assay.

Results and Discussion. metformin induced cell cycle arrest in Go/G1 phase that was accompanied by a strong decrease of cyclin D1 and c-Myc expression and by inhibition of pRB phosphorylation in all the CRC cell lines analysed. The antiproliferative activity of the drug resulted mediated by suppression of mTOR and IGF-1/AKT pathways. In particular, we observed inactivation of mTOR and of its downstream targets S6 and 4EBP1, both in an AMPK dependent and independent way, and also downregulation of the activity of IGF1R. Differently from what was observed in other cancer cell lines, we found that metformin did not induce apoptosis, autophagy or senescence. In support of these results, a clonogenic assay showed that metformin did not arrest growth of cells, but only slowed down cell ability of forming colonies.

Conclusion.our findings highlight that metformin inhibits the proliferation of CRC cell lines as a consequence of promoting cell cycle arrest in the Go/G1 phase, but does not induce cell death. Further investigations are needed to better elucidate the mechanisms altered by the drug in CRC and caution should be used when treating cancer patients with metformin.

No conflict of interest.

367 A new strategy to prevent anthracycline-induced cardiotoxicity while improving anti-cancer activity

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Introduction. The anthracycline doxorubicin (dox) is one of the most widely used anticancer agents in the clinic, but its full potential has not been realised because of dose-dependent cardiotoxicity. The main cancer-associated target of dox is topoisomerase IIα (topo IIα) in which poisoning of the enzyme leads to lethal DNA double strand breaks. Recently the cardiac isoform topo IIβ has been revealed as a major mediator of dox-induced cardiotoxicity. When dox treatment is combined with formaldehyde-releasing prodrugs (FRP) this results in an enhanced level of tumour cell kill, but paradoxically, lowers the level of cardiomyocyte kill. In this study we examined the modes of dox-induced DNA damage in cancer and cardiomyocyte cell lines to determine how this is modulated by FRP.

Materials and Methods. Cells consisted of rat and mouse cardiomyocyte cell lines (H9c2 and P19) and rat and mouse breast carcinoma cell lines (4T1.2 and MTLn3). Dox was used either alone or in combination with the FRP AN-7. Dox-DNA covalent adducts were monitored by the incorporation of [14C] dox into DNA while the comet assay was used to detect DNA strand breaks (presented as Olive Tail Moment [OTM]). Reactive oxygen species (ROS), mitochondrial membrane potential and cell death were assessed using DCF-DA, JC-1 and Sytox green dyes respectively. Preliminary studies were conducted in mice to assess the contribution of single agent and combination treatment to heart toxicity.

Results and discussion. Treatment with 2 μM dox produced low background levels of DNA covalent adducts in all cell lines while combined addition with 100 μM AN-7 caused high levels of adducts in both cardiac cell lines (~ 40 adducts per 10 kb). DNA-adducts were also potentiated by AN-7 in cancer cell lines, but were lower relative to cardiomyocytes (ranging from 2 to 15 adducts per 10kb). In contrast, high levels of DNA strand breaks were induced by single agent dox treatment (OTM of approximately 2-3 in all cell lines) while combination with AN-7 significantly reduced DNA breaks, an effect particularly more pronounced in the cardiac cell lines. As expected dox produced ROS in cardiomyocyte cell lines, however this level was unaffected by AN-7. Dox decreased mitochondrial membrane potential in all cell lines but this was exacerbated by AN-7 combination treatment only in the cancer cell lines; accordingly cell death was only potentiated in the cancer cell lines. Preliminary in vivo studies in mice revealed that combined AN-7 and dox treatment diminished dox-induced myocardial cell injury as measured by cardiac troponin release and electron microscopy.

Conclusion. Collectively these results indicate that dox-induced topo II poisoning is prevented by AN-7 co-administration. In these circumstances the major DNA damage observed is switched to formation of formaldehyde-mediated covalent adducts bypassing dox topo IIβ poisoning and protecting cardiomyocytes. Therefore treatment with AN-7 and related drugs represents a promising cardioprotective strategy in the context of anthracycline chemotherapy.

No conflict of interest.

368 Next generation sequencing to predict metastatic melanoma therapy response

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Introduction. BRAF mutations, present in 50% of melanomas, cause the over-activation of the MAPK/ERK pathway affecting cell proliferation and survival. Most of them result at codon 600 that is a molecular target for BRAF inhibitors treatment. Unfortunately, the reactivation of MAPK signalling or activation of an alternative signalling pathway, as PI3K/AKT/mTOR, deriving from different mechanisms of acquired tumor drug resistance (as secondary mutations in NRAS or MEK1) cause disease progression within 6–9 months after the therapy beginning. Next Generation Sequencing (NGS) technologies facilitate a screening of multiple genes starting from a small amount of material and drastically decrease the time and cost associated with a comprehensive genome analysis. Our aim was to verify that NGS approach could be useful to identify mutations able to predict response/resistance to metastatic melanoma therapy.

Material and method. A series of 25 patients, treatment naïve and with confirmed histological stage IV of metastatic melanoma was enrolled at the Oncology Department of the IRCCS 'Giovanni Paolo II' in Bari, Italy. We developed an Ampliseq Custom panel for Ion Torrent PGM Sequencer to analyze the coding region of 11 target genes with a coverage of 93.85%. The melanoma custom panel size was 39.08Kb and contained 303 amplicons. The analysis, performed with both single or barcoded samples, required 20ng (2 pools) of FFPE DNA. Torrent Variant Caller and Ion Reporter software v4.2 were used for the variant calling. BRAF codon 600 and NRAS codon 61 mutations were confirmed in Sanger sequencing and ARMS. The correlation between the detected mutations with clinic-pathological features and time to progression has been analyzed.

Results and discussion. BRAF, KIT, MITF, PIK3CA and MC1R resulted the most mutated genes. Moreover we identified different mutation patterns related to the different therapy response. As regards Ion PGM sequencer specificity in variant calling, we have compared the NRAS and BRAF results obtained with Ion PGM and with ARMS/Sanger sequencing methods, demonstrating a higher accuracy of the NGS technology (2 cases BRAF false negative with Sanger method and 1 case NRAS false negative with ARMS method).

Conclusion. Our study highlighted that the Ion PGM approach could be useful to improve metastatic melanoma patients therapeutic setting.

No conflict of interest.

369 MiR-125b as potential prognostic circulating biomarker in metastatic melanoma patients treated with Temozolomide/Fotemustine

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Background. Melanoma is the fifth leading cause of death. A number of therapeutic approach have been evaluating in the clinical setting, such as BRAF inhibitors and immunotherapies. However, chemotherapy is still considered the standard treatment for metastatic melanoma (MM) by many oncologists because biological therapies have a limited effectiveness in terms of progression free survival. Temozolomide (TMZ), an alkylating agent, has been recently introduced for the treatment of MM. Also nitrosureas have been evaluated for the treatment of MM, in particular Fotemustine (FM). Recently, it has been showed that the administration of sequential non-therapeutic low dose TMZ before full dose FM has high activity in MM patients. TMZ acts on the activity of the DNA repair enzyme MGMT, but a lack of correlation

between MGMT promoter methylation and efficacy of TMZ treatment has been observed, highlighting the need to identify different non-invasive biomarkers, as miRNAs. Few data are available on the role of miRNAs as prognostic markers among which miR-125b has been extensively studied in melanoma. However, no data are available on circulating miRNAs. For this reason, we focused on miR-125b variation expression, in whole blood in response to TMZ/FM.

Materials and Methods. A cohort of 18 patients affected by MM have been enrolled. The treatment schedule included oral TMZ administered at a single dose of 100 mg/m2 on days 1 and 2 followed by intravenous FM at a dose of 100 mg/m2 on days 2, 4 hrs after TMZ. Treatment cycles were repeated every 3 weeks.. Tumor assessments were obtained at screening and every three cycles. Whole blood samples were collected prior to treatment and again after clinical evaluation. Total RNA was extracted and miR-125b expression was evaluated through qRT-PCR.

Results and Discussion. Paired t-test results showed an upregulation of miR-125b after treatment (p=0.006). We compared miR-125b expression just before and after treatment, stratifying patients according to clinical response. Interestingly, we found a significant upregulation of miR-125b in patients responding to therapy (p=0.0134). These results seemed to indicate that patients overexpressing miR-125b before treatment with TMZ/FM might have a better prognosis.

Conclusion. In conclusion, the results of our exploratory study encourage to extend analysis in a greater cohort in order to confirm miR-125b as a prognostic marker

No conflict of interest.

371 Activity of a STAT3 inhibitor in diffuse intrinsic pontine gliomas

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Introduction. Chemotherapy treatments have failed to improve the outcome of patients with diffuse intrinsic pontine gliomas (DIPG), a disease characterized by a highly invasive phenotype and a likely conserved blood brain barrier. Because the activation of the STAT3 pathway has been related to enhanced migration and invasion in adult gliomas, and because its presence has been demonstrated in glioma stem cells, we hypothesized that it could be a suitable target in DIPG.

Material and method. A panel of 6 DIPG cell models (in spheroid and adherent culture conditions) established at our institution from DIPG patients at diagnosis was studied by western blot for STAT3 and STAT3 phosphorilation (p-STAT3). In vivo, p-STAT3 was assessed by immunohistochemistry in DIPG tumors (HSJD-DIPG-007 model) engrafted in the brainstem of Nod.Scid mice. The activity of the STAT3 inhibitor WP1066 was studied in the cell models from different perspectives: inactivation of the pathway (western blot), cytotoxicity (MTS assays), migration (wound healing assay and transwells) and invasion (transwells and expression of matrix metalloprotease-9, MMP9). The effect of the STAT3 activator interleukin 6 (IL-6; 40 ng/mL) on such properties was also assessed.

Results and discussion. We found p-STAT3 by western blot in all the DIPG cultures, consistent with an activated STAT3 pathway in vitro. p-STAT3 positive cells were found diffusely in the brainstem and cerebellum of DIPG xenografts. Such results encouraged us to test in vitro the activity of WP1066 (a molecule in clinical trials for adult glioma). WP1066 cytotoxic activity (IC50) in the cell lines was in the range 3-5 µM, consistent with published results in glioma cell lines. WP1066 inhibited STAT3 phosphorilation, migration, MMP-9 expression and invasion at sub-cytotoxic concentrations (0.01 to 1 µM). IL-6 activated STAT3 to p-STAT3, enhanced migration and invasion of the cells and abolished the effects of the drug.

Conclusion. Our results point at the STAT3 pathway as an important target for therapeutic inhibition in DIPG. Because the blood brain barrier activity might preclude drug penetration in vivo, future studies will focus on the in vivo activity and pharmacokinetics of WP1066 in preclinical DIPG models.

No conflict of interest.

372 Combined therapy with Histone deacetylase inhibitor and chemotherapy overcomes drug resistance in lung cancer cells

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Introduction. In the last two decades, reversal of platinum resistance in lung cancer patients had raised great interest for clinicians and scientists. Histone deacetylase inhibitors (HDACis) were described to reduce glycolytic flux, through inhibition of the expression of glucose transporter type 1 (GLUT 1), in lung tumor cells.The expression of related metabolic markers such as GLUT-1 and hexokinase, in different types of lung tumor cell lines previously treated with LBH (Panobinostat®) alone or combined with platinum-based chemotherapy (CDDP), will be investigated and correlated with treatment response. This metabolic characterization will allow us to identify the metabolic adaptation associated to Panobinostat effects which can be exploited in a new combined therapy to enhance lung cancer cell apoptosis.

Material and Method. The cellular viability of NSCLC, A549 and Calu-1, cell lines was measured by MTT assay, using LBH (1.0-80.0 nM) and CDDP (5.0-80.0 nM). The apoptotic effect mediated by combination of LBH and CDDP was accessed by AnexinV and cleavage of caspase-3 and PARP. The expression of genes related to energy metabolism was performed by qPCR and the protein levels by western blotting.

Results and Discussion. We observed decrease in cell viability after LBH and CDDP treatment in a time- and dose-dependent manner. However, A549 cells were more sensitive to CDDP than Calu-1 cells. The combined treatment using low doses of LBH and CDDP increased the apoptosis induced byCDDP in the both cell line in about 20% after 24 and 48h, suggesting an additive effect that overcomes the CDDP resistance in Calu-1 cell line. The apoptotic profile was confirmed by increase in caspase-3 and PARP cleavage after 24h of LBH/CDDP treatment.To check if the combination is affecting the glycose uptake, we assessed Glut1 mRNA levels. LBH/CDDP, decreases the mRNA levels of Glut1, which is inversely related to hexokinase I/II mRNA and protein levels in A549 cells, suggesting disruption in energy metabolism trough decrease in glycose uptake.

Conclusion. Taken together, our results suggest that targeting HDAC we are capable to overcome chemotherapy resistance by altering energy metabolism. Combinatorial therapies could improve lung cancer treatment strategies in the future.

No conflict of interest.

373 A Goodpasture antigen-binding protein kinase inhibitor to treat drug-resistant invasive lung and breast cancer

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Introduction. GPBP (Goodpasture antigen-binding protein) is an exportable non-conventional protein kinase regulating type IV collagen organization. Type IV collagen is a major component of the extracellular matrix that interacts with cancer stem cells (CSC) and forms a protective shield against anti-tumor therapies. CSC are recognized as being responsible for drug resistance and invasiveness. Expression of COL4A3BP, the gene coding for GPBP and for cytosolic ceramide transporter CERT, has been associated with multidrug resistance and poor prognosis in breast and lung cancer patients. To investigate COL4A3BPactivity causing these adverse effects we generated a small-molecular-entity to specifically inhibit GPBP kinase activity.

Material and Method. Yeast two-hybrid system was used to identify a five-residue motif stabilizing GPBP aggregates displaying enhanced kinase activity. A series of peptidomimetic compounds featuring a terphenyl structure were synthesized and tested. The compound 3-[4"-methoxy-3,2'-dimethyl-(1,1';4',1")terphenyl-2"-yl] propionic acid referred to as T12, displaying good toxicokinetics and inhibitory activity, was selected for therapeutic assays using different models for non-small cell lung cancer (A549 and patient-derived cancer cells) and breast cancer (murine 4T1).

Results and Discussion. T12 was shown to compromise the viability of tumors exhibiting predominant mesenchymal-invasive phenotype and thus sharply reduced metastasis formation. In contrast, T12 had no effect on tumors with predominant epithelial phenotype, and required doxorubicin, an inducer of epithelial-to-mesenchymal transition (EMT), to display anti-tumor activity in animal models. The evidence also suggests that T12 efficiently destabilized the privileged niche of mesenchymal drug-resistant CSC by inhibiting GPBP kinase activity and type IV collagen expression and function. Consistently, down-regulation of COL4A3BP or type IV collagen expression compromised cancer cell viability in three-dimensional cultures only after EMT induction. Finally, treatment with N26, a GPBP-blocking monoclonal antibody, had similar anti-tumor effects than T12 in animal models.

Conclusion. The general mechanism for multidrug-resistance and poor prognosis associated with COL4A3BP expression depends at least in part on GPBP kinase activity. T12 emerges as a First-in-Class drug candidate to specifically treat drug-resistant and metastatic lung and breast cancer.

Conflict of interest: Ownership: Fibrostatin SL. Advisory board: Fibrostatin SL. Corporate-sponsored research: Fibrostatin SL

Other substantive relationships: T12 and chimeric N26 are drug candidates being developed by Fibrostatin SL, a biotech company. J Saus, F Revert and F Revert-Ros are co-founders and stockholders of Fibrostatin SL

374 Lysosomotropic REV-ERBß antagonism as an innovative anticancer strategy

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Introduction. In a continuous effort to identify novel anticancer strategies, inhibition of autophagy is being emerging as a promising approach. We recently reported that the circadian nuclear receptor REV-ERBβ plays an unexpected role in sustaining cancer cell survival when autophagy flux is compromised. Our studies also identified a chemical compound, ARN5187, with a dual inhibitory activity toward autophagy and REV-ERBβ, which showed a higher anticancer activity than the clinically relevant autophagy inhibitor, chloroquine (CQ), against breast cancer BT-474 cells.

Material and Method. Dual REV-ERBβ/autophagy inhibitors and CQ were analyzed as follow: lysosomotropy was evaluated analyzing reduction of lysosomal pH. Autophagy inhibition was tested by immunoblot with autophagy marker proteins, fluorescent-tagged LC3 proteins and transmission electron microscopy (TEM). REV-ERBβ antagonistic activity was assessed evaluating the ability of compounds in releasing REV-ERB-mediated transcriptional repression by luciferase-based assays and qRT-PCR on endogenous targets. In vitro anticancer activity of compounds was assessed in breast cancer cells with a different ERBB2 and ER status, liver, skin, and

prostate tumoral cells. Human mammary epithelial HMEC cells were used to test the toxic activity of compounds in non-cancer cells.

Results and Discussion. Biological evaluation of ARN5187 analogues with improved anticancer activity compared to our hit revealed that the higher cancer cytotoxicity of compounds was mainly related to an enhanced antagonistic potency toward REV-ERBβ. Our analysis indicated that dual REV-ERBβ/autophagy inhibitors shared similar lysosomotropic activity with CQ and equally autophagy. Hence, we compared the anticancer activity of our most potent compound, 30, and CQ against different human tumor tissue cells. Indicating that a combined autophagy and REV-ERBβ inhibitory activity improves cytotoxicity deriving by the singular inhibition of autophagy, 30 was significantly more effective than CQ in killing all tested cells. In addition, 30 did not affect the viability of non-cancer human mammary epithelial HMEC cells at the tested doses.

Conclusion. Dual inhibition of REV-ERBβ and autophagy may be a promising novel anticancer strategy. Our first class of dual REV-ERBβ/autophagy inhibitors represents a valuable scaffold for progressing new multi-target anticancer agents.

Conflict of interest: Ownership: Grimaldi B, Torrente E and Scarpelli R are payed by the Istituto Italiano di Tecnologia and are co-inventors in a patent that includes compounds mentioned in the abstract owned by the Istituto Italiano di Tencologia.

375 Interaction between mitochondrial and nuclear DNA in cisplatin resistance: Use of transmitochondrial hybrids

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Introduction. Cisplatin is a first-line chemotherapeutic agent for several types of tumours. CDDP cytotoxicity has been originally ascribed to formation of inter- and intra-strand nuclear DNA cross-links that, by hindering both RNA transcription and DNA replication, lead to cell cycle arrest and apoptosis1. More recent data suggest that only 5-10% of CDDP is bound to DNA, whilst other nucleophilic molecules, such as phospholipids, cytoskeletal and membrane proteins, and mtDNA are targeted2. Unfortunately, the therapeutic effectiveness of cisplatin is limited by the onset of drug resistance, whose mechanisms are still not fully understood. Our previous research showed that cisplatin-resistant ovarian cancer cells (C13), when compared to the sensitive counterpart (2008), are characterized by a reduced respiratory chain activity as well as a metabolic switch towards a lipogenesis3. Here we used cytoplasmatic transmitochondrial hybrids to investigate if mtDNA mutations might be responsible of the metabolic switch observed in cisplatin-resistant cells.

Methods. Experiments were parallely performed on ovarian cancer cells and in their derived cybrids. Transmitochondrial cybrid (H2008 and HC13) were generated by fusion of enucleated 2008 and C13 with the mtDNA-less (ρ0) osteosarcoma cells. We firstly sequenced the entire mtDNA of all cell lines and therefore we measured the mtDNA copy number by real-time PCR. We controlled CDDP sensitivity of cybrid cell lines by trypan blue exclusion assay and by flow cytometer. After that we analyzed some mitochondrial parameters by flow cytometry, in live microscopy and oxygraphy. Finally we measured the protein expression of HIF and c-Myc nuclear transcription factors by western blotting.

Results and Discussion. The mitochondrial profiling showed no major differences between H2008 and HC13 suggesting that the metabolic remodelling of C13 is independent from mtDNA and might be controlled by nuclear factors. Thus, the activity of transcription factors involved in the metabolic reprogramming of cancer cells, such as HIF-1α and c-Myc, was evaluated. Of note, a different protein expression of both HIF and c-Myc was identified.

Conclusions. Even if the complete metabolomic fingerprint of ovarian cancer cells remains to be further elucidated, preliminary results prompt to further investigate the metabolic reprogramming as a critical goal to identify pharmacological targets to overcome cisplatin resistance.

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No conflict of interest.

376 Depletion of mitochondrial DNA modulates cellular sensitivity to cisplatin

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Introduction. Cisplatin is one of the most important chemotherapeutic agents in ovarian cancer treatment, but unfortunately the onset of resistance is the main problem of this therapy. Although a plethora of studies regarding CDDP resistance has been performed, the molecular mechanisms are not still understood. The increase of anaerobic glycolysis, even in the presence of oxygen (Warburg effect), is the first observation showing the alteration of energetic metabolism used by cancer cell as a strategy to adapt and proliferate independently from the substrate availability1. This evidence suggested to validate the hypothesis that similar metabolic strategies might be involved in CDDP resistance. Thus, the aim of this study was to investigate alterations of mitochondrial function and metabolism in order to identify alternative pathways exploited by cancer cells to escape CDDP cytotoxicity and prevent this phenomenon.

Material and Method. The analysis of mitochondrial function were conducted measuring oxygen consumption, mitochondrial mass and potential by flow cytometry. In order to investigate the impact of mitochondria on CDDP-resistance, human ovarian cancer cells sensitive (2008) and resistant (C13) to cisplatin were depleted of their mtDNA (rho0-clones) as previously described2. Cell viability of 2008 cells and resistant C13 to cisplatin and respective rho0 clones was determined by MTT assay and by Trypan blue exclusion assay.

Results and discussion. Compared to 2008, C13 cells were more glucose-dependent for survival, showed diminished mitochondrial mass as well as a significantly reduced respiratory chain activity correlated to lower oxygen consumption and mitochondrial potential and different susceptibility to various metabolic stresses. Therefore, these results suggest that mitochondria impairment may be involved in CDDP resistance mechanisms, in relation to the observed metabolic transformation events such as changes in glycolytic rate and mitochondrial respiratory pattern. Cell viaibility assays showed that cisplatin IC50was higher in parental 2008 than 2008-rho0 cells, but similar between C13 cells and C13-rho0.

Conclusion. The study of the processes that influence mitochondria impairment of cancer cells can be useful to develop more effective treatments and potentially improve the clinical impact of platinanting drug.

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No conflict of interest.

377 Efficacy of metronomic topotecan and pazopanib combination therapy in preclinical models of primary and late stage metastatic triple-negative breast cancer

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Introduction. Metronomic chemotherapy has shown promising activity in numerous preclinical studies and also in some phase II clinical studies involving various tumor types and it is currently undergoing phase III trial evaluation. Triple-negative breast cancer (TNBC) is an aggressive subtype with limited treatment options and very poorer survival than other types. We evaluated the potential therapeutic impact and molecular mechanisms of topotecan administered in a continuous low-dose metronomic manner, alone or in concurrent combination with pazopanib on a triple-negative, primary and metastatic breast cancer model.

Materials and Methods. Proliferation and apoptotic assays were performed on a serially selected metastatic variant of the triple negative MDA-MB-231 breast cancer-cell line, 231/LM2-4 and human umbilical vein endothelial cells (HUVECs). Cell lines were exposed to topotecan, pazopanib, sunitinib, or combinations, for 144 hours in hypoxic conditions. VEGF, HIF1α and ABCG2 gene expression was performed with real-time PCR and topotecan intracellular concentrations were measured by high-performance liquid chromatography. Mice with primary tumors or advanced metastatic disease were treated with topotecan and pazopanib alone or in combination.

Results and discussion. A significant antiproliferative and proapoptotic activities were determined by metronomic topotecan on both endothelial and cancer cells, enhanced by pazopanib. Metronomic topotecan and pazopanib combination treatment greatly inhibited the expression of HIF1α, ABCG2 and VEGF genes in hypoxic cancer cells, together with an increase in the intracellular concentration of the active form of topotecan. In vivo administration of metronomic topotecan and pazopanib combined treatment significantly enhanced antitumor activity compared to the single drugs alone and markedly prolonged survival with a significant decrease in tumor vascularity, proliferative index, and an induction of apoptosis.

Conclusion. Our results suggest a potential novel therapeutic option for the treatment of metastatic triple-negative breast cancer patients.

No conflict of interest.

378 Evolution of pediatric solid tumors towards a limited drug distribution phenotype

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Introduction. The inadequate penetration of chemotherapy to the solid tumor cells may account for an important mechanism of drug resistance. However,

the in vivo evaluation of drug distribution in tumor compartments (intracellular, extracellular and vascular) has been rarely studied. We have recently developed a reproducible microdialysis-tumor homogenate method that overcomes the limitations of the microdialysis technique in hydrophobic drugs and provides a powerful tool to characterize compartmental anticancer drug distribution. In this work, we hypothesized that tumors under clinical treatments evolve towards a drug-impenetrable phenotype. We have focused on the study of the distribution of the active metabolite of irinotecan, SN-38, in patient-derived xenografts (PDX) of highly aggressive pediatric solid tumors.

Material and methods. Two PDX models of neuroblastoma (HSJD-NB-001 and -002), two of Ewing Sarcoma (HSJD-ES-002 and -006) and two of alveolar rhabdomyosarcoma (HSJD-aRMS-001 and -002), each pair from the same patient at different stages of treatment (early and late, respectively), were established at Hospital Sant Joan de Deu Barcelona. We used a microdialysis-tumor homogenate method for the study of the intratumor distribution of SN-38 administered in the tumor-bearing animals by continuous infusion (steady state plasma levels). Dialysate samples from the tumor extracellular fluid were taken overnight during 16-19 hours, followed by terminal blood and tumor homogenate samples. We calculated the volume of distribution of unbound SN-38 (Vu,tumor; high values of this parameter indicate high distribution of the drug in the intracellular compartment of the tumor, whereas low values suggest that the drug is predominantly distributed in the extracellular space) in each tumor model. The values obtained from each pair of PDX models were compared using a t-test.

Results and discussion. The Vu,tumor values were 33.5 ± 14.9 and 9.7 ± 9.8 mL/g tumor for the neuroblastoma PDX models (early and late, respectively), 10.9 ± 5.5 and 0.9 ± 0.7 mL/g tumor for the Ewing sarcoma models, and 8.3 ± 1.7 and 5.4 ± 2.4 mL/g tumor for the rhabdomyosarcoma models; mean ± SD of 7-9 experiments. The values were significantly different between each pair of samples (P=0.0013, P=0.0004 and P=0.0128, respectively).

Conclusion. Our results suggest that drug penetration issues might be involved in chemoresistance of pediatric solid tumors upon treatment.

No conflict of interest.

379 1H-NMR to detect intracellular metabolites in living human ovarian cancer cell lines

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Introduction. Currently, the first-line treatment for ovarian cancer is a combination of a platinum-based drug, such as cisplatin or carboplatin, coupled with paclitaxel [Joerger M. et al., (2007); Vella N. et al., (2011)]. Even if the clinical benefits of cisplatin are largely recognized, the therapeutic effectiveness of the drug is limited by the severity of its side effects (ototoxicity, nephrotoxicity and neurotoxicity) [Pasetto L.M. et al., (2006)], and by the potential progression of tumor cells to a cisplatin-resistant state [Koberle B. et al., (2010)] whose mechanisms are poorly understood. Interestingly, it is emerging that cisplatin resistance can be achieved by rewiring key metabolic pathways, which increase the cell defense from the cytotoxic effects of the drug. Recently NMR-based metabolomic is used to characterize cellular metabolites and interpret them in terms of metabolic changes taking place in a wide range of situations. These include differences in cell properties (e.g., drug resistance, cell cycle stage, specific growth conditions and genetic characteristics) and changes induced in response to different perturbations (e.g., disease, drug exposure and irradiation).

Methods. We developed an experimental protocol to acquire the spectra from a living cell population. To shed light on platinum resistance mechanisms, we used 1H-NMR spectroscopy to analyze the metabolic profile of human ovarian carcinoma cells, sensitive (2008) and resistant (C13) to cisplatin.

Results and Discussion. 1H-NMR spectra indicate a different metabolic fingerprint of the two cells lines with particular regard to lipids and GSH content.

Conclusion. Even if here we show only preliminary results, our data suggest that 1H-NMR might be a new and usefull methodological approach to study the mechanism involved in cisplatin resistance.

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No conflict of interest.

380 Role of miR-483-3p in platinum drug-resistance of human ovarian carcinoma cells

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Introduction. In spite of the efficacy of platinum compounds, drug resistance represents a major limitation to the cure of ovarian carcinoma. Altered expression of microRNAs (miRNAs) may contribute to tumor drug-resistance by regulating different aspects of cell response. The aim of this study was to analyze the expression pattern of miRNAs in platinum sensitive/resistant cells using genome-wide and functional approaches, in relation to alterations possibly linked to platinum drug-resistance.

Material and Method. Gene and miRNA expression profiling was performed using the Illumina microarray platform. Functional studies were carried out by transfection of an inhibitor or precursor of miRNA. Quantitative Real-Time PCR (qRT-PCR) was used to measure mRNA levels. Protein levels were examined by western blot. Putative targets of miRNA were in silico selected using miRecords software. Gene Ontology analysis was performed with the DAVID software.

Results and Discussion. The expression pattern of miRNAs in ovarian carcinoma cells resistant to platinum compounds (IGROV-1/Pt1 and IGROV-1/OHP) as compared to parental cells (IGROV-1) was examined. Six miRNAs were up-regulated in both resistant variants, among which we found miR-483-3p, which has been implicated in apoptosis and proliferation regulation. Validation studies using qRT-PCR confirmed increased levels of miR-483-3p in the resistant cells. Functional studies carried out by transfecting a synthetic precursor of miR-483-3p in parental cells, documented a consistent and marked up-regulation of the miRNA levels at different times. Growth inhibition and colony forming assays indicated that miR-483-3p over-expression conferred mild levels of cisplatin resistance to IGROV-1 cells. A reduced proliferative rate upon miRNA over-expression in IGROV-1 cells was observed, besides a down-regulation of predicted miR-483-3p targets (CDK6 and PRKCA). Cisplatin sensitivity of IGROV-1 cells decreased in the presence of a PKC-alpha inhibitor. Gene Ontology analysis revealed a prevalent modulation of PRKCA in platinum-resistant cells, thus supporting the interference of miR-483-3p with factors sustaining tumor cell proliferation.

Conclusion. Increased expression of miR-483-3p appears to confer low levels of cisplatin resistance by interference with the proliferative potential of ovarian carcinoma cells. Because low levels of resistance have been proposed to be clinically relevant, our results may be of translational value.

No conflict of interest.

381 Vorinostat synergizes with EGFR inhibitors in NSCLC cells by increasing ROS through the upregulation of the major mitochondrial porin VDAC1 and the modulation of c-myc-Nrf2-Keap1 pathway

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Introduction. In EGFR wilde type non small cell lung cancer (NSCLC) patients, activation of alternative pathways contribute to the limited efficacy of the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. The nuclear factor-erythroid 2-related factor 2 (Nrf2) and its repressor Kelch-like ECH-associated protein 1 regulator (Keap1), involved in the response to redox stress and often dysfunctional in NSCLC, have been recently suggested as potentially involved in EGFR-TKI resistance. To improve the activity of EGFR TKIs we investigate a combinatorial strategy with the histone deacetylase inhibitor vorinostat, an antitumor agent we have previously shown to exert its lethality by inducing oxidative stress.

Material and Method. We used both established and patient-derived NSCLC cells. Synergy was assessed by Chou and Talalay method. Apoptosis and reactive oxygen species (ROS) were measured by cytofluorimetric analysis. DNA damage and mitochondrial homeostasis regulators were evaluated by western blotting. Knockdown of voltage-dependent anion-selective channel protein 1 (VDAC1) expression was obtained by a specific siRNA.

Results. Simultaneous exposure to vorinostat and gefitinib or erlotinib, induced strong synergistic antiproliferative and pro-apoptotic effects paralleled by accumulation of ROS content. Co-administration of the scavenger N-acetyl cysteine significantly reduced lethality, suggesting a causal role for oxidative damage in the induction of apoptosis. The alteration of mitochondria homeostasis induced in the combination setting was highlighted by an increase of the cytochrome c cytoplasmic fraction paralleled by a reduction of the mitochondrial fraction, leading to the accumulation of ROS, DNA damage and the consequential induction of apoptosis. Moreover by knockdown experiments we suggested that the upregulation of VDAC1, the major mitochondrial porin of outer mitochondrial, induced by vorinostat and further increased by the combination, is functionally involved in the oxidative stress-dependent apoptosis. Finally the pro-survival and the antioxidant capacity of the cells were also inhibited by the combination as shown by the complete deactivation of AKT and the downmodulation of c-myc and of its downstream target Nrf2, respectively.

Conclusion. All together, these results demonstrated for the first time that alteration of redox homeostasis is new mechanism underlying the observed synergism between vorinostat and EGFR TKIs in NSCLC.

No conflict of interest.

382 Preclinical activity of the repurposed drug Auranofin in classical Hodgkin Lymphoma

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Classical Hodgkin lymphoma (cHL) is considered a highly curable disease; however, about 20% of patients cannot be treated with standard first-line chemotherapy and have a bad outcome. Therefore, new drugs or drug combinations are needed to reduce toxicity and to increase the efficacy for refractory/resistant patients. Recently, the drug repurposing, or the discovery of new useful activities for 'old' clinically used drugs, was proposed as a new more efficient and less expensive approach to discover novel agents for cancer therapy.

Auranofin (AF) (Ridaura®) is an oral gold-containing trietylphosphine approved for curing rheumatoid arthritis. It is now considered a new therapeutic option for Cronic Lymphocytic Leukemia (CLL), and was recently approved by FDA, as a single agent for Phase II clinical trial in CLL and in Ovarian Cancer, or in combination with Sirolimus in Lung Cancer.

The aim of our study is to evaluate the efficacy of AF in the cHL cancer setting using preclinical models and different approaches including in vivo studies.

We demonstrated that AF inhibited in a dose dependent manner proliferation and clonogenic growth of cHL cells; induced apoptosis, caspases 3 activation, Bcl-2, Bcl-XL down-regulation, but Bax up-regulation; inhibited thioredoxin reductase (TrxR) enzymatic activity and increased Reactive Oxygen Species (ROS) accumulation; inhibited NF-kB activity and its target genes IRF4 and CD40, the iperexpressed molecule CD30 and the Discoidin Domain Receptor 1 (DDR1); down-modulated Notch1 receptor but not its ligand Jagged1; decreased IL-13, TNF-alpha, TGF-beta1, VEGF, CCL5, CCL17 and IL-6 release. Supernatants from cHL cells treated with AF reduced their capability to recruit peripheral blood mononuclear cells and to induce tubulogenesis in endothelial cells. AF had a synergistic effects when used in combination with doxorubicin, gemcitabine and cisplatin; overcame drug resistance mediated by CD40 engagement and by cHL-Mesenchymal stromal cells. Finally, AF treatment led to a 70% reduction of L-540 tumor xenografts growth in nude mice with no significant weight reduction.

These preclinical findings, demonstrating that AF could not only kill cancer cells but also affect micro-environmental interactions, suggest that AF used alone or in combination with standard chemotherapeutic drugs, may represent a promising future therapeutic strategy for refractory/relapsed cHL.

No conflict of interest.

383 Aurora kinases A and B are potential therapeutic targets in KRAS-induced lung cancer

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The most frequent genetic change found in lung tumors are activating point mutations in the KRAS gene, which have been causally linked to the oncogenic process. Unfortunately, different approaches to target RAS proteins for therapy have been unsuccessful. Therefore, in order to select better targets for lung cancer therapy, key cancer-relevant KRAS downstream pathways will need to be identified. The overall objective of this study was to identify novel therapeutic targets in KRAS-mediated lung cancer. This project was based on the following hypotheses: (1) KRAS activates mitotic kinases Aurora A and/or B; and (2) Aurora A and/or B are relevant therapeutic targets in KRAS-induced lung cancer. These hypotheses were formulated on the basis of published studies showing that Aurora A directly phosphorylates RAS effector pathway components, and Aurora A and B both cooperate with oncogenic RAS to promote malignant transformation. In order to test this hypothesis, we first determined whether oncogenic KRAS induces Aurora kinase expression. For that purpose, we used three different cell-based models: (1) an immortalized primary lung epithelial cell line and its isogenic KRAS-transformed counterpart, (2) H1703 lung cancer cell line engineered to express oncogenic KRAS inducibly, and (3) KRAS positive lung cancer cell lines H358 and A549 stably expressing inducible shRNAs targeting KRAS. In all cases, KRAS expression positively correlated with Aurora A and Aurora B expression. In order to validate Aurora A and/or B as therapeutically relevant KRAS targets in lung cancer, we used genetic and/or pharmacological approaches in the abovementioned cells to inactivate Aurora A or B. In KRAS positive H358 and A549 cell lines, inducible shRNA-mediated knockdown of Aurora A or B, as well as treatment with a dual Aurora A and B inhibitor, decreased growth, viability, migration, invasion and oncogenicity in vitro and induced cell death. In addition, Aurora A inhibition in the A549 KRAS-transformed cell line efficiently reduced tumor growth in vivo. More importantly, in the primary isogenic model and in the H1703 KRAS-inducible cell line, dual pharmacological inhibiton of Aurora A and B reduced growth, viability, migration, invasion and oncogenicity in vitro and induced cell death in an oncogenic KRAS-dependent manner. This suggests that Aurora kinase inhibition therapy can specifically target KRAS transformed cells. In conclusion, our results support our hypothesis that Aurora kinases are important KRAS targets in lung cancer and suggest Aurora kinase inhibition as a novel approach for KRAS-induced lung cancer therapy.

No conflict of interest.

384 Trabectedin differentially affects ETS fusion genes binding to IGF-1R promoter in Ewing sarcoma and prostate cancer

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Introduction. Aberrant expression of ETS transcription factors, including FLI1 and ERG, due to chromosomal translocations has been described as a driver event in initiation and progression of different tumors. Interest in ETS-driven diseases has been further supported by finding that DNA-binding chemotherapeutics reverse transcriptional program of oncogenic transcription factors. Such effect was described for trabectedin, a tetrahydroisoquinoline molecule binding the N2 of guanine in the minor DNA groove. In this study, effects of trabectedin were investigated on IGF-1R gene regulation in Ewing sarcoma (EWS) and prostate cancer (PCa), characterized by EWS-FLI1 and TMPRSS2-ERG fusion genes, respectively.

Material and methods. The study was performed in TC-71 and 6647 EWS cell lines or TMPRSS2-ERG-positive VCaP PCa cells. Recruitment of EWS-FLI1 or TMPRSS2-ERG to the promoters of several target genes including IGF-1R, TGF2βR, and PIM-1 was analyzed by Chromatin Immunoprecipitation (ChIP) assay upon treatment with trabectedin. A combination of trabectedin and anti-IGF1R/IR dual inhibitor OSI-906 was tested in cell lines. Western blotting analysis was performed to evaluate IGF-1R expression upon treatment with trabectedin in EWS cells.

Results. Trabectedin was found to displace EWS-FLI1 and TMPRSS2-ERG from the promoters of canonical target genes TGF2βR and PIM-1, respectively. However, trabectedin also caused increased recruitment of EWS-FLI1 on IGF-1R promoter in EWS cells. The effect was dose- and time-dependent and correlated with up-regulation of IGF-1R protein levels. Conversely, trabectedin induced TMPRSS2-ERG detachment from IGF-1R promoter in PCa cells. In keeping with the differential effects of trabectedin on IGF-1R promoter occupancy, combination of trabectedin with OSI-906 gave synergistic effects in EWS cells while subadditive effects were registered in VCaP cells.

Conclusions. This study evidences a cellular-dependent activity of trabectedin on DNA binding of the chimeric genes. Either EWS-FLI1 or TMPRSS2-ERG directly upregulate IGF-1R gene (Amaral AT, Garofalo C 2015; Mancarella C, under review). Trabectedin changed the recruitment of chimeric genes on IGF1Rpromoter depending on the cellular context with a clinical impact. In fact, the results suggest that combination of trabectedin and anti-IGF-1R inhibitors represents a potential therapeutic option for EWS but not for PCa patients. (Grants: AIRC IG-14049 and FIRB RBAP11884M_005 to KS)

Conflict of interest: Other substantive relationships: Maurizio D'Incalci has received honoraria related to PharmaMar boards

386 Chemosensitisation of human breast carcinoma cell line MDA-MB-435S to vincristine and paclitaxel by silencing integrins alpha v and alpha 4

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Introduction. Integrin signalling regulates numerous cellular processes in tumor cells including tumor cell adhesion, migration, invasion and survival. Integrins play an important role in breast cancer metastasis. In addition, metastatic cancer cells are highly resistant to antitumor drugs. The aim of this study was to investigate the role of integrins αvβ3, αvβ5, α3β1 and α4β1 expressed on MDA-MB-435S cells in sensitivity to different antitumor drugs and cell migration, one of the necessary requirements for efficient cell invasion and metastasis.

Materials and Methods. For silencing of integrin subunits β3, β5, αv, α3 and α4 in human breast carcinoma MDA-MB-435S cell line, the siRNA sequences specific for β3, β5, αv, α3 and α4 were transfected using Lipofectamine RNAiMAX Reagent. Flow cytometry analysis was used to analyse the expression of integrins αvβ3, αvβ5, αv, α3β1 and α4. Confocal microscopy was used to visualise paxillin, phospho-paxillin or F-actin fibers using specific antibodies or phalloidin-FITC, respectively. Cell survival was assessed using MTT assay. Boyden chambers (pore size 8 μm) were used to measure migration.

Results and Discussion. While decreased expression of integrins αvβ3, αvβ5 or α3β1, achieved by β3, β5, αv or α3-specific siRNA transfection, reduced sensitivity of MDA-MB-435S cells to cisplatin, no change was observed in MDA-MB-435S cells upon knockdown of α4β1 using α4 siRNA transfection. Conversely, decreased expression of integrins αvβ5 or α4β1 upon integrin subunit β5, αv or α4 siRNA transfection sensitized MDA-MB-435S cells to vincristine or paclitaxel, but there was no change in cell survival upon knockdown of integrins α3β1 or αvβ3 upon α3 or β3 siRNA transfection. The cytotoxic activity of camptothecin and doxorubicin was not altered upon transfection of any of analysed siRNAs. The down-regulation of integrins αvβ3 and αvβ5 achieved by αv siRNA transfection strongly decreased migration, amount of stress fibers and focal adhesions, whereas the down-regulation of integrin α4β1 upon α4 siRNA transfection moderately increased cell migration with simultaneous

slight increase in amount of stress fibers and focal adhesions.

Conclusion. We suggest that transfection of at least some breast carcinoma cells with integrin αv siRNA could enhance activity of vincristine or paclitaxel and simultaneously inhibit their metastatic potential. These new insights should be useful when devising strategies for more efficient treatment.

No conflict of interest.

387 Role of the receptor tyrosine kinase Axl in the aggressive features of platinum-resistant ovarian carcinoma cells

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Introduction. Drug resistance is a limitation to the efficacy of platinum-based therapy in ovarian carcinoma and is associated with an aggressive cell behavior and an increase of the metastatic potential of tumor cells. The Tyr-kinase receptor Axl is known to play a pro-metastatic/anti-apoptotic role. The aim of this study was to examine whether the inhibition of Axl interferes with drug resistance and cell aggressive features in platinum-drug resistant ovarian carcinoma cells.

Material and Method. Loss-of-function studies based on RNA interference were carried out by transfection of small interfering RNAs (siRNAs). Quantitative Real-Time PCR (qRT-PCR) was used to measure mRNA levels. Protein levels were examined by western blot and Antibody Arrays.

Results and Discussion. We found that mRNA and protein expression of Axl was increased in the platinum-resistant IGROV-1/Pt1 and IGROV-1/OHP cells as compared to the parental IGROV-1. IGROV-1/Pt1 cells displayed reduced sensitivity to targeted agents and increased migratory/invasive capabilities and were therefore used to examine the role of Axl in cell aggressiveness. When the IGROV-1/Pt1 cells were transfected with siRNAs against Axl, marked and persistent reduction of mRNA/protein were obtained. Following silencing of Axl, IGROV-1/Pt1 cells displayed reduced growth and less invasive/migratory capabilities as compared to control siRNA-transfected cells. Axl silencing did not produce significant changes in cisplatin-sensitivity, whereas it tended to reduce gefitinib-sensitivity and Akt/p38 activation. The treatment with a pharmacological inhibitor of Axl reduced the migratory/invasive capabilities of the IGROV-1/Pt1 cells.

Conclusion. These findings suggest that the increased levels of Axl in ovarian carcinoma cells contribute to their aggressiveness, because the inhibition of this factor by two different approaches results in a reduction of growth, migration and invasive capabilities. Axl silencing did not reduce resistance to cisplatin, but tended to reduce sensitivity to gefitinib, suggesting that the inhibition of specific cell survival factors generates the activation of compensatory mechanisms for maintaining tumor cell survival. Thus, sensitization of drug-resistant cells may require the concomitant inhibition of multiple survival pathways. These results provide a rationale for testing multi-target inhibitors of survival pathways in combination with cisplatin.

No conflict of interest.

388 Role of SR-BI and multidrug-resistance P glycoprotein in controlling cholesteryl ester uptake from high density lipoproteins: Are HDLs promising carriers for anticancer drugs?

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Introduction. High cholesteryl ester (CEs) contents and low levels of plasma HDL have been observed in cancer patients and in tumour bearing animals. Accordingly, the CE-HDL uptake via the scavenger receptor class B type I (SR-BI) is enhanced in tumour cells, suggesting that this pathway may contribute to malignant proliferation. In addition, SR-BI, and P-glycoprotein-MDR1 (P-gp) have been shown to participate in the regulation of membrane cholesterol trafficking and CE metabolism/transport. In order to investigate the requirement for CE-HDL by cancer cells and tumours, we investigated the cholesterol metabolism in MDR1-resistant lymphoblastic CCRF-CEM cells.

Material and Methods.CEM were made resistant by stepwise exposure to low (LR, 50 nM) and high (HR, 500 nM) doses of vincristine. P-gp activity (3H-vinblastine), CE content, CE synthesis (14C-oleate), neutral lipid and DiI-HDL uptake (fluorescence), SR-BI and P-gp protein expression (western blotting) were determined. To better evaluate the relationship between CE metabolism and P-gp activity, the P-gp inhibitors cyclosporine (CLS, 2,5μM) and verapamil (VPM, 10μM), were used. Moreover, to further verify the CE-HDL uptake in tumour cells we used HDL-like nanoparticles (rHDLs) containing lipophilic anti-cancer drug (valrubicin),

Results and Discussion. No difference was found in the CE contents and synthesis between parental and resistant cells, however the membrane control of CE-HDL was quite different. In fact, lipid droplets and CE-HDL uptake were lower in the resistant cells, in spite of the SR-BI over-expression. The P-gp inhibitors reactivated membrane cholesterol traffic as demonstrated by CE-HDL uptake increase, further SR-BI over-expression and higher neutral lipid content. The interrelationship between P-gp and CE-HDL uptake was supported by the lower cytotoxic effect of rHDL in resistant compared to parental cells. Furthermore, the cytotoxic efficacy of rHDL increased when P-gp activity was inhibited.

Conclusions. Our data suggest that HDLs supply CEs to cancer cells via SR-BI. Moreover, these results suggest that (i) P-gp protein is involved in the control of CE-HDL uptake, (ii) anticancer drugs and CE-HDL uptake share, at least in part, the same transport mechanisms. The dependency of CE-HDL by tumour cells makes HDL a promising carrier for anti-cancer drugs. The possible involvement of P-gp in this pathway opens a novel strategy especially for MDR1 cells.

No conflict of interest.

389 Wnt/ß-catenin signalling inhibition is a potential new therapeutic strategy in soft tissue sarcomas

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Introduction. Soft Tissue Sarcomas (STS) are malignant tumors of mesenchymal origin that affect around 400,000 individuals per year in the entire world with more than 50 recognized subtypes. This group of tumours is characterized by molecular alterations in cellular processes such as cell cycle regulation and apoptosis in which β-catenin has been recently involved. β-catenin is the key molecule in the Wnt/APC/β-catenin signalling pathway and it regulates the transcriptional program activated by the secreted protein Wnt. The aims of this study were: 1) to evaluate the role of the Wnt/APC/β-catenin pathway in the pathogenesis of STS; 2) to study its role as a functional target of therapeutic agents.

Materials and Methods. A panel of STS cell lines and patient samples were used. Cells were treated with different inhibitors of the Wnt/APC/β-catenin pathway (XAV939, PKF118-310, LGK974) for further analysis. These compounds were combined with chemotherapeutic agents used in STS treatment such as Doxorubicin and Trabectedin. Cell viability (CellTiter 96® AQueous One Solution, Promega; xCELLigence System; ACEA Biosciences) and cell cycle analysis (BD FACScan Verse) were performed. Expression and cellular localization of different proteins were studied by means of Western Blot, quantitative PCR and immunocytochemistry.

Results and Discussion. Nuclear β-catenin as well as expression of downstream effectors were detected in 85% the cell lines studied, but no correlation with histology could be demonstrated. Inhibition of the Wnt/APC/β-catenin pathway resulted in decrease of cell proliferation by apoptosis induction or G1 arrest. The most effective mechanism for inhibiting the pathway was the abrogation of the β-catenin-TCF binding. Response to these compounds led to a decrease of nuclear β-catenin and downregulation of key target genes such as CDC25A. Finally, combination of chemotherapeutic agents with these inhibitors resulted in a synergistic response, suggesting that they may complement standard agents in the treatment of STS.

Conclusions. The inhibition of β-catenin and its transcriptional program represents a new promising therapy for those sarcomas not responding to other treatments. Clinical trials should be addressed in order to validate this new therapeutic strategy in soft tissue sarcomas.

No conflict of interest.

390 Synergistic antitumor activity of sorafenib and Akt inhibitors

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Introduction. Hepatocellular carcinoma (HCC), the major histological subtype of liver cancer, is one of the leading causes of cancer-related deaths worldwide. Patients with HCC are often diagnosed at advanced stage, where chemotherapy is the only treatment option. Sorafenib, a multi-kinase inhibitor with anti-angiogenic functions, is the only FDA-approved molecular-targeted agent for the treatment of patients with advanced HCC. Yet, Sorafenib shows limited overall survival benefit associated with resistance and tumor recurrence. Since Sorafenib targets angiogenic VEGFR and PDGFR kinases and RAF/MEK/ERK signaling, the primary mechanism of resistance to Sorafenib and tumor recurrence in HCC patients emerges to be the compensatory PI3K/AKT signaling. In this study, we analyzed the synergistic effects of Sorafenib and AKT inhibitors on HCC cell growth, determined possible mechanisms underlying synergistic mechanism of action by transcriptome analysis, and further showed that combination therapy leads to tumor regression in HCC xenografts in vivo.

Material and Method. Cytotoxic activities of the PI3K/AKT pathway small molecule inhibitors were shown by SRB and RT-CES assays in HCC cell lines having normal or hyperactive AKT kinase. Their effects on cell cycle progression, migration and apoptosis were shown by flow cytometry, wound healing assays, western blots and immunofluorescence. Transcriptome profiling of cells that were treated with AKT inhibitor and Sorafenib as single agents and with a combination of both were performed with microarray experiments. Therapeutic efficacy of combination therapy was shown in vivo in athymic mice bearing HCC xenografts.

Results and Discussion. Sorafenib and 6 PI3K/AKT pathway inhibitors suppressed cell growth in a dose- and time-dependent manner. Particularly the isoform specific

AKT inhibitors (AKTi-1,2 and AKTi-2) were effective in the blockage of cell migration and they enhanced pro-apoptotic effect of Sorafenib significantly (more than 3 fold) through complete inhibition of p-Akt, p-Erk, cyclinD1 and Rb and activation of p21. The striking synergistic cytotoxicity induced by combination of Sorafenib and AKTi-2 in vitro was further analyzed at transcriptome level using microarray analysis and the synergistic molecular mechanisms of action was revealed (~6000 altered genes at p<0.01). The enhanced anti-growth efficiency in combination therapy was associated with down-regulation of compensatory signal transduction, which is present in mono-treatments. Finally, we showed that combining Sorafenib (30 mg/kg) with AKTi-2 (7.5 mg/kg) enhances anti-tumor efficacy of Sorafenib in vivo and results in necrosis and tumor regression.

Conclusion. Our results provide in vitro and in vivo experimental evidence of the therapeutic potential of combination therapies with Sorafenib and AKT inhibitors for the treatment of advanced HCC.

No conflict of interest.

391 Antibody dependent cellular cytotoxicity: An effective strategy to target cutaneous melanoma cells that acquired resistance to BRAF inhibition

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Introduction. About 50% of cutaneous melanomas (CM) harbor BRAF mutations that constitutively activate the MAPK pathway. Small molecule inhibitors of BRAF (BRAFi) and MEK are clinically effective in BRAF-mutant metastatic CM, but patients almost invariantly develop an early resistance. The latter rely on heterogeneous mechanisms, which, however, display shared traits including an altered signaling and a frequent de novo expression of multiple receptor tyrosine kinases (RTK). However, the direct involvement of specific RTK in mediating resistance to BRAFi is still controversial, with contrasting data on the ability of small molecule inhibition to restore sensitivity to BRAFi. On these grounds, rather than dissecting the molecular mechanisms of resistance, we focused our attention on the possibility to exploit the frequent expression of RTK in BRAFi-resistant CM to target them by immunologic means.

Material and method. Vemurafenib resistant cultures were generated by drug adaptation of 7 BRAF-mutant CM cell lines established in our Institution; RNAsquencing identified genes modulated following acquisition of drug resistance; expression of the RTK EGFR was measured by flow cytometry; drug cytotoxicity was measured by MTT assays; signaling was evaluated by western blotting; antibody dependant cell cytotoxicity (ADCC) was evaluated by a flow cytometry method.

Results and discussion. RNAsequencing identified overexpression of different RTK in vemurafenib-resistant (VR) cells. Three out of 7 VR cultures had de-novo membrane expression EGFR proteins, while 2 parental cell cultures were constitutively EGFR-positive. Phosphorylation of EGFR was detected in VR cells and was reduced following incubation with erlotinib, gefitinib or the anti-EGFR monoclonal antibody (mAb) cetuximab. Despite this observation, incubation of the 3 EGFR-positive VR cultures with different doses of these drugs did not restore their sensitivity to vemurafenib, ruling out a key role of EGFR signaling in resistance. Notably, however, cetuximab was able to mediate ADCC toward all the EGFR-positive VR CM cell cultures tested, with a maximum average specific cell lysis of 48% (p< 0.01) for PBMC to target ratio of 80:1.

Conclusion. Though preliminary, our data support the rationale use of clinically available mAbs targeting RTK as a therapeutic option in combined approaches for CM patients relapsed to targeted therapies.

No conflict of interest.

392 Identification of thyroid tumor cell vulnerabilities through a siRNA-based functional screening

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Introduction. The non-oncogene addiction paradigm indicates that the tumorigenic state often depends on the activity of a wide variety of non-oncogenic genes and pathways, essential to support the phenotype of cancer cells but not required to the same degree for the viability of normal cells. We faced this concept in order to unveil nodal points for therapeutic intervention for thyroid carcinoma, which represents the most frequent endocrine cancer, with an incidence rapidly increasing. A fraction of thyroid tumor develops resistance to standard therapy and progresses to more aggressive forms with poor prognosis; moreover, target therapy often results unsuccessful. Therefore, we need to better understand the mechanisms of thyroid carcinogenesis and to identify novel targets for therapeutic intervention.

Material and Methods. We screened a commercial small interfering RNA (siRNA) oligonucleotide library (Ambion's Silencer® Select Human Druggable Genome siRNA Library V4) targeting the human druggable genome in the thyroid cancer BCPAP cell line (carrying the BRAFV600E mutation) in comparison with immortalized normal human thyrocytes (Nthy-ori 3-1). 309 mother 96-well-plates, containing 27,093 siRNA oligos targeting 9,031 genes, have been screened in duplicates in both cell lines. The effect of each siRNAs on the cell viability was analyzed seven days after transfection by a high-throughput colony formation assay. Based on screening results, some selected hits were prioritized for technical confirmation. Then, using commercially available siRNAs with different sequences, we validated some of them in BCPAP and in other thyroid cancer cell lines.

Results. Data obtained from the screening in the two cell models revealed a panel of 386 hits that resulted as BCPAP specific survival genes. We selected 28 of them for technical confirmation. We confirmed 15 genes that are involved in: cell cycle control, vesicular transport, glucose and fatty acid metabolism, and intracellular signal transduction. We performed validation studies on some hits using independent siRNAs. Interestingly, they resulted lethal not only for BCPAP cells, but also for the other thyroid cell lines.

Conclusions. This work identified putative non-oncogenes essential for sustaining the oncogenic phenotype of thyroid tumor cells, but not of normal cells. We found that these genes play a role in the biology of thyroid cancer and could represent promising targets for new therapeutic strategies.

No conflict of interest.

394 The role of ALDH1 in chemoresistance of colon cancer cells

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Introduction. Malignant tumours consist of subpopulations of tumour cells. Cancer stem cells (CSC) represent a subpopulation of cells with increased tumorigenic and chemoresitant properties. There were several key stem cells markers identified in colon cancer: CD133, CD44, ALDH1, LGR5 and others. In our study we focused on the ALDH1 expression in colon cancer cells HT-29, HCT-116 and LS-180.

Material and method. Human colon cancer cells HT-29, HCT-116 and LS-180 were retrovirally transduced to stably express green fluorescent protein (EGFP). Was prepared 5-flourouracil (5-FU) resistant cell line derived from colorectal carcinoma HT-29/GFP/FUR proliferating in clinically relevant plasma concentration of 5-FU. RNA from cells was isolated and analysed by qPCR. Small interfering RNA (siRNA) silenced ALDH target gene was introduced to the cells by nucleofection with a NeonTM transfection system (Invitrogen). Properties of cells with siRNA were analysed. Aldefluor assay, cell proliferation assay, Annexin V assay and qPCR for confirmation of RNA silencing were used to analyse the properties of cells.

Results and Discussion. Expression level of different isoenzymes of aldehyde dehydrogenase in tested cells was identified. We detected connection between the activity of enzyme ALDH1A1 and a resistance to chemotherapy used in the treatment of colon cancer. The chemoresistant HT-29/GFP/FUR cell line is characterized by significantly increased expression of ALDH1A3 but decreased expression of ALDH1A1 in comparison to parental cells. Molecular inhibition of ALDH1A3 in this chemoresistant cells partially reversed chemoresistance.

Conclusion. We confirmed a connection between the expression of CSC markers and a resistance to chemotherapy. Silencing of the ALDH1A1 gene can sensitise the cancer cells to some chemotherapeutics in vitro. This provides the basis for the development of new approaches to the treatment of cancer patients.

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No conflict of interest.

395 Overcoming acquired EGFR inhibitor resistance in NSCLC with targeted beam irradiation in combination with targeted agents

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Introduction. Non-small cell lung cancer (NSCLC) patients undergo primary, adjuvant or neoadjuvant radiotherapy treatment for NSCLC and image-guided radiotherapy (IGRT) allows more accurate treatment plans with reduced side effects. The small animal radiation research platform (SARRP) allows the treatment of animal models of cancer with planned protocols similar to those utilised in the clinic.

NSCLC patients with activating mutations in the epidermal growth factor receptor (EGFR) gene are treated with EGFR tyrosine kinase inhibitors (EGFR-TKI). However, resistance emerges in the majority of patients due to a number of well-documented mechanisms. The HCC827 NSCLC cell line, which harbours an activating EGFR mutation (del E746-A750), was used to generate EGFR-TKI resistant models and treated with irradiation (IR) and/or relevant targeted agents to evaluate the efficacy of combination treatment in EGFR inhibitor resistance.

Material and Method. Acquired resistance to EGFR-TKI was generated through repeated exposure to EGFR-TKIs. Resistant tumour/cell material was characterised for further mutations in the EGFR gene and also for c-MET and Axl over-expression and/or genomic amplification by quantitative PCR. Subcutaneous xenografts were established in nude mice and the mice were treated with either IR alone (3Gy/mouse/

day) or in combination with a targeted agent. Response to treatment was evaluated by tumour growth inhibition.

Results & discussion. EGFR-TKI resistant HCC827 cell lines were generated and c-MET amplification was identified in a number of clones. Resultantly Crizotinib, a c-MET inhibitor, was selected for combination evaluation. Treatment of parental HCC827 xenografts with targeted IR or Erlotinib resulted in tumour regression. However, in the c-MET driven resistant model, both the IR and Erlotinib response was significantly attenuated resulting in tumour re-growth. Combination testing with Crizotinib restored the efficacy of both IR and Erlotinib to naïve treatment levels confirming the role of c-MET in resistance.

Conclusion. Resistance to EGFR-TKI and IR in a HCC827 model is driven by c-MET amplification. Treatment with Crizotinib restored sensitivity to both EGFR inhibitors and IR demonstrating that pre-clinical models of resistance are invaluable in assessing novel agents targeting the EGFR pathway and the development of new combination strategies which seek to prevent or overcome resistance.

No conflict of interest.

396 Cancer cells with alternative lengthening of telomeres are more sensitive to DNA damage by anti-sense telomere transcripts

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Most cancer cells use telomerase as telomere maintenance mechanism (TMM) to overcome replicative senescence. Alternative lengthening of telomeres (ALT) is used as TMM in around 5% of all human cancers, with high prevalence in sarcoma and glioblastoma. ALT cells are proficient and depend on homologous recombination to elongate telomeres. If recombination can be therapeutically targeted in ALT cells remains unknown. Long non-coding transcripts from telomeres called TERRA were identified to bind and block telomerase, but function in ALT cells was not well characterized. To this end, we expressed TERRA transcripts in ALT cells to study TMM, in vitro cell growth and viability.

Adeno- and lentivirus constructs (AV and LV) were established for transient and stable recombinant expression of approximately 130 units of UUAGGG repeats in sense (S) or anti-sense (AS) orientation in ALT tumor cell models (n=3). Cells expressing eGFP were controls for growth, MTT cell viability, clonogenicity and senescence-associated beta-galactosidase assays. Relative telomere length (TL) and expression was analyzed by RT-qPCR, mean TL by TRF analyses, telomerase activity by TRAP assay, ALT by C-circle assay and ALT-associated PML bodies (APBs).

ALT cell models expressed recombinant TERRA-S/AS to similar extents and caused elevation or reduction of endogenous TERRA levels. Population doubling time, cell viability and senescence were not affected by TERRA-S/AS expression. However, clonogenicity and TL were reduced in early passages of LV TERRA-AS cell clones and in transient AV TERRA-AS treated ALT cells. At the same time ALT characteristics like heterogeneous TL, c-circles and APBs disappeared in TERRA-AS, but not in S or control cells. Further, ALT cells with recombinant TERRA-AS expression or after incubation with modified TERRA-AS oligonucleotides showed strong hypersensitivity to zeocin, a DNA damaging agent. Telomerase was not become activated even after long time passages.

Our results suggest an essential function of TERRA in ALT mechanism that can be targeted by TERRA anti-sense strategies to sensitize ALT cancer cells for therapeutic drugs without activation of telomerase. Established ALT cell models can be used for screening of most effective drug combinations.

No conflict of interest.

397 Acquired AKT drug resistance is associated with altered cap-dependent protein translation

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Background. The PI3K/AKT/mTOR pathway is a key driver of cell growth, proliferation and survival and is deregulated in multiple forms of cancer. Targeting the pathway is therefore a potential therapeutic strategy, and several small molecule inhibitors of AKT are currently in clinical trial, including MK2206 and AZD5363. Resistance has been a major barrier to the success of many molecularly targeted therapies, however, mechanisms of AKT inhibitor resistance are not yet well defined. We aimed to model acquired AKT inhibitor resistance preclinically to elucidate potential driving mechanisms prior to its emergence in the clinic.

Materials and Methods. We generated acquired resistance to AZD5363 in several cell line models by continuous stepwise exposure to CCT129254, a closely-related precursor of AZD5363. We have focused our attention on A2780 254R, a PTEN-deficient A2780 ovarian carcinoma cell line with acquired resistance to AZD5363, and utilized a variety of techniques including exome sequencing, gene expression microarray analysis and cross screening of molecular targeted drugs to identify potential mediators of resistance.

Results and Discussion. A2780 254R demonstrated resistance to the ATP-competitive inhibitor AZD5363 and importantly, cross-screening against a panel of signal transduction inhibitors revealed even greater resistance to the allosteric AKT inhibitor MK2206, but not to direct PI3K or mTOR inhibition. While alterations to direct AKT substrates were minimal, there were striking pathway changes downstream of mTORC1 including markedly elevated p70S6K phosphorylation and reduced 4EBP1 protein expression. Consistent with this, we identified altered dynamics of eIF4F complex formation and elevated cap-dependent protein translation refractory to treatment with AKT inhibitors.

Conclusions. We propose that alterations to signaling downstream of AKT can increase cap-dependent protein translation and drive resistance to both ATP-competitive and allosteric AKT inhibitors currently in the clinic. Our future work aims to validate these novel findings and develop strategies to overcome resistance.

Conflict of interest: Other substantive relationships: M.D.G. is a consultant for Astex Pharmaceuticals

398 Axl receptor kinase is up-regulated in metformin resistant LNCaP prostate cancer cells

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Backgroud. Recent epidemiologic studies show that metformin, a widely used anti-diabetic drug might prevent certain cancers. Metformin also has an anti-proliferative effect in preclinical studies of both hematologic malignancies and solid tumors. Many clinical studies testing metformin as an anti-cancer drug are in progress.Not all tumor types are sensitive to metformin, and development of acquired resistance in tumors initially sensitive to this drug would be expected.To understandthe mechanism(s) of inherent and acquired resistance and its mechanism of action as a anticancer drug, we developed metformin resistant LNCaP cells.

Methods. To simulate the clinical situation, cells were exposed repeatedly to an IC50 oncentration of metformin (2.5 mM), allowing the cells to regrow between treatments. A gene expression array was performed, comparing parenteral and resistant cells.

Results. After 10 exposures to repeated IC50 concentrations of metformin, the LNCaP cells became 4-fold resistant to metformin. Further exposure to drug, or treatment with increasing concentrations of drug did not result in an increase in resistance. Resistance was stable, as cells cultured in the absence of metformin were still resistant. The expression array revealed that Edil2, Ereg,Axl,Anax2,cd44 and Anex3 were the top upregulated genes and calbindin2 and TPTE9 (*transmembrane phosphatase with tensin homology) and IGF1R were down regulated.

The metformin ressitant subline (MetR) as well the castrate resistant cell lines, DU145 and PC-3, were also more resistant to taxotere, compard to the androgen sensitive lines LNCaP and CWR22, that do not overexpress Axl. Forced expression of Axl in LnCaP cells decreased metformin and taxotere sensitivity and knockdown of Axl in MetR cells increased sensitivity to these drugs.Inhibition of Axl kinase activity by R428, a small molecule Axl inhibitor in early clinical trails, also sensitized MetR resistant cells to metformin. MetR cells had an increased proliferation rate and invasion ability as compared to the parenteral cells and expressed markers of EMT.

Conclusions. Axl over expression is associated with increased resistance to metformin and taxotere. Inhibitors of Axl may enhance tumor responses to metformin as well as to other chemotherapeutic agents in cancers that over express Axl.

No conflict of interest.

400 Base Excision Repair modulation of cisplatin activity in KRAS mutated NSCLC cells

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Introduction. KRAS is one of the best characterized genes in cancer but still remains practically undruggable. KRAS mutations in NSCLC are supposed to be associated to a poor prognosis and poor response to chemotherapy but this feature lacks a mechanistic evidence so far. In tumors, KRAS is mutated mostly at codons 12, but this process has as result a pool of mutations differing in the base replacement and the amino acid substitution. We hypothesized that different KRAS mutations in NSCLC patients may differently impact on drug sensitivity. We generated isogenic NSCLC clones expressing different KRAS mutations to determine the response to cisplatin, the election drug for the first line treatment of NSCLC in the clinic. The G12C clone was less sensitive in vitro and in vivo to cisplatin, but MAPK and PI3K pathways were similarly modulated by cisplatin in all the clones.

Material and method. DNA damage response was determined by western blotting, immunofluorescence and Real-Time PCR. Platinum adducts on DNA were revealed by DRC-ICP-MS.

Results and discussion. Cisplatin was able to enter the cells and reach its major target, the DNA, in all the KRAS mutant clones and, even if a slight difference was observed in cisplatin intracellular uptake and processing, this did not explain the different behaviour of the clones after cisplatin treatment. At early time points after cisplatin exposure, a similar amount of DNA adducts was observed in all the clones; however, in the cells carrying the G12C mutation a much faster decrease was observed 24 hours after treatment start. In addition, the G12C clone did not show H2AX activation and G2/M cell cycle phase arrest compared to all the others. Systematic analyses of DNA damage repair systems involved in cisplatin adducts removal revealed that Base Excision Repair (BER) could be responsible of KRAS G12C clone resistance to cisplatin: in fact, concomitant treatment of cells with different BER inhibitors at non toxic concentrations was able to almost completely restore the sensitivity of KRAS G12C mutant clone to cisplatin.

Conclusion. The results seem to indicate that KRAS G12C mutation could stimulate BER, promoting platinum removal from DNA. These preclinical findings could represent the starting point to design more tailored therapies and new trials in the clinical setting.

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No conflict of interest.

401 Caveolin-1 levels are crucial for the anti-apoptotic role of Fas in chemoresistant colon cancer cells

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Introduction. Caveolin-1 (Cav-1), a structural component of caveolae (a subtype of lipid rafts), negatively regulates many signal transduction pathways by sequestering receptors at the plasma membrane. We have demonstrated a pro-survival functional shift of the death receptor Fas in chemoresistant colon cancer cells. Since high Cav-1 protein levels are found in metastatic clones and MDR-phenotypes in cancer cells, we have assessed the impact of Cav-1 expression modulation on drug sensitivity and Fas functionality in chemoresistant colon cancer cell lines.

Material and Method. Cell lines: HT29 and HCT116 human colon cancer cell lines and their derived oxaliplatin or 5FU-resistant partners. Protein expression: western blot and immunocytochemistry. Stable Cav-1KD cell lines: lentiviral Cav-1 siRNA infection and cell sorting. Drug resistance: MTT viability assay at 72h. Gene expression: qPCR. Treatments: Oxaliplatin (OXA) (15 μM for HT29; 2μM for HCT116); 5FU (20 μM for HT29; 4μM for HCT116); DAC (0,5 μM); CH11 (Fas agonist antibody; 150 ng/ml for 24h). Apoptosis detection: Annexin-V binding.

Results and Discussion. We have found that high basal levels of Cav-1 are a common trait of most of the OXA and 5FU-resistant cells. OXA treatment increases Cav-1 expression and induces a weak Cav-1/Fas colocalization at the plasma membrane. Stable knock-down of Cav-1 in resistant cells restores sensitivity to oxaliplatin by downmodulating the p38MAPK pathway. In contrast with Cav-1 expressing cells, the specific activation of Fas in Cav-1 KD cells differentially activates the PI3K/AKT pathway and is able to increase the apoptotic response mediated by this death receptor. Moreover, pharmacological modulation of Cav-1 and Fas gene expression by DAC shows an opposite but coordinated response to this demethylating agent. These results point out a functional anti-apoptotic link between Cav-1 and Fas in resistant cells that, by tailoring the PI3K/AKT and the p38 MAPK pathways, contributes to the drug-resistant phenotype.

Conclusion. Cav-1 levels are relevant to drug resistance in colon cancer cell lines and contribute to the functional pro-survival shift of the death receptor Fas. Pharmacological down-modulation of Cav-1 levels in resistant colon cancer cells could help to reverse drug resistance and improve second line treatment effectiveness.

No conflict of interest.

402 The novel multi-kinase inhibitor EC-70124 acts a dual inhibitor of NF-kB and STAT3 signaling in prostate cancer stem cells

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Introduction. Cancer stem cells (CSCs) contribute to treatment failures and disease recurrence because of their resistance to current treatments. Pathways controlling CSCs may provide ideal targets for development of new anticancer therapies. Over-activity of the STAT3 and NF-kB signaling has been reported in CSCs in prostate cancer. EC-70124 is a novel multi-kinase inhibitor generated by combinatorial engineering of the biosynthetic pathways of glycosylated indolocarbazoles like staurosporine and rebeccamycin. In this study, we analyzed the efficacy of EC-70124 in prostate cancer cell lines focusing on its effects on the prostate CSC subpopulation.

Materials and Methods. Cell viability and proliferation were assessed using colorimetric, clonogenic and prostate-sphere forming assays in LNCaP, VCaP, 22RV1, DU145 and PC3 cell cultures. Activity of NF-κB and STAT3 in cell cultures and tumor xenografts was assessed using luciferase reporters. Gene expression and protein levels were measured by immunoblotting and RT-qPCR. In vivo activity was tested in nude mice bearing subcutaneous xenografts of DU145 and 22Rv1 cells.

Results and discussion. Biochemical assays showed that EC-70124 was highly effective in inhibiting IKK-beta and JAK activity, the upstream activating kinases in the NF-kB and STAT3 pathway, respectively. In cellular assays EC-70124 reduced STAT3 and NF-κB activity, phosphorylation of STAT3 and IkB-alpha, and expression of STAT3 and NF-kB target genes at nanomolar concentrations and after short incubation times. EC-70124 effectively inhibited cell viability and proliferation of prostate cancer cell lines with IC50 of 100-400 nM. Moreover, EC-70124 was a potent inhibitor of CSCs in vitro in prostate-sphere formation and self-renewal assays. STAT3 and NF-kB activity and expression of target genes was greatly affected in the CSC-enriched population. Importantly, treatment with EC-70124 (40 mg/kg, PO, daily for 2 weeks) inhibited growth of prostate tumor xenografts in nude mice and was associated with suppression of STAT3 and NF-kB signaling and reduction of CSCs in tumor tissue.

Conclusion. EC-70124 acts as a dual inhibitor of STAT3 and NF-κB signaling in prostate cancer cells and is an effective anticancer agent both in vitro and in vivo with the ability to block maintenance and self-renewal of prostate CSCs. EC-70124 may provide the basis for new therapeutic approaches for prostate cancer treatment.

Conflict of interest: Other substantive relationships: Entrechem SL, Co-authors (PC, LEN, FM) are employees of the company

403 Glutathione (GSH) underlies carboplatin resistance of ovarian clear cell carcinoma (OCCC)- related to hepatocyte nuclear factor 1b (HNF1b)

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Epithelial ovarian cancer (EOC) accounts for 90% of all ovarian malignancies and is the leading cause of death from gynecological cancer. The high mortality is mainly due to the late diagnosis and cell resistance to platinum based therapy. Within EOC, ovarian serous carcinoma (OSC) is the most prevelant histological type whereas clear cell carcinoma (OCCC) is rare but more chemoresistant, in an extraovarian presentation. Hepatocyte nuclear factor 1b (HNF1b) de novo expression is a molecular hallmark in OCCC, being responsible in part by these cells morphological pattern due to glycogen accumulation. Carboplatin, a platinum-based antineoplastic agent (DNA damaging and reactive oxygen species (ROS) generating drug) is one of the standard chemotherapeutic agents used in EOC treatment.

In the present study we aimed to explore: 1) if glutaminolysis is a key pathway for GSH production in OSC (OVCAR3 cell line) and OCCC (ES2 cell line); 2) if GSH synthesis underlies chemoresistance to carboplatin; 3) the association of HNF1b with GSH biovailability in OCCC, and 4) if the inhibition of GSH would sensitize cells to carboplatin, both in vitro and in vivo. Glutaminolytic profile was defined by nuclear magnetic resonance (NMR), GSH was quantified by high-performance liquid chromatography (HPLC), cell death was determined by fluorescence-activated cell sorting (FACS) (Annexin V and propidium iodide labeling) and HNF1b and GCL expression was evaluated by immunofluorescence. Murine (BALB\c-SCID) 6 week female (~20 grams) model of subcutaneous and peritoneal tumors was established in order to test buthionine sulphoxamine (BSO) (20mM) and carboplatin (100 mg/Kg) effects.

We observed that both OVCAR3 and ES2 cell lines perform glutaminolysis, although ES2 uses this pathway exclusively to synthesize other amino acids and GSH. In turn, OVCAR3 besides the lower levels of amino acids and GSH can still produce intermediates from tricarboxylic acid (TCA) cycle and from phosphatidylcholine degradation. ES2 cells are more resistant to carboplatin induced cell death than OVCAR3 cells and the abrogation of GSH synthesis by BSO increases ES2 sensitivity to carboplatin. Moreover, HNF1b regulates the expression of gamma-glutamylcysteine ligase (GCL), a key enzyme in glutathione synthesis, and ES2 shHNF1b generated cell line decreases GCL expression, reaching the levels detected in OVCAR3. In vivo, OCCC adjuvant administration of BSO with carboplatin (n=3) reduces the size of subcutaneous tumors and peritoneal tumor dissemination, whereas carboplatin or BSO individual administration had no effect in tumor burden.

This study shows that the maintenance of an internal loop driven by HNF1β and GCL sustains high GSH levels in ES2, leading to carboplatin resistance. Moreover, our in vivo results show that GSH chemical depletion can be useful to sensitize OCCC to chemotherapy.

Key words: clear cell carcinoma (OCCC), chemoresistance to carboplatin, glutaminolysis, glutathione (GSH), buthionine sulphoxamine (BSO), HNF1b, gamma-glutamylcysteine ligase (GCL).

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404 Identification of amplified genes in lapatinib resistant HER2-positive breast cancer cells

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Background. Despite the clinical success of HER2-targeted therapies such as trastuzumab and lapatinib for the treatment of HER2-positive breast cancer, the majority of patients with metastatic cancer develop progressive disease, due to either innate or acquired resistance. The aim of this study was to investigate mechanisms of lapatinib resistance in an acquired lapatinib resistant cell line model.

Methods.HCC1954 cells were exposed to 1 μM lapatinib continuously for >6 months. Proliferation assays were performed to examine sensitivity of HCC1954 parental cells

(HCC1954-par) and lapatinib conditioned cells (HCC1954-L) to three HER2-targeted tyrosine kinase inhibitors. Gene copy number alterations were examined by array CGH and gene expression was assessed by microarray. Protein alterations were examined by Western blotting.

Results and Discussion. HCC1954-L cells are resistant to lapatinib compared to HCC1954-par (IC50 = 2.7 ± 0.08 μM v 420 ± 2 nM), neratinib (IC50 = 312 nM v >10 nM) and afatinib (IC50 = 1.26 ± 0.09 μM v >10 nM). The HCC1954-L cell line accumulated 15 significant chromosomal alterations (13 amplification events and 2 deletions). A number of genes which are located on the HER2 amplicon, including HER2, growth factor receptor-bound protein 7 (GRB7) and StAR-related lipid transfer domain protein 3 (STARD3) were further amplified in HCC1954-L cells compared to HCC1954-par cells. In addition genes previously associated with cancer but not implicated in HER2-positive breast cancer were amplified in the resistant cells, including RecQL helicase (RECQL1: 0.77 amplification), UDP glycosyltransferase 8 (UGT8: 0.74 amplification), acid ceramidase (ASAH1: 0.74 amplification) and phosphodiesterase 3A (PDE3A: 0.77 amplification). Using Western blotting we found that expression of RecQ1, UGT8 and PDE3A proteins is also higher in HCC1954-L than HCC1954-par cells.

Conclusions. Gene expression and copy number aberration analysis of acquired lapatinib resistant cells implicates genes involved in metabolic survival pathways in lapatinib resistance.

Conflict of interest: Other substantive relationships: JC and NOD have received research funding from GlaxoSmithKline

405 Effect of Palbociclib (CDK 4/6 inhibitor) on breast cancer cell growth

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Introduction. Cyclin D1 is implicated in breast cancer growth. Cyclin D1 amplifications occur in 15% of breast cancer while its overexpression occurs in 50%, mainly estrogen receptor (ER) positive subtype. Phosphorylation of pRb is mediated in G1 by CDK4 and CDK6 interacting with cyclin D1. Palbociclib (PD0332991) is a highly selective inhibitor of the cyclin D1-CDK4/6; it suppresses DNA replication at low concentrations in RB proficient human cancer cells, including breast cancer.

In order to evaluate the in vitro effects of Palbociclib treatment on breast cancer cells, we performed proliferation assays on 3 human breast cancer cell lines and 9 breast cancer cultures of cells obtained from breast cancer patients with different receptor (ER/PgR, HER2) expression profiles.

Material and method. Breast cancer tissue specimens were collected, processed, and cultures of primary cells were obtained; human breast cancer cell lines MDA-MB-231, MCF7 and BT474 (American Type Culture Collection) are used as control. CDK 4/6 inhibitor Palbociclib (PD0033291, Pfizer), dissolved in DMSO, was used at increasing concentration of 31.25 nM, 62.5 nM, 125 nM, 250 nM, 500 nM and 1uM. Cells were daily treated in vitro for 6 days; Mtt assay and growth curves were performed to study the effect of Palbociclib on proliferation. Western Blot analysis of Rb an phospoRb were performed.

Results and discussion. We observed different responses to Palbociclib among the primary breast cancer cells analysed. A 40% inhibitory effect on the cancer cells growth (40% inhibition at 1 uM drug concentration for 6 days treatment) was found on ER positive breast cancer cells. Western Blot analysis revealed that phosphorylation of Rb was decreased in treated breast cancer cells.

Conclusion. The cell-cycle machinery is frequently disrupted in cancer. In breast cancer tumors with functional RB, CDK4/6 inhibition suppress cell growth and DNA replication. The evaluation of in vitro response might be useful in understanding the molecular mechanisms of cell cycle progression.

No conflict of interest.

406 Oleocanthal, a phenolic compound of extra-virgin olive oil, inhibits cell growth and induces apoptosis in hepatocellular carcinoma cells

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Introduction. The Mediterranean diet is associated with beneficial health properties. The principle source of dietary fat in the Mediterranean diet is extra-virgin olive oil (EVOO). Oleocanthal (OLC) [(-)-deacetoxyligstroside aglycone] is a naturally occurring minor phenolic component isolated from EVOO, which showed potent anti-inflammatory activity via its ability to inhibit cyclooxygenase enzymes COX-1 and COX-2. Although structurally dissimilar, OLC showed anti-inflammatory activity comparable to the non-steroidal anti-inflammatory drug (NSAID) ibuprofen, a non-selective inhibitor of COX-1 and COX-2. It is well-established that chronic inflammation plays an essential role in the pathogenesis of several types of cancers, including hepatocellular carcinoma (HCC). We have previously shown that NSAIDs affect the growth of HCC cells, the aim of this study was to characterize the potential anti-cancer effects of OLC in HCC cells.

Material and method. A panel of well-characterised human HCC cell lines, e.g., HepG2, Huh7, Hep3B and PLC/PRF/5 cells, was used in this study. Cells were treated with OLC and ibuprofen, and cell viability was evaluated using MTS assays. Apoptosis was evaluated by measuring caspases activities, Hoechst staining and Western blot analyses of protein involved in apoptotic process. ROS production by HCC cells was determined using the cell-permeable fluorescent probe H2DCFDA.

Results and discussion. OLC treatment in a dose-dependent manner inhibited cell viability in all cell lines studied, with an IC50 ranging from 29 μM in Hep3B cells to 75 μM in Huh7 cells at 72hours, whereas ibuprofen IC50 was always > 100 μM in all cell lines. Moreover, OLC inhibited colony formation. Induction of apoptosis, as evidenced by PARP cleavage, activation of caspases 3/7, chromatin condensation and fragmentation, was observed only after treatment with OCL but not with ibuprofen. Finally, OLC treatment in dose dependent-manner induced intracellular ROS production. Therefore, OLC results to be more effective than ibuprofen in cell growth inhibition and apoptosis induction in HCC cells.

Conclusion. We found that OLC treatment exerts a potent anti-tumoral activity against HCC cells. Taken together, our findings provide preclinical support of the chemopreventive and chemotherapeutic potential of EOVV against HCC.

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No conflict of interest.

407 Cellular and molecular characterization of 5-Fluorouracil (5-FU) induced toxicity on human cardiomyocytes and endothelial cells

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Introduction. 5-fluorouracil (5-FU) and its prodrug Capecitabine are pyrimidine antimetabolite widely used in the treatment of several solid tumors (gastro-intestinal, gynecological, head and neck, breast carcinomas). Therapy with fluoropyrimidines is associated with a wide range of adverse effects, including diarrhea, dehydration, abdominal pain, nausea, stomatitis, and hand- foot syndrome. Since the cellular and molecular mechanisms associated with 5-FU-induced cardiovascular toxicity still require deepen investigation, we examined the effects of 5-FU on primary cell cultures of human cardiomyocytes (HCM) and human endothelial cells (HUVECs), which in turn represent the two crucial components of the cardiovascular system.

Material and Methods. We performed MTT and LDH assay to evaluate 5-FU abilities to interfere with cell proliferation, while the induction of apoptosis was assessed by flow cytometry. Cell cycle was also analysed. The disruption of mitochondrial transmembrane potential and cytoplasmic accumulation of reactive oxygen species (ROS) were tested by fluorescent probes. In addition we assessed the ability of 5-FU to induce autofagic vesicles (AVOs) by flow cytometry and transmission electron microscopy (TEM). Finally we studied the cardiovascular toxicity of 5-FU in vivo using a murine model for colon cancer xenograft.

Results and Discussion. We observed autophagic features at the ultrastructural and molecular levels, mainly in 5-FU exposed cardiomyocytes, while reactive oxygen species (ROS) elevation characterized the endothelial response. These were prevented by the ROS scavenger N-acetyl-cysteine (NAC). We found induction of a senescent phenotype on both cell types treated with 5-FU. In vivo, using a xenograph model of colon cancer, we showed that 5-FU treatment induced ultrastructural changes in the endothelium of various organs at therapeutic doses.

Conclusion. Altogether, our data suggest that 5-FU can affect, both at the cellular and molecular levels, two key cell types of the cardiovascular system, potentially explaining some manifestations of 5-FU-induced cardiovascular toxicity.

No conflict of interest.

408 Development of novel anti metastatic cancer therapeutics

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The Rho GTPase Rac is a pivotal regulator of cancer cell migration and invasion; processes required for metastatic progression. We previously characterized the small molecule EHop-016 as a novel Rac inhibitor in metastatic breast cancer cells, and recently reported that EHop-016 was effective at reducing tumor growth, metastasis, and angiogenesis in nude mice at 25 mg/kg bodyweight (BW) (Castillo-Pichardo, et al. 2014). We also determined the pharmacokinetics and bioavailability of EHop-016, and reported that EHop-016 is rapidly cleared from mouse plasma with a half-life of ~4 hrs and ~30% bioavailability (Humphries-Bickley, et al., 2015). To improve the bioavailability and efficacy of EHop-016, we synthesized and screened a number of derivatives, from which EHop-016A was identified. At nM concentrations (100-250 nM), EHop-016A has a dramatic effect on breast cancer cell morphology by inducing the detachment of cells from the extracellular matrix (ECM) with a concomitant loss in focal adhesions, cell surface actin structures, and cell polarity, as well as reduced cell migration. Consequently,

EHop-016A inhibits cell viability with a GI50 of 150nM and 75nM in MDA-MB-435 and MDA-MB-231 human metastatic cancer cell lines respectively. Caspase 3/7 assays and western blotting for BCl2 Homology proteins, demonstrated that the reduced cell viability in response to EHop-167 is probably due to anoikis: apoptosis due to dissolution of integrin-mediated cell to ECM attachments. In addition, EHop-167 reduced the mammosphere formation of MDA-MB-231 cells, indicating an inhibitory effect on breast cancer stem-cell like properties that are not dependent on cell-ECM attachments. Therefore, EHop-167 may act as an inhibitor of malignant cancer cells with metastatic potential. These results support further in vitro and in vivoevaluation of this new small molecule compound as a potential anticancer agent.

No conflict of interest.

409 In vitro resistance to 3rd generation EGFR tyrosine kinase inhibitors

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Introduction. Non-small cell lung cancer (NSCLC) patients with activating mutations in the epidermal growth factor receptor (EGFR) gene generally show good responses to therapy with 1st generation EGFR tyrosine kinase inhibitors (TKIs, e.g. gefitinib). Unfortunately resistance emerges during treatment, in most cases due to a T790M gatekeeper mutation. 2nd generation EGFR TKIs (e.g. afatinib) are active against this resistance conferring mutation and, more recently, 3rd generation TKIs have been developed (e.g. AZD9291) which selectively inhibit mutant (including T790M) but not wild-type EGFR, resulting in reduced adverse effects. In order to identify resistance mechanisms that are common to all classes of EGFR TKIs, we aimed to generate cell lines resistant to EGFR TKIs of all generations.

Material and methods. EGFR mutant NSCLC cell lines were exposed to AZD9291 or gefitinib (1 to 2 μM) for up to 14 days and EGFR-TKI resistant cells lines were isolated. Proliferation under increasing doses of EGFR TKIs was determined using a resazurin-based viable cell dye assay and IC50 values calculated. Apoptosis was analysed either by western blotting for PARP cleavage or a live cell nuclear condensation assay.

Results and Discussion. We have generated resistant cell lines with >100 fold increase in IC50 values for AZD9291, afatinib, gefitinib and erlotinib (PC9, HCC827). In addition we derived a resistant H1975 cell line (which is intrinsically resistant to 1st generation EGFR TKIs due to a T790M gatekeeper mutation) with a greater than 100-fold increase in IC50 for AZD9291 (1.4 μM as opposed to 7 nM in resistant and parental H1975, respectively). The majority of resistant cell lines showed a markedly reduced level of pEGFR that was insensitive to EGFR TKI treatment, which also had no effects on pMAPK or pAKT levels. Additional mutations in EGFR exons 19-21 were not detected. To date, little is known about the mechanisms of resistance to 3rd generation EGFR TKIs and the cell lines generated in the present work (with cross-resistance to 1st, 2nd and 3rd generation EGFR-TKIs) will provide important preclinical tools to expand this research. RNA-sequencing and RPPA analysis are currently ongoing to investigate novel mechanisms of resistance. Furthermore, these studies will enable us to identify signalling pathways that can be targeted to delay or potentially prevent the emergence of resistance, to further improve the effectiveness of EGFR-TKIs.

No conflict of interest.

410 High expression of EGFR and low expression of ERBB3, within AXL-high/MITF-low melanomas, identify tumors intrinsically resistant to BRAF inhibition

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Introduction. The discovery of the BRAFV600E substitution as the most recurrent mutation in skin cutaneous melanoma (SKCM) rapidly led to the development of BRAF inhibitors (BRAFi). However patients sharing the same driver mutation show diverse response to BRAFi. In SKCM, heterogeneity at the transcriptional level was highlighted by gene expression profiling studies. Indeed, a 'Melanoma Phenotype-Specific Expression' (MPSE) signature defines two phenotypes based on the reciprocal expression levels of MITF transcription factor and AXL receptor tyrosine kinase (RTK). Low MITF/AXL ratio has been recently shown to predict early resistance to BRAFi. Melanomas with high expression of AXL, however, include both BRAFi resistant and sensitive tumors. Therefore, we here assessed whether expression of all RTK genes could stratify melanomas in different groups allowing the identification of BRAFi intrinsically resistant tumors with higher accuracy.

Material and methods. Gene expression and pharmacological data for 58 melanoma cell lines, retrieved from CCLE, were used to identify subtypes and assess their differential sensitivity to BRAFi PLX4720. A gene signature, identified by ClaNC algorithm, was used to validate the subtypes in public datasets of SKCM cell lines (n = 187) and clinical samples (n = 361). Gene Set Enrichment and Ingenuity Pathway Analysis were applied to identify altered pathways in each subtype. Melanoma cell lines were established in vitro from surgical specimens. Western blot analysis and growth inhibition were used for biochemical and drug susceptibility assays.

Results and discussion. Clustering of CCLE cell lines according to RTKs and integration with the MPSE classification revealed three SKCM subtypes. We derived a gene signature distinguishing with high accuracy these three subtypes that were validated in an independent dataset of melanoma cell lines and also in a cohort of clinical samples. We further confirmed this classification by Western blot in a panel of patient-derived cell lines. One of the subtypes identified here for the first time displayed the highest and lowest expression of EGFR and ERBB3 and included BRAF-mutant tumors all intrinsically resistant to BRAFi PLX4720, according to CCLE pharmacogenomics data and to in vitro growth inhibition assays. Pathway analysis revealed activation and inhibition of specific biological mechanisms in resistant tumors.

Conclusion. Based on transcriptional features we defined a robust classification of SKCM cell lines and clinical samples that allows identification of a subtype of tumors displaying intrinsic resistance to targeted therapy with BRAFi with higher accuracy in comparison to the AXL/MITF dual classification. These data offer insights into melanoma heterogeneity, provide definition of molecular features characterizing resistant tumors and have implications for development of alternative treatments in predefined subsets of unresponsive patients.

No conflict of interest.

411 An Egyptian plant extract Factor XY exhibits potent cytotoxic effects against bladder and prostate cancer

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Introduction. We have identified a candidate extract, Factor XY (FXY), from a native Egyptian plant that exhibits potent efficacy against prostate cancer, and bladder cancer. We are investigating the cytotoxic effects and mechanism of action of FXY on both on cultured and in mouse models using luciferase labeled cells.

Materials and Methods. An organically extracted compound referred to as FXY was tested for phyto-cytotoxic activity on prostate cancer cells (PC-3 and C4-2B), and on multiple bladder cancer cell lines. A dose escalation ranging from 0.01μg/ml - 100μg/ml was analyzed by MTS assay. IC50s for each cell line were determined. FXY was tested to authenticate its activity against cancer stem cells using sphere formation assay at a concentration of 25μg/ml. Two cell lines that showed high sensitivity to FXY, the UM-UC-9 and UM-UC-10, were utilized in SCID mouse models of bladder cancer (injected subcutaneously and orthotopically). In addition, the prostate cancer cell line PC-3 was injected into the left ventricle (intra-cardiac) to establish disseminated growth. All animals were treated with FXY by oral gavage at a dose of 4mg/kg body weight.

Results and Discussion. All the cell lines examined exhibited IC50 ranging from 2-20μg/ml in vitro. Western blot of the treated cells showed marked Inhibition of the protein p65 at 30 minutes post treatment, and a total inhibition of HER-2. In the sphere forming assays, FXY resulted in inhibition of secondary and tertiary sphere formation. In both mouse models, FXY treated animals exhibited statistically significantly lower tumor burden weight as compared to their respective controls.

Conclusion. FXY exhibits strong efficacy (low IC50 values) against multiple prostate and bladder cancer cell lines. We will continue to evaluate the mechanism(s) of action of FXY on bladder cancer.

No conflict of interest.

412 miR-199 enforced expression inhibits tumor growth in a HCC transgenic mouse model

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Introduction. Hepatocellular carcinoma (HCC) is the most common primary liver cancer and one of the most deadly tumors worldwide. With the exception of the multi-kinase inhibitor Sorafenib, there are no effective systemic treatments available for this disease. New perspectives in HCC treatment emerged in the last decade with microRNAs (miRNAs), a large class of short RNAs frequently altered in human cancer and affecting crucial cancer-associated pathways. miR-199 has been reported to be consistently down-regulated in HCC and is involved in HCC pathogenesis through the regulation of several target genes as mTOR and c-Met.

Material and Methods. The expression of miR-199 in HCC cell lines and in mice tissues was analyzed by quantitative RT-PCR. The apoptotic effect of miR-199 on HCC cell lines were assessed by MuseTM Annexin V. Mimic-199 oligonucleotides were introduced in vivo by intra-peritoneal injection and Sorafenib was administered orally. Livers of transgenic mice were histologically examined to assess number and extent of lesions. Results and Discussion. In this study, we firstly demonstrated how the enforced expression of miR-199 through miRNA-mimic oligonucleotides or Adeno Associated Viruses (AAV) leads to inhibition of cell viability and increase of apoptosis in HCC cell lines. According to these data, we subsequently proved that a long-term administration of miR-199 mimics in tumors arising in a HCC transgenic mouse model leads to a reduction in number and size of liver nodules in comparison with the untreated control animals. Interestingly, results obtained with miR-199 administration were comparable to results obtained in mice treated with Sorafenib.

Conclusion. This work suggest that miR-199 replacement might have a significant therapeutic value and could provide new therapeutic opportunities for HCC treatments.

No conflict of interest.

413 A genetic signature of drug synergy: XPO1 inhibition and cisplatin overcome chemoresistance in ovarian cancer

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Introduction. Ovarian cancer (OvCa) is one of the leading causes of death from gynecologic malignancy with more than 190,000 new cases each year worldwide. Most patients present with advanced stage disease and treatment is surgical debulking followed by combination platinum- / taxane-based chemotherapy. Unfortunately, the majority of women will die from recurrence and chemoresistance. Therefore, novel therapeutic strategies are urgently needed. Exportin-1 (XPO1) is the sole nuclear exporter of a number of tumor suppressors and cell cycle regulators and is overexpressed in OvCa. We have previously demonstrated that the Selective Inhibitors of Nuclear Export (SINE), KPT-185 and KPT-330, inhibit XPO1-mediated nuclear export and result in the increased apoptosis, decreased cell proliferation of OvCa cells and marked survival advantage in mouse models of OvCa. Based on these findings, we hypothesized that combining XPO1 inhibition with cisplatin would overcome platinum resistance.

Materials & Methods. Cell viability was measured via MTTs with immortalized OvCa cell lines and patient-derived OvCa lines (PD-OvCa) that were treated with +/- cisplatin and/or +/- XPO1 inhibition (using KPT reagents and leptomycin B). Effects of different treatments on the transcriptome were measured by microarray analysis. Ingenuity Pathway Analysis (IPA) â was used to define altered pathways, upstream regulators and genes causal to phenotypic changes. Real-time PCR and western blots were used to confirm all findings.

Results. Treatment of all OvCa and PD-OvCa cell lines with low dose KPT-185 (IC15) and cisplatin resulted in synergistic activity and a >4-fold increase in sensitivity to cisplatin. IPA analysis of transcriptome changes identified a 105 genes signature unique to only the combination of KPT-185 and cisplatin. Further IPA signature analysis revealed 18 upstream regulators of the signature that highlighted statistically significant increases in cell death via apoptosis and necrosis and decreased cell proliferation.

Conclusion. We present the first gene signature and explanation for the synergistic effect of combination XPO1 inhibition/cisplatin in OvCa. The synergistic increase in platinum sensitivity results from reciprocal effects on cell death and cell proliferation. These results provide a promising foundation for overcoming cisplatin resistance through targeted XPO1 inhibition and future studies to enhance this effect.

No conflict of interest.

414 HER3 and MEK dual targeting may overcome HER3-dependent chemo-resistance of colon cancers

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The medical treatment of colorectal cancer (CRC) has evolved greatly in the last years, involving combined chemotherapy protocols and, more recently, new biologic agents. Nevertheless, prognosis remains adverse for patients with metastatic disease, and a significant portion of early-stage patients develop recurrence after chemotherapy. Clinical trials are now directed to evaluate new drug combinations, and treatment schedules, in order to overcome the mechanisms of chemo-resistance.

By the use of patient-derived or established colon cancer cell lines carrying different genetic background, we found that the tyrosine kinase receptor HER3 is strongly involved in the mechanisms of resistance to 5-FluoroUracil and Oxaliplatin drugs.

By the use of a monoclonal antibody targeting HER3, named U3-1287, we found down-regulation of HER3 phosphorylation, HER3 internalization and degradation in all cell lines. Functionally, U3-1287 inhibits tumor cell proliferation inducing growth arrest at the G1 phase of the cell cycle, and reduces tumor mass in a xenograft model.

Even though U3-1287 administration is highly efficient in abrogating the HER3-dependent activation of PI3K pathway in colon cancer cells, we found that it induces a compensatory mechanism, involving the increase of HER2 receptor expression that in turn activates MAPK pathway.

To overcome U3-1287-induced activation of MAPK, we used a combined therapy with U3-1287 antibody and the MEK-inhibitor Trametinib. We found, as expected, that Trametinib inhibits MAPK but also induces the phosphorylation of HER3 receptor that in turn activates PI3K pathway; the combined therapy results in the complete abrogation of both PI3K and MAPK pathways, and in a significant reduction of cell survival in vitro in all cancers cell lines.

These data identify a new combination strategy that, independently from the genetic background of the cells, may overcome the mechanisms of resistance to chemotherapy in HER3-overexpressing colon cancers.

No conflict of interest.

415 DNA double-strand break repair in primary cultures of patient-derived ovarian carcinoma xenografts

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Introduction. Epithelial ovarian cancer (EOC) represents the most fatal gynecological malignancy. The DNA repair capacity is a key determinant for the cellular response to DNA damaging agents and is inversely correlated with their cytotoxicity. It has been reported that half of high grade EOC display defects in DNA repair, accounting for the sensitivity of this tumor to the first line chemotherapy. The aim of the present work was to evaluate the functional DNA repair activity, focusing on DNA double strand-break (DSB) repair, in EOC cell lines with different sensitivity to cisplatin (DDP) and in EOC primary cultures obtained from patient-derived ovarian carcinoma xenografts (PDX), recently established in our laboratory.

Material and method. Primary cultures derived from intraperitoneal ascites or from subcutaneous EOC xenograft solid masses were mechanically disaggregated and cultured in RPMI. The experiments were performed within the third in vitro passage.

To functionally evaluate DSB repair pathways, cells were transfected with various reporter substrates for EGFP-based quantification of specific DSB repair activities: homologous recombination (HR), total non-homologous end-joining (tNHEJ), and micro-homology mediated NHEJ (mmEJ) pathway. Briefly, 10^6 cells were transfected with different plasmid mixtures, containing the meganuclease expression plasmid (pCMV-I-SceI) together with one of the recombination substrates, and wtEGFP plasmid in split samples for determination of transfection efficiencies. Cellular fluorescence was quantified by flow cytometry and recombination frequencies were calculated from the fraction of wtEGFP positive cells normalized for transfection efficiency.

Results. Among cell lines, OVCAR5 showed very low DSB repair frequencies in all the three sub-pathways investigated. Interestingly, OVCAR433 showed the highest DSB repair frequencies and also the lowest sensitivity to DDP. MmEJ repair frequencies were lower than HR and tNHEJ both in cell lines and in primary EOC cultures derived from PDX. In EOC primary cultures tNHEJ frequencies were 2 to 10 times higher than HR.

Conclusion. We were able to analyze distinct DSB repair activities in EOC primary cultures obtained from PDX.

The application of such DNA repair assays to a panel of PDX xenografts with known sensitivity to DDP treatment will allow to assess the power of these tests to predict the response to chemotherapy.

No conflict of interest.

418 Molecular determinants of response to PI3K and K-RAS pathways inhibitors

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Introduction. Genes involved in the PI3K/AKT/mTOR pathway and K-RAS are among the most frequently altered genes linked to an aggressive and resistant cancer phenotype. PI3K pathway is an attractive target due to its well documented primary role in tumor development and progression and the multiple connections linking it with other pro-survival pathways, such as MAPK pathway. K-RAS is frequently mutated in largely diffused tumors, such as NSCLC, and mutations in this gene are considered negative predictive factors. Due to the high number of effectors and the presence of a complex network of feedback loops linking many of the proteins belonging to these pathways, it is emerging that the best way to determine a significant inhibition of the signaling cascade, and hence a therapeutic benefits, should be the combination of two or more drugs.

The purpose of the study was to investigate the effects of new molecules targeting the PI3K and the K-RAS pathways at different levels in a panel of NSCLC cell lines with a different genetic background, in order to identify the molecular determinants of response to the treatment and to find a combination of drugs with greater antitumor activity without concomitant increase in toxicity.

Material and methods. The effect of the different drugs (such as isoform-specific or pan inhibitors of PI3K, mTOR, MEK, ERK) on cell viability was evaluated with in vitro cytotoxicity assays and the activity of the inhibitors at molecular level was determined with western blot. A siRNA-based high throughput screening was used to identify new molecular targets whose inhibition could increase the anticancer efficacy of the treatment.

Results and discussion. Cellular systems differing in their PI3K/AKT/mTOR and K-RAS status show different sensitivity to single agents inhibiting these two pathways at distinct molecular levels. The activity of these compounds is correlated with the modulation of downstream targets, as observed through western blot analysis. In particular, cancer cells harboring mutations in K-RAS, which respond to PI3K inhibitors, seem to be resistant to a MEK inhibitor (MEK-162), with the exception of H727 cell line. To characterize the molecular determinants of MEK-162 activiy, we are using two strategies: 1. generation of H727 cells resistant to this drug and 2.use of a siRNA-based high throughput screening on resistant cell lines treated with MEK-

162 to identify genes in synthetic lethality with MEK inhibition which could restore sensitivity.

Conclusions. The preliminary findings reported herein indicate that specific mutations in PI3K and K-RAS pathways are associated with a different response to target-oriented anticancer drugs. Synthetic lethality assays and more detailed molecular analysis are ongoing in order to the define the best combination of inhibitors.

No conflict of interest.

419 Anti-VEGF therapy elicits metabolic evolution of tumors

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Introduction. Anti-VEGF therapy has been shown to cause metabolic perturbations in tumors, including severe impairment of glucose and ATP levels. We investigated therapeutic effects of anti-VEGF therapy in experimental tumors with different glycolytic phenotypes as well as the possible modulation of metabolic features of tumor cells by anti-VEGF therapy.

Materials &**Methods.**Tumor xenografts and spontaneous tumors were treated with anti-VEGF antibodies. In situ markers, induced metabolic bioluminescence imaging, extracellular flux analysis and mass spectrometry were used to characterize metabolic changes in tumors treated with anti-VEGF therapy or ex vivo cultures of tumor cells. [18F]FLT and [18F]FAZA PET were used to track proliferation and hypoxia in tumor xenografts, respectively.

Results & Discussion. Protracted anti-VEGF therapy induced both vascular regression and necrosis in tumor xenografts; however, highly glycolytic tumors became more rapidly resistant than poorly glycolytic tumors to anti-angiogenic therapy. By PET imaging, tumors chronically treated with anti-VEGF therapy were associated with increased hypoxic and highly proliferative tumor areas. Importantly, protracted anti-VEGF therapy selected for highly glycolytic cells and that this metabolic switch was stable and associated with increased tumor aggressiveness and resistance to VEGF blockade in serially transplanted mice. Mechanistically, this metabolic switch was associated with increased STAT3 activity, and it could in part be reverted by STAT3 inhibitors.

Conclusion. These results support the hypothesis that in xenograft models the highly glycolytic phenotype of tumor cells - either primary or secondary - is a cell-autonomous trait which confers resistance to VEGF blockade. Moreover, the observation that some metabolic traits of tumors can be stably modulated by anti-angiogenic therapy suggests evolutionary dynamics of tumor metabolism, which could be therapeutically targeted.

No conflict of interest.

420 Rapid induction of doxorubicin resistance in connected microenvironment elucidates novel molecular mechanisms

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Introduction. Emergence of drug resistance is the most critical cause of failure in cancer treatment. Developing alternative medicine usually requires good grasp of molecular mechanisms, which is hampered by difficulties in obtaining drug-resistant cells. Serial passage of cell culture with increasing drug concentration usually takes several months or years. Working with tissues from relapsed tumor patient is impractical to most researchers.

Materials and Method. Here we introduce a microfluidic device based on nanochip technology that consists of approximately 500 hexagonal micro-compartments of continuous concentration gradient. Genetic mutations and expression alterations leading to drug resistance were investigated by whole exome and transcriptome sequencing, respectively.

Results and Discussion. We verified that U-87 glioblastoma cells cultured on the nanochip under doxorubicin media showed drug resistance as early as seven days. Subsequent exome sequencing has identified 61 candidate mutations. Gene ontology terms for molecular function were statistically enriched in 'nucleoside binding' and 'ATP-dependent helicase activity'. This is in excellent agreement with the previous knowledge that doxorubicin inhibits the enzyme topoisomerase II by intercalating between two base pairs of DNA double helix. From the mutation and expression data, we were able to identify three mechanisms of resistance development to doxorubicin. Firstly, FLNA (Filamin A) that was known to regulate the influx and efflux of topoisomerase II poisons was mutated by a frame-shift insertion. Secondly, mutation of three genes (NLRP13, NSD1, CARD6) and overexpression of inflammatory cytokines (CCL2, CXCL1, CXCL2, IL6, IL8, IL1B, TNF) indicate independently the role of NF-kB via the NOD-like receptor signaling pathway. Lastly, overexpression of aldo-keto reductase enzymes, converting doxorubicin into doxorubicinol, is likely to contribute to resistance development as well. Consequences of loss-of-function variants were verified by siRNA knockdown experiments.

Conclusion. In this work, we have demonstrated that a microfluidic device of concentration gradient induces cancer drug resistance rapidly in vitro and that subsequent analysis of mutation and expression data reveals the molecular mechanisms of resistance development successfully. This combination of nanochip and deep sequencing technologies would provide a promising platform for overcoming cancer drug resistance.

No conflict of interest.

421 Acquisition of drug-resistance causes mesenchymal-to amoeboid transition (MAT) in epithelial ovarian cancer cells (OCCs)

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Introduction. Low survival rates in advanced stage ovarian cancer patients is attributed to acquisition of drug resistance against widely used chemotherapy drugs cisplatin (Cis) and paclitaxel (Pac). While molecular mechanisms underlying drug resistance is a subject of broad interest, how these molecular level changes alter the phenotype of drug resistant cells and its implication remains unclear. Here, we have performed a comparative study of the biophysical changes which occur in cisplatin resistant (Cis LR) and paclitaxel resistant (Pac LR) ovarian cancer cells (OCCs) compared to drug sensitive (CTL) OCCs. Further, we have studied the effect of microenvironment in modulating these changes.

Materials and Methods. Cellular spread area, contractility and cortical stiffness of cells was measured using phase contrast microscopy, trypsin de-adhesion assay and AFM (atomic force microscopy). Random-cell motility and wound-healing assay was performed to measure single and collective cell motility. Collagen degradation assay was done to measure proteolytic invasiveness. 3D collagen invasion assay and FDM (fibroblast derived matrix) remodelling was done. Immunofluorescence localization was done for important molecules involved directly/indirectly in invasion mechanism (vinculin, actin filaments and phospho-MLC). Drug inhibition studies was performed to study cross-talk and detailed mechanism of invasion. Statistical significance was measured by using t-test for all the experiments.

Results & Discussion. Compared to CTL cells, both CisLR and PacLR cells were more rounded, and possessed higher baseline contractility and lower cortical stiffness. Moreover, compared to CTL cells which exhibited high levels of collagen degradation mediated by proteases, PacLR cells showed no visible proteolytic degradation. In contrast, CisLR cells exhibited a combination of proteolytic degradation and force driven remodelling, with a cross-talk between both modes. In presence of ECM (extra-cellular matrix) these phenotypes show two-fold increase, suggesting ECM aggravates invasion in these cells.

Conclusion. Together, our results are indicative of mesenchymal to amoeboid transition (MAT) in untreated ovarian cancer cells upon acquisition of drug resistance, and raise the possibility that targeting molecular players involved in effecting this transition may reverse drug resistance.

No conflict of interest.

422 Cellular & molecular mechanisms of drug resistance in cancer cells mutated for Fbxw7 - tumour suppressor gene

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Introduction. The cellular homeostasis is controlled by the ubiquitin-proteasome system, which regulates the proteins turnover. FBXW7 is a known tumour suppressor gene, the fourth most commonly mutated gene in colorectal cancer (CRC) and frequently mutated in a variety of other epithelial tumours (1, 2). FBXW7 functions as an E3 ligase, thus, loss of FBXW7 function is likely to lead to the accumulation of its downstream substrates. This may disrupt a variety of critical signalling pathways resulting in acquisition of the hallmarks of cancer. Hence, elucidating FBXW7 mechanism(s) of action could be important for cancer therapy. We have tested the hypothesis that FBXW7 depletion results in the induction of cancer stemness properties via drug resistance in CRC mediated by the epithelial-mesenchymal transition (EMT) and self-renewal changes.

Materials and Methods. a) Cytotoxicity and viability analysis of normal and FBXW7-deleted CRC cells, treated with 5-fluorouracil, oxaliplatin & cisplatin (3), tested by fluorometric, colonosphere & stem-cell associated gene reporter assays.

b) Cell response and morphologies assays of intestinal mini-guts derived from normal and fbxw7-deleted mice (4) for testing drug efficacy.

c) Western blotting, qRT-PCR & immunofluorescence assays used for molecular mechanism analysis.

Results and Discussion. Our data suggest that:

· Deficiency of FBXW7 in CRC cells is associated with maintenance of CRC stemness and drug resistance through EMT-induced activity.

· Intestinal organoids are an interesting and physiologically relevant surrogate system for large- and mid-scale in vitro testing of intestinal epithelium-damaging drugs.

Conclusion. Our findings could be clinically important for understanding the biological nature of CRC cancer initiating cells with FBXW7 mutation(s) owning rapid expansion and therapeutic resistance in CRC. Furthermore, new mini-gut

approaches may have the potential to generate new diagnostic and predictive tests for the stratification of patients and improved disease management.

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No conflict of interest.

423 The c-Met targeting antibody MM-131 reverses HGF-induced tumor cell resistance to standard-of-care drugs

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Introduction. MM-131 is a novel, bispecific antibody designed to potently inhibit the c-Met receptor by co-targeting the widely expressed tumor antigen EpCAM. MM-131 was designed to overcome ligand-dependent and ligand-independent c-Met activation and has been shown to substantially reduce tumor growth in a variety of preclinical models. Here, we examined the ability of hepatocyte growth factor (HGF), the ligand for c-Met, to render cancer cells less sensitive to standard-of-care anti-cancer drugs, and evaluated the effect of combining MM-131 with these drugs in preclinical models.

Material and **Methods.** Using a 3D cell culture system and cell viability as a readout, we assessed how HGF modifies the activity of standard-of-care drugs in a panel of lung, colorectal and gastric cancer cell lines. For each cell line and drug, the effect of adding HGF and MM-131 was calculated by fitting a four-parameter logistic function to each dose-response curve and comparing the curve parameters. The resulting data were used to quantify the effects of HGF and MM-131 and to build a predictive model of MM-131 activity. Model predictions and MM-131-drug combinations were then tested in vitro and in mouse xenograft models.

Results and Discussion. By systematically screening a panel of cancer cell lines, we found that HGF frequently renders cancer cells insensitive to standard-of-care drugs at clinically relevant doses. With few exceptions, neither HGF nor MM-131 substantially affected the EC50 of the anti-cancer drugs. Rather, HGF tended to increase cell proliferation, which was generally reversed by the addition of MM-131. In addition, the rescuing capacity of the ligand was generally more pronounced for cytostatic, as compared to cytotoxic, drugs. Notably, c-Met amplified cell lines were not desensitized to standard-of-care drugs by the addition of HGF, but the addition of MM-131 nevertheless sensitized these cell lines to drug treatment.

Conclusions. In this preclinical study, we found that HGF frequently reduces the sensitivity of cancer cell lines to standard-of-care agents and that the c-Met targeting antibody MM-131 reverses this resistance when HGF is present. In addition, combining MM-131 with standard-of-care drugs in ligand-independent cell lines generally results in greater activity than either drug alone. These findings suggest that MM-131 may provide therapeutic benefit when administered in combination with standard-of-care therapy in cancers in which HGF is present or c-Met is amplified.

No conflict of interest.

424 Activation of MAPK via loss of the tumour suppressor gene NF1 confers resistance to the SRC inhibitor Saracatinib (AZD0530) in epithelial ovarian cancer

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Introduction. Epithelial ovarian cancer is the 5th most common cancer affecting women in the UK, and the most lethal of all the gynaecological cancers. 70% of patients with EOC will be diagnosed with high grade serous ovarian cancer (HGSOC), and the majority of these women will present with late stage disease. Although these women initially respond well the standard of care, which includes debulking surgery and a platinum and taxane base chemotherapy regime, the majority will relapse with platinum refactory disease. SRC tyrosine kinase has been shown to be overexpressed in late stage poor prognosis ovarian tumours, hence SRC inhibitors are currently entering clinical trial in this disease. However, recent results from the SAPPROC trial indicated no benefit of the addition of the SRC inhibitor saracatinib to weekly paclitaxel in platinum resistant ovarian cancer patients. This work aims to identify mechanisms of resistance to SRC inhibitors.

Methods. A tumour suppressor screen siRNA screen was performed in human foreskin fibroblast cells, and used 3 independent siRNAs to silence 178 clinically relevant tumour suppressor genes (TSG) to determine the loss of which TSG confers resistance to saracatinib. NF1 was then silenced using 2 independent siRNAs and by using a short hairpin targeting NF1. A stable SRC knockdown cell line was generated using a short hairpin. A SRC inhibitor resistant cell line was generated by culturing TOV112D cells in the presence of saracatinib for a period of 6 months. Sensitivity to saracatinib (AZD0530), and the MEK inhibitor trametinib (GSK1120212) was determined by 10 day colony formation assay. Levels of NF1 expression, and MAPK activation were determined by western blotting.

Results. The TSG siRNA screen identified the loss of NF1 expression as a potential mechanism of resistance to saracatinib. This finding was validated by silencing NF1 in two independent ovarian cell lines, and testing sensitivity to saracatinib. Following NF1 silencing, there was an increase in resistance of both cell lines to saracatinib. Furthermore, a stable SRC knockdown cell line exhibited loss of NF1 protein and mRNA expression. The stable SRC knockdown cell line exhibited increased activation of MEK and ERK, consistent with loss of NF1 mediated suppression of RAS. Moreover, although these stable SRC knockdown cells exhibited resistance to saracatinib, they were more sensitive to trametinib, indicating a reliance on MAPK signalling. A novel saracatinib resistant ovarian cell line also exhibited activation of MAPK signalling, and importantly, combination of saracatinib with trametinib exerted synergistic effects in this model system.

Conclusion. Activation of MEK signalling via loss of NF1 induces resistance to the SRC inhibitor saracatinib in ovarian cancer cells and so, a combination of MEK and SRC inhibitors may be of benefit in the treatment.

No conflict of interest.

425 Flubendazole as potential anti-neuroblastoma therapy option

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Introduction. Flubendazole was shown to exert anti-leukaemia and anti-myeloma activity through inhibition of microtubule function.

Material and method. Flubendazole was tested for anti-cancer in cancer cell lines and in the chick chorioallantoic membrane assay. Protein levels were determined by Western blot and flow cytometry. RNAi-mediated depletion was used to inhibit gene expression.

Results and discussion. Neuroblastoma was identified as highly flubendazole-sensitive cancer entity in a screen of 321 cell lines from 26 cancer entities. Flubendazole also reduced the viability of five primary neuroblastoma samples in nanomolar concentrations thought to be achievable in humans and inhibited vessel formation and neuroblastoma tumour growth in the chick chorioallantoic membrane assay. Resistance acquisition is a major problem in high-risk neuroblastoma. 119 cell lines from a panel of 140 neuroblastoma cell lines with acquired resistance to various anti-cancer drugs were sensitive to flubendazole in nanomolar concentrations. Tubulin-binding agent-resistant cell lines displayed the highest flubendazole IC50 and IC90 values but differences between drug classes did not reach statistical significance. Flubendazole induced p53-mediated apoptosis. The siRNA-mediated depletion of the p53 targets p21, BAX, or PUMA reduced the neuroblastoma cell sensitivity to flubendazole with PUMA depletion resulting in the most pronounced effects. The MDM2 inhibitor and p53 activator nutlin-3 increased flubendazole efficacy while RNAi-mediated p53-depletion reduced its activity.

Conclusion. Flubendazole represents a potential treatment option for neuroblastoma including therapy-refractory cells.

No conflict of interest.

426 The Resistant Cancer Cell Line (RCCL) collection: Cell lines with acquired drug resistance

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Introduction. The heterogeneity and individuality of cancer diseases is tremendously high. Recent genomic investigations revealed a tremendous genetic complexity in the cells from solid cancer diseases. Cancer cell (sub)populations may differ substantially between primary tumours and metastases as well as within primary tumours. This heterogeneity is a consequence of cancer clonal evolution processes. Among other models, comprehensive cancer cell line collections will be required to address this wide complexity. Resistance acquisition to anti-cancer therapies represents a major obstacle to the development of effective anti-cancer therapies. Major cancer cell drug resistance mechanisms have been discovered in drug-adapted cancer cell lines including the ABC transporters ABCB1 (also known as P-glycoprotein or MDR1) and ABCC1 (also known as MRP1) and clinically relevant resistance mechanisms to so-called 'targeted therapeutics' (e.g. EGFR tyrosine kinase inhibitors, oncogenic BRAF inhibitors, anti-androgens).

Material and method. Initially chemosensitive cancer cell lines are adapted to growth in the presence of clinical concentrations of anti-cancer drugs.

Results and discussion. The Resistant Cancer Cell Line (RCCL) collection consists of >1000 cell lines from 15 different cancer entities with acquired resistance to a broad range of cytotoxic and targeted anti-cancer drugs (www.kent.ac.uk/stms/cmp/RCCL/RCCLabout.html).

Conclusion. The RCCL collection is a readily available tool for the studying of drug-induced cancer cell resistance mechanisms, the investigation of anti-cancer agents, and the examination of drug-induced clonal evolution processes.

No conflict of interest.

427 Targeting the MDM2/MDM4 interaction interface as a promising approach for p53 reactivation therapy

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Introduction. Restoration of the wild-type p53 tumour-suppressor function is as an attractive anti-cancer strategy. Various strategies aimed to release p53 from its key negative regulators, MDM2 and MDM4, have been developed. However, most of these approaches selectively target only one of these molecules, leaving the other free to operate. Although p53 re-activation is observed in many wild-type TP53 cancer cells exposed to these molecules, only few of them die by apoptosis. Since, recent studies indicate that MDM2/MDM4 heterodimerization is required for efficient inactivation of p53 (Pant et al., Huang et al., PNAS 2011; Tollini et al., Cancer Cell 2014), we developed a method that targets the activity of MDM2 and MDM4 simultaneously.

Material and method. Computational and mutagenesis analyses were used for the analysis of the heterodimer binding interface, and the identification of a peptide that binds MDM2 by mimicking the MDM4 C-terminus. Confocal miscroscopy, flow-cytometry, immunoprecipitation and cell fractionation were used to test the peptide localization and acivity. Peptide efficacy was confirmed in vivo by xenograft experiments and the mechanism of action was analysed through whole genome profiling and chromatin immunoprecipitation experiments.

Results and Discussion. We identified a peptide that by competing for MDM4/MDM2 interaction, activates p53. The peptide selectively targets the MDM2/MDM4 nuclear complexes and causes increased chromatin-occupancy by p53. It induces p53-dependent apoptosis in vitro in different tumor cell lines compared to control peptides and reduces tumour growth in vivo. Genome-wide transcriptomic analysis evidenced the activation of p53-apototic targets. Particularly, interfering of MDMs interaction results in specific activation of p53-mediated apoptotic oxidative stress response, a pathway that has received attention as one of the 'Achilles' Heel' of tumor cells (Trachootham et al., Nat Rev Drug Discov, 2009; Green et al., Science 2014), supporting the targeting of this complex as a cancer cell-specific strategy.

Conclusion. These data identify the MDM2/MDM4 interaction interface as a valuable molecular target for therapeutic re-activation of p53 oncosuppressive function. The particular features of this approach (high specificity derived by the peptide sequence and selective activation of a subcellular pool of p53) confers to it increased selectivity and potentially reduced toxicity compared to the current approaches.

No conflict of interest.

428 CCDC6 protein expression is reduced in Non Small Cell Lung Cancers (NSCLC) by post-translational regulation mechanisms: New therapeutic implications

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Introduction. CCDC6 gene rearrangements and point mutations that could cause the functional inactivation of the encoded protein have recently been identified in NSCLC. Moreover, we recently reported that CCDC6 expression levels are reduced in 30% of NSCLC. We hypothesize that CCDC6 protein reduction in NSCLC could be ascribed to post-translational modifications that could affect protein stability.

Material and methods. NSCLC H1975 and H460 and colon cancer HCT116 WT, CCDC6 -/- , FBXW7-/- and USP7 -/- cell lines were utilized for sensitivity test, immunofluorescence and biochemical assays. 62 primary human NSCLC tissue samples, after informed consent, were employed for IHC staining. A statistical analysis was performed.

Results and discussion. CCDC6 protein is regulated in a cell cycle dependent manner. Cdk1 and Gsk3 mitotic kinases phosphorylated CCDC6 on serine and threonine residues, respectively, and direct the recruitment of CCDC6 to the FBXW7 E3 Ubl. Mutation of the phosphoresidues of CCDC6 degron motifs for the FBXW7 E3 Ubl recognition sites affected CCDC6 cell cycle regulated turnover. Moreover, the de-ubiquitinase enzyme USP7 appeared responsible of the fine tuning of the CCDC6 stability. In the analyzed primary samples the low expression of CCDC6 was significantly correlated to the reduced levels of the de-ubiquitinase enzyme USP7. The different level of CCDC6 that we observed in NSCLC might be due to impaired turnover of the protein. In fact in cell lines harbouring low CCDC6 levels we were able to stabilize the protein expression levels by utilizing the proteasomal inhibitor MG132, by silencing FBXW7 by shRNA or by overexpressing an USP7 construct. HCT116 cells knocked down for CCDC6 or USP7 resulted sensitive to PARP inhibitor Olaparib, since CCDC6 defects affected HR repair, as we previously reported. Moreover the combination of cisplatinum and olaparib had synergistic effects on these cells. The reconstitution of CCDC6 in USP7-/- and CCDC6-/- HCT116 cells was sufficient to revert the olaparib sensitivity suggesting that CCDC6 is a critical determinant for the Olaparib response.

Conclusion. As USP7 reduction is related to chemo- and radio- therapy resistance in lung adenocarcinomas, the identification of CCDC6 as a novel USP7 substrate provides the rational for novel personalized therapy in NSCLC patients carrying USP7 deficiency.

No conflict of interest.

429 Cytokine mediated host-tumour interactions lead to treatment resistance

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Introduction. Most treatment resistant cancers have hyper activation of RAS and PI3K pathways. Tumours deficient for the negative regulators of RTK signalling, such as PTEN and Sprouty2 (SPRY2), often fail to respond favourably to therapeutic interventions. These highlight the significance of receptor tyrosine kinase (RTK) signalling in the survival of tumours to cytotoxic anti-cancer treatments. A better mechanistic understanding of these pathways using appropriate in vitro and in vivo models can lead to identification and development of synergistic therapeutic regimens to combat treatment resistance. Using prostate cancer as a model disease, we have identified RTK driven cytokine axis that mediates host-tumour interactions which enable tumours to become treatment resistant.

Materials and Methods. Using an integrated approach involving human genetic analysis, in vitro and in vivo functional studies, and murine cancer genetic modelling we show that sprouty2 deficiency drives development of treatment resistant prostate cancer.

Results and Discussion. Clinically relevant SPRY2 deficiency caused hyper activation of RTK signalling through HER2/EGFR axis. HER2 activation induced p38 stress kinase mediated the expression of IL6 cytokine. IL6 in an autocrine manner increased the key steroid enzymes involved in hormone biosynthesis and in a paracrine manner mobilised the fatty acids and cholesterol, the key substrate for hormone synthesis, from the host adipose tissue to the tumour. The treatment resistant tumours increased the cholesterol and lipid accumulation while the tumour bearing mice showed signs of cachexia with systemic increase in free fatty acids and cholesterol. Abrogation of activated IL6 axis or lowering of systemic cholesterol levels rendered these tumours sensitive to hormone ablation and inhibited cancer cachexia in tumour bearing mice. Thus, the tumours target energy reserves such as adipose tissue to fuel their bioenergetic requirements through RTK induced cytokine axis. Similar understanding of metabolic interactions between host and tumour at a physiological level may aid in development of adjuvant therapies to decrease treatment resistance in aggressive cancers.

No conflict of interest.

430 Investigation of the effects of HMG-CoA reductase inhibitors and mTOR inhibitors alone or as a novel combination in breast cancer cells

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Introduction. Breast cancer is a molecularly heterogeneous disease. Statins e.g. simvastatin, inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme of the mevalonate pathway. Translation of the gene (HMGCR) transcript is regulated by eukaryotic translation initiation factor 4E (eIF4E). Mammalian target of rapamycin (mTOR) inhibitors e.g. rapamycin and metformin, lower the levels of free eIF4E, thus reducing HMG-CoA reductase production.

Aims: To study the influence of HMG-CoA reductase inhibitors and mTOR inhibitors alone, or in combination, on the proliferation of breast cancer cells.

Materials and Methods. MDA-MB-468, MCF7 and Hs578T cells were treated with rapamycin or metformin. The sensitization concentration (Cs) and time-point were determined for each cell line. Sensitization is defined as a cell viability decrease, statically maintained through 3 consecutive higher concentrations.

MDA-MB-468 and MCF7 cells were exposed to various concentrations of simvastatin. Cell viability was measured using MTT assays after 24, 48 and 72 hours, and respective IC50 values were recorded.

Subsequently, MDA-MB-468 cells were treated with 0 and 35ng/mL (Cs) rapamycin and protein was isolated after 24, 48 and 72 hours. Phosphorylation of eurkaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) was measured by Western Blotting.

Results and discussion. In all cell lines, sensitization was achieved with rapamycin but not with metformin. MDA-MB-468 and MCF-7 cells are simvastatin sensitive, with IC50 attained at 6.3μM, 24 hours and 9μM, 48 hours respectively.

Rapamycin (35ng/mL) caused hypophosphorylation of 4E-BP1. Preliminary data from MDA-MB-468 cells treated with a combination of rapamycin and simvastatin have shown increased viability loss compared to simvastatin-treated cells.

Ongoing research: Further analysis of simvastatin treatment and simvastatin/rapamycin combinations on appropriate cell lines, using cell proliferation and protein expression as end points.

Conclusion. MDA-MB-468 (TNBC) and MCF7 (ER+ PR+ HER2-) response to simvastatin

is dose and time dependent. Furthermore, eIF4E-regulated processes may be influenced by sensitization concentrations of rapamycin via a phosphorylation-dependent pathway acting on 4E-BP1. Rapamycin pre-treatment may reduce the simvastatin dose required to attain IC50. The combinatory approach exploits pathways converging on the same target, to identify the lowest drug concentrations providing optimum response.

No conflict of interest.

431 ErbB3 targeting to overcome resistance to tyrosine kinase inhibitors

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Introduction. Lung malignancy is the leading cause of worldwide cancer related death. In recent years personalized medicine has been developed for tumors harboring genetic driver mutations. Targeting the epidermal growth factor receptor (EGFR) has played a central role in advancing NSCLC research, treatment, and patient outcome over the last several years. In lung cancer, 10-15% of NSCLC contain activating mutations in the EGFR kinase conferring hypersensitivity to the oral TKIs gefitinib and erlotinib. Unfortunately, response is not durable and all the patience develop resistance through various mechanisms. Second and third-generation EGFR-TKIs are under development and are designed to have more potent inhibition of EGFR and to be more selective for T790M resistant mutation. Elevated expression of hepatocyte growth factor (HGF), a ligand of the MET receptor tyrosine kinase, or duplication of Met gene are alternative mechanisms of acquired resistance to first generation EGFR-TKIs. ErbB3 has been shown to play a pivotal role in the development of resistance and our group has previously demonstrated that anti-ErbB3 antibodies together with first generation EGFR TKIs synergistically affect NSCLC with T790M acquired resistance in vitro and in vivo. In the present study we have evaluated the activity of anti-Erbb3 antibodies in combination with third-generation EGFR-TKIs in sensitive cell line and in an in vitro model of HGF induced resistance.

Materials and Method. We used HCC827, which are NSCLC cell lines harboring EGFR exon 19 deletion, and expressing MET receptor. Activity of anti-ErbB3 antibodies and EGFR-TKIs (WZ4002), as monotherapy and in combination treatment, was analyzed by cell proliferation assay. ErbB3 phosphorylation and downstream MAPK/ERK 1/2 and PI3K/Akt signaling were analyzed upon HRG and/or HGF induction by western blot of cell extract from samples treated with anti-ErbB3 and WZ4002, alone or in combination.

Results and discussion. HCC827 sensitivity to WZ4002 has been confirmed by cell proliferation assay while anti-ErbB3 monotherapy has no effect on cell proliferation. When dosed in combination, anti-ErbB3 antibody and EGFR TKI act synergistically showincreasing percentage of cell death. Western blot of cell extract from cell treated with the combination show a marked increase in the inhibition of both PI3K/Akt and MAPK/ERK 1/2 transduction pathways respect to cell treated with single agents. The addition of HGF to the culture medium stimulates the EGFR-TKIs resistant mechanism mediated by the MET receptor and this has been confirmed in cell proliferation assays where HGF-treated cells showhigh degree of resistance to WZ4002 up to 10 uM. The addition of 10 ug/ml of anti-ErbB3 antibody is able to partially restore sensitivity to EGFR TKI as showed by cell proliferation assay.

Conclusions. Preliminary data confirmed the ability of anti-ErbB3 antibody to synergize with EGFR TKI to inhibit cell proliferation of already sensitive cell line and to partially restore sensitivity in a model of HGF mediated mechanisms of acquired resistance. Further studies need to be carried out but data suggest that the introduction of anti-ErbB3 antibodies to EGFR-targeted therapies would reduce chances of tumor relapse occurrence.

No conflict of interest.

433 Different responses of human ovarian carcinoma cells to the gene therapy mediated by genetically modified mesenchymal stem cells

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Introduction. Human ovarian carcinoma represents a disease with high mortality. Adipose tissue-derived mesenchymal stem cells (AT-MSC) can serve as vehicles for therapeutic genes. We engineered AT-MSC to express yeast cytosine deaminase::uracil phosphoribosyltransferase (CD::UPRT), capable of converting the prodrug 5-fluorocytosine (5-FC) into highly toxic – 5-fluorouracil (5-FU). Synthesized toxic compound induces apoptosis and together with bystander effect increases the elimination of tumor cells. We compared the cytotoxic effect of CD::UPRT-MSC/5-FC on human ovarian cell lines - chemoresistant SKOV-3 and chemosensitive A2780 in vitro and in vivo.

Material and methods.SKOV-3 and A2780 were cocultured alone or with AT-MSC or CD::UPRT-MSC in the presence of 5-FC. The cytotoxic effect was evaluated using fluorimetric assay. SKOV-3 and A2780 cells were seeded according to CellPlayer™ 96-well Kinetic 3-D Spheroid Protocol (Essen BioScience) with different ratio of CD::UPRT-MSC in the absence/presence of 5-FC. The efficiency of elimination of tumor cells was evaluated by chemoluminescence assay and kinetic assay (IncuCyte ZOOM™ Kinetic Imaging System). Athymic female nude mice were injected with SKOV-3 or A2780 ± CD::UPRT-MSC cells subcutaneously and treated with 5-FC. Subsequently mice were injected with SKOV-3 or A2780 ± CD::UPRT-MSC cells intraperitoneally and treated with 5-FC.

Results and discussion. We showed different response of cytotoxic treatment mediated by CD::UPRT-MSC/5-FC on SKOV-3 and A2780 cells. In the 3-D culture only 40% of SKOV-3 but even 90% of A2780 cells were destroyed after CD::UPRT-MSC/5-FC treatment. Subcutaneous xenografts of SKOV-3 or A2780 cells were eliminated by using CD-MSC/5-FC treatment on nude mice, when therapeutic AT-MSC were co-injected along with SKOV-3 or A2780 cells and the treatment started consequently. The 3-D experiments predicted the effect of treatment on nude mice, when tumor cells were injected intraperitoneally. Residual SKOV-3 cells caused that all mice had to be sacrificed due to moribund state at the same day, regardles of the treatment. The overall survival of animals injected with A2780 is much higher in the treated group in comparison to untreated control.

Conclusion. We performed three types of analysis, which showed different efficiency of CD::UPRT-MSC/5-FC treatment on SKOV-3 and A2780 cells. The best in vivo performance predicts the 3-D model.

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434 Differential effects of acteoside in normal and cancer cells

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Introduction. Malignant transformation is a gradual process fuelled by the deregulation of various cell signaling pathways. Natural products and their extreme structural diversity offer a unique source for the isolation of novel anti-tumor agents. Herein, we comparatively analyzed the differential effects of acteoside in cancer and non-malignant cells.

Materials and Methods. Acteoside was purified from Lippia citriodora; its purity was established by high performance liquid chromatography, nuclear magnetic resonance and mass spectrometry. The effects of the compound were studied in human cancer cell lines; in a cell model of progressive mouse skin cancer, as well as in normal human diploid fibroblasts. Also, acteoside was tested either as a dietary supplement in Drosophila flies or for its in vivo anti-tumor activity in a melanoma (B16.F1) grafted mouse cancer model.

Results and Discussion. Exposure of human cancer cells to acteoside induced dose- and time-dependent cytostatic and cytotoxic effects, which were p53-independent. Moreover, acteoside acted synergistically with different cytotoxic agents, namely, oxidants, chemotherapeutic drugs or proteasome inhibitors, and it also sensitized human cancer cells being resistant to doxorubicin. These effects seemed specific to tumor cells, since treatment of normal human fibroblasts with acteoside induced no apparent cytotoxic effects, and, in fact, moderately enhanced cell growth. Similarly, analyses in a mouse skin cancer model showed that metastatic cancer cells were significantly more sensitive to the compound as compared to pre-malignant cells. Moreover, acteoside significantly suppressed in vivo tumor growth in melanoma-bearing mice, with no evident signs of toxicity in normal tissues. Supportively, dietary administration of acteoside in Drosophila melanogaster flies revealed no toxic effects; it slightly improved healthspan and also protected flies from exogenously imposed oxidative stress. At the molecular level, acteoside enhanced cellular antioxidant responses, affected proteasome peptidase activities and differentially increased oxidative stress in tumor cells. Regarding possible intracellular targets, our preliminary analyses indicate that acteoside, likely, exerts an inhibitory effect on protein kinase C. **Conclusion.**Acteoside holds promise as a natural compound with selective cytotoxic activity against tumor cells.

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No conflict of interest.

435 Synthesis and characterization of polyehtylene glycol coated magnetic nanoparticles and their use for anti-cancer drug delivery

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Introduction. Although conventional chemotherapy is the most valid method to cope with cancer, it has many drawbacks such as decrease in production of blood cells, inflammation of the lining of the digestive tract, hair loss etc. Reasons for its side effects are that drugs used in chemotherapy are distributed evenly within the body of a patient and cannot distinguish the cancer cells from the healthy ones. To decrease the negative effects of chemotherapeutic drugs and to increase their efficiency, many drug delivery systems have been developed until now. Magnetic nanoparticles have an important potential for cancer treatment. The most significant feature of magnetic nanoparticles is that they can be manipulated for targeting to tumor side by application of external magnetic field. Also, their small size and their capability to carry drug by surface modification are other important characteristics for

drug delivery. Objectives of this study are to synthesize magnetic Fe_3O_4 nanoparticles (MNPs), polyethylene coated magnetic nanoparticles (PEG-MNPs), folic acid conjugates- polyethylene coated magnetic nanoparticles (FA-MNPs) and doxorubicin loaded formulation (Dox-FA-MNPs) of FA-MNPs, and to investigate cytotoxicity of FA-MNPs and Dox-FA-MNPs on HeLa cells.

Materials and Methods. Magnetic Fe_3O_4 nanoparticles (MNPs) were synthesized via thermal decomposition. PEG coated MNPs (PEG-MNPs) and FA-MNPs were successfully synthesized and characterized by one or more of TEM, FTIR and TGA analysis. Doxorubicin (Dox) loading capacity of FA-MNPs was investigated by spectrometer. Internalization of FA-MNPs by HeLa cells were observed by Prussian blue staining under light microscopy. Biocompatibility of FA-MNPs and anti-proliferative effects of Dox-FA-MNPs on HeLa cells were analyzed by XTT cell proliferation assay.

Results. The results show that synthesis of MNPs, PEG-MNPs and FA-MNPs were successfully achieved. MNPs had a special shape and small size. Doxorubicin was successfully loaded to 500 µg/mL FA-MNPs. Internalization experiments showed that FA-MNPs were taken up by HeLa cells. FA-MNPs and Dox-FA-MNPs were given to HeLa cells for investigation of their cytotoxicity. Cell proliferation assay results showed that Dox-FA-MNPs significantly decreased the proliferation of HeLa cells when compared to FA-MNPs.

Conclusion. These nanoparticles are suitable for targeted drug delivery and could be used in clinic for further trials.

No conflict of interest.

436 Therapeutic potential of a novel C-terminal inhibitor of Hsp90 encapsulated in liposomes

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Introduction. Breast and prostate cancers are often resistant to standard chemo- and targeted therapies. Inhibition of Hsp90 would be an alternative treatment when combined with some targeted therapies that could improve the response rate. However, some toxicity observed with the N-terminal domain inhibitors reduces the efficacy of this approach. Using C-terminal inhibitors of Hsp90, some of pitfalls would be overcome since the inhibition mechanism is different. Here we report the biological effects obtained after encapsulation in pegylated liposomes of 6BrCaQ (6-bromo-3-[4-methoxyphenylcarboxamide]-quinolein-2-one), a non-water-soluble molecule very effective in cell cycle inhibition, apoptosis induction and migration inhibition on several cancer cell lines.

Methods. 6BrCaQ liposomes were prepared by lipid film hydration and extrusion. Cell proliferation and cell migration capacities of breast and prostate cancer cell lines were evaluated by flow cytometry and videomicroscopy, as was apoptosis. The biological effect was also analysed in real-time with the xCELLigence system by following Cell Index (CI). Western blotting was used to analyse expression of Hsp90 and Hsp70 and degradation of client proteins.

Results and Discussion. Our results demonstrate an improvement of the efficacy of 6BrCaQ against MDA-MB-231 and PC-3 cells by its encapsulation in pegylated liposomes. Liposomes are nanometer-sized and the Hsp90 inhibitor is incorporated into the phospholipid bilayer. A decrease in CI value is observed with liposomal 6BrCaQ whereas liposomes or 6BrCaQ alone are devoid of effect. Liposomal 6BrCaQ induces a G2/M arrest combined with a cleavage of caspase-3 at a lower concentration than free drug. The cytotoxicity of liposomes is confirmed by real-time analysis of confluence. The migratory activity of PC-3 cells (MCP-1-dependent) is blocked by the Hsp90 inhibitor. Concerning chaperone expression, although Hsp70 is weakly induced by liposomal 6BrCaQ, Hsp90 is downregulated. We also have evidence of the specificity of the inhibition of Hsp90 through the downregulation of CDK4, a client protein.

Conclusion. We provide ample evidence that inhibition of the C-terminal domain of Hsp90 induce anti-proliferative, proapoptotic and anti-migratory mechanisms in cancer cells. The cytotoxic activity of 6BrCaQ is improved by increasing its apparent water-solubility through liposomal encapsulation so that we can envisage in vivo studies to evaluate its therapeutic potential.

No conflict of interest.

437 Improving precision of BRAF-targeted therapies to personalize melanoma treatments

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Introduction. For the first time there are treatment options for malignant melanoma - a disease that was essentially considered incurable at the systemic stage. Despite significant survival benefit, drugs against the hallmark melanoma oncogene BRAF fall short of providing sustainable treatment as eventual progression invariably occurs. In contrast, most colon and papillary thyroid patients, and some melanoma patients with BRAF tumors only show marginal benefit from this therapeutic modality, suggesting that modulators of treatment responses exist. Specifically, during the genesis of melanoma the oncogene BRAF(V600*) has recently emerged as a major regulator of altered metabolic homeostasis. To this end, we have recently demonstrated a molecular rationale for the altered metabolism seen in melanoma cells driven by a restricted melanocyte-lineage program involving the melanocyte-lineage factor MITF and the downstream mitochondrial biogenesis co-regulator - PGC1-alpha. Building upon the intersection of the metabolic state and response to BRAF-targeted drugs, we have sought drivers of clinical efficacy.

Materials and Methods. Through metabolic and integrated pharmacogenomic analyses performed using multiple melanoma cell lines and across human cancer cell lineages, show that the effect of BRAF- and MEK-inhibitors directly relate to the inherent metabolic state of the cancerous cells. In addition, using pharmacogenomic analyzes we arrive at defining cues that predicts heightened treatment benefit, which we demonstrate in clinical cohorts can segregate patients who enjoy long-term overall survival from those who have early progression during treatment.

Results and Discussion. Specifically, we have derived a pharmacogenomic approach to identify drivers of BRAF-targeted drug responses in melanomas that extends to personalized prospective stratification in the clinic. We provide data to suggest that heightened sensitivity to this class of precision medicines is inherently linked to the cellular metabolic state, a premise guided by the paradoxical pre-existing clinical resistance to BRAF-targeted agents seen in a fraction of melanomas and most other human tumors.

Conclusions. Optimizing oncogene-targeted precision medicines towards personalized treatments which we provide data to accomplish in melanoma, will in addition to improving patient benefit directly, also help to identify and curb resistance mechanisms in order to extend the usefulness of these drugs towards sustainable treatment.

No conflict of interest.

438 Bispecific CD73 x CD38 monoclonal antibody as a potential tool to overcome adenosine-mediated immunosuppression

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Introduction. Different tumors exploit the ectoenzyme CD73 to produce adenosine (ADO), one of the escape mechanisms from immune defense. ADO was recently reported to be produced not only from ATP, but also from NAD+ by an alternative pathway relying upon CD38 and CD203a/PC-1.

CD73 and CD38 are characterized by a peculiar distribution in normal and pathological tissues and have become independent targets in mAb-mediated therapy.

Aim of this work was the analysis of the possibility that a murine bispecific mAb (bsmAb) may block the enzymatic functions of CD73 and CD38.

Material and method. The bsmAb was generated using the mild reducing agent 2-mercaptoethanesulfonic acid sodium salt (MESNA), followed by dialysis under oxidising conditions in order to allow antibody to reform. CD73 x CD38 bsmAb was selected for of its ability to bind distinct targets to interfere with ADO generation.

Result and discussion. The anti-CD73 mAb identified was obtained from an existing hybridoma, which underwent several rounds of cloning by limiting dilution. The clones were selected for the ability to bind CD73 and simultaneously for the inhibitory effects exerted on the enzymatic activity, blocking ADO generation.

Second step was focused on CD38. None of the mAbs of our panel (7) were able to block the enzymatic functions of CD38. So, we considered the possibility to produce a bsAb with one arm specific for CD73 and the other one for CD38.

The CD73 x CD38 bsmAb (IgG1 x IgG2a) obtained inhibits the ectoenzymatic functions of the CD73 molecule on human B lymphoblastoid lines from healthy donors. After ligation, bsmAb inhibits ADO generation in modalities dependent from antibody concentration.

The functional effects of the bsmAb were also investigated on mixed lymphocyte cultures, taken as representative of alloimmune response. The results obtained show a significant reduction of the proliferation after treatment with the bsmAb.

Conclusion. Different tumors exploit CD73 and CD38 to drive an escape mechanisms from immune defenses: growth and metastasis may be blocked by mAb-specific treatments. The CD73 x CD38 bsmAb can become a powerful drug able to modulate the immune response in tumors and transplantation and a first candidate for humanization.

Ongoing efforts focus on the use of an inhibitory CD73 x CD38 bsmAb to determine whether an antibody-mediated pharmacological inhibition of ADO can be used in therapy to avoid tumor evasion and diffusion.

No conflict of interest.

439 A phase II study of metronomic chemotherapy (MC) with bevacizumab in advanced non-squamous non-small cell lung cancer (NSCLC): A novel strategy to delay drug resistance

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Introduction:Targeting vascular endothelial growth factor(VEGF) has shown modest and transient improvement in advanced NSCLC and other solid tumors. The incorporation of antiangiogenic agents to MC regimens has been shown to further enhance efficacy in preclinical models.The objectives of this pilot study were to determine preliminary clinical efficacy,and explore potential correlations of angiogenic biomarkers with the combination of MC with bevacizumab in advanced NSCLC.Material and methods:Untreated patients(pts) with stage IV non-squamous NSCLC with performance status 0-1 were treated with a 4-week cycle of paclitaxel(80mg/m2-D1,8,15),gemcitabine(G)(200-300mg/m2-D1,8,15) and bevacizumab(10mg/kg-D1,15) for 6 cycles.Patients without progressive disease or significant toxicities received maintenance bevacizumab every 2 weeks.Plasma for multiples angiogenic and antiangiogenic biomarkers(including but not limited VEGF,sVEGFR2,BFGF,PLGF,PDGFa,Angio-1, Angio-2,Thrombospodin-1)were collected at different intervals during therapy.Results and discussion:33 evaluable pts were enrolled(median age,59yrs;female,60%;> 5% weight loss,70%,brain mets,9%;never/light smoking,24% .Efficacy endpoints: ORR-73%(CR-1,PR-23) ,6 pts with SD;median PFS-9 m(95%CI, 7-10);median OS-30m(95%CI,18-37);and 2-year survival-55%. Worst hematologic and non-hematologic toxicities:gr 3 neutropenia(n=1);gr 3/4 nausea/vomiting(N=1);gr 3/4 fatigue(N=2);ischemic colitis(N=1);cerebral ischemia(N=1);gr 3/4 pneumonitis,related to G(N=2); and gr 3/4 proteinuria(N=3).Statistical significant correlations of biomarkers(baseline values Vs efficacy parameters include ORR:-sVEGR2,endocan,IL-8,TGFb-1, Thrombospondin-1,and Angio-1;PFS: Angio-2,and IL-8; and OS: PLGF and PDGFa. Further correlations at different time intervals will be presented. Conclusions:While conclusions are limited by the size of the trial,the results are consistent with the hypothesis that the addition of bevacizumab to MC may result in enhanced anti-angiogenic effect and clinical benefits in advanced NSCLC.Possible biologic explanation of these observations may be related to a delayed acquisition of resistance to VEGF therapy via up regulation of alternative pro-angiogenic pathways.

No conflict of interest.

440 Transcriptional control of pancreatic cancer and small molecule modulators of transcription

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Introduction. Pancreatic cancer (PC) is one of the most deadly forms of human cancer with 5-year survival less than 5%. Presently the nucleoside analog gemcitabine is used for front line disease management but its low response rate and the frequent development of drug resistance, which is poorly understood, limits its effectiveness. A large body of literature indicates that the STAT3, STAT5, c-Myc, HIF-1a and NF-kB pathways are active drivers of pancreatic cancer development and progression.

Material and method. We aimed to reassess the role and function of selected transcription factors in a panel of PC cell lines and assess the activity of WP1066, our small molecule modulator of transcription, and its close congeners. Our reevaluation studies of the oncogenic function of STAT3, STAT5, c-Myc and NF-kB were initially carried out in 3 established pancreatic cancer cell lines (PANC-1, Colo357, and MIAPaCa2) and 2 primary tumor cell lines (MDAPATC53 and MDAPATC50) isolated from patient tumor specimens with known clinical features and available relevant in vivo models. PC cells were separately treated with cytokines (IL-6 and IFN-α), gemcitabine and TNF-α and fractionated into cytoplasmic and nuclear portions. Levels of p-STAT3, p-STAT5, c-Myc, and NF-kB were assessed using Western blot, MesoScaleDiscovery, DNA binding activity, and confocal methods.

Results. Collectively, our data showed that STAT3 was constitutively activated (p-STAT3Y705) is exclusively located in the nucleus of the ColoFG357 and MiaPaCa2 cell lines while c-Myc was present in the nucleus of all tested cell lines. IL-6 and IFN-α treatment induced p-STAT3Y705, and their combined use resulted in synergistically higher levels of p-STAT3Y705 and in nuclear localization in all five PC cell lines. In contrast, and rather surprisingly, the tyrosine-phosphorylated STAT5Y694 and NF-kB were mostly localized in the cytoplasm in all five cell lines. Consistent with these results, the DNA binding activity of p-STAT5 and NF-kB is very low compared to p-STAT3 and c-Myc.

Conclusion. Our findings contribute to unraveling the functional significance of p-STAT3, c-Myc, HIF-1a, and NF-kB as oncogenic transcription factors during pre- and post- gemcitabine treatment and the critical need to concurrently inhibit several targets and develop new class of agents affecting directly and concurrently transcriptional processes.

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441 Chronic myeloid leukemia stem cells are sensitive to the pharmacological inhibition of hypoxia-inducible factor-1a

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Introduction. Chronic myeloid leukaemia (CML) is a clonal haematopoietic stem cell (HSC) disorder due to the 9;22 reciprocal chromosomal translocation, which results in the generation of BCR/abl 'fusion' oncogene and the expression of the BCR/ABL oncoprotein, a constitutively-active tyrosine kinase (TK). Chronic-phase CML patients are treated with TK inhibitors (TKi) such as imatinib- mesylate (IM). However, rather than definitively curing CML, TKi induce a state of minimal residual disease (MRD) due to the persistence of leukaemia stem cells (LSC). We proposed that the expression of BCR/ABL is suppressed in LSC (but not in progenitor cells), so that LSC are independent of BCR/ABL signaling and refractory to TKi. This suppression is facilitated in the hypoxic environment of stem cell niches where haematopoietic stem cells (HSC) as well as LSC reside in bone marrow. Hypoxia-Inducible Factor-1α (HIF-1α) is a key regulator of cell adaptation to hypoxia and of HSC and LSC maintenance. This study aims to address the in vitro and in vivo effects of the pharmacological inhibition of HIF-1α on LSC maintenance.

Materials and Methods. Two CML cell lines and primary CML cells from human or mouse BM were incubated in hypoxia or normoxia and treated with HIF-1α inhibitor (HIFi) or IM, alone or in combination; the maintenance of LSC in culture was evaluated using the culture-repopulation ability (CRA) and colony-formation ability (CFA) assays; shRNA-mediated HIF-1α knockdown was performed to confirm the results obtained with HIFi; the effects of HIFi were also tested in vivo, using a mouse model of CML where the disease was induced by BCR/abl-carrying retrovirus.

Results. HIFi treatment and shRNA-mediated knockdown of cell lines or primary cells incubated in hypoxia led to apoptosis of cell bulk and suppressed CRA. CRA, on the contrary, was not affected by IM treatment. The HIFi/IM combination exhibited a synergistic effect. HIFi reduced CFA of primary cells. HIFi treatment of BCR/abl-transduced mice decreased leukaemia cell number in BM and in particular the LSC-containing population (lineage–/Sca-1+/c-Kit+), without affecting the HSC.

Conclusions. Using two CML cell lines, primary cells and a mouse model of CML, we demonstrated that IM-resistant LSC are instead sensitive to HIFi. On this basis, we propose the HIFi/IM combination as a novel therapeutic approach targeting CML cell bulk as well as LSC in order to, at one time, induce remission and prevent MRD.

No conflict of interest.

442 A role for ABCG2 beyond drug transport: Regulation of autophagy

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Background. ABCG2 (BCRP) is an ABC transporter that was first identified by virtue of its overexpression in a breast cancer cell line selected for resistance to anthracyclines. Since then, its overexpression has been linked to MDR in hematological malignancies as well as colon, esophageal, endometrial, pancreatic and lung tumors. In addition, ABCG2 is highly expressed and plays a protective role in normal stem cells as well as in cancer stem cells (CSCs); interestingly, one study suggests that ABCG2 may be required to maintain 'stemness' and prevent differentiation.

Although the best studied role for ABC drug transporters involves their ability to enhance cell survival by effluxing drug substrates from target cells, a number of studies suggest that ABCG2 can also protect normal, tumor and/or stem cells against apoptosis in the presence of stressors that are not substrates for the transporters. These studies strongly argue for a key role for ABCG2 in cell survival, much broader than its currently established role in drug efflux. However, the mechanism by which ABCG2 promotes survival of cells in the presence of stressors that are not ABCG2 substrates is unknown. We now show that the multidrug transporter, ABCG2, is a regulator of autophagy, and that its expression in tumor cells in vitro enhances autophagy flux, thereby providing a transient 'resistance' to stress inducers.

Methods. Standard cell/ molecular biology methods were utilized: cell culture and transfection, western blot analysis, immunohistochemistry, shRNA.

Results. We have addressed the role of ABCG2 in tumor response to a variety of stressors that are not ABCG2 substrates (nutrient deprivation, ionizing radiation, rapamycin). Using several pairs of cell lines and their ABCG2-overexpressing sublines, we show that cells overexpressing ABCG2 were more resistant to these stressors; this resistance was associated with an elevated level of autophagy flux, as measured by a higher rate of p62 degradation and greater accumulation of LC3-II when compared to parental cells. Knockdown of ABCG2 reduced autophagy flux in resistant cells to a level similar to that observed in parental cells, confirming that the enhanced autophagy was ABCG2-dependent; inhibition of autophagy diminished this survival advantage. Importantly, ABCG2 expression conferred a survival advantage to cells exposed to environmental and therapy-related stressors cells were more resistant to nutrient deprivation and radiation-induced cell death.

Conclusion. Our data indicate that ABCG2 is a regulator of autophagy, and that its expression in tumor cells in vitro enhances autophagy flux, thereby providing a transient 'resistance' to non-substrate stress inducers. If validated in vivo these studies may allow us to 1) better predict those tumors in which autophagy-

targeted therapy could be successful, and 2) identify more therapeutic targets in the autophagy pathway that can be drugged alone or in combination in different tumor milieus

No conflict of interest.

443 PORCN inhibition is effective therapy for Wnt-addicted genetically defined cancers

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Introduction. Wnt signaling is implicated in multiple cancers. Recent identification of genetic mutations in upstream regulators of Wnt sensitivity may define a subset of these cancers that will respond to treatment with upstream Wnt inhibitors. Wnt secretion can be blocked by small molecule inhibition of the membrane bound O-acyl transferase PORCN that is required for the post-translational modification of all Wnts.

Results. We developed an orally bioavailable small molecule PORCN inhibitor, ETC-1922159 (ETC-159) that blocks the palmitoleation and hence the activity of all human Wnts. ETC-159 is highly effective in blocking the growth of multiple human cancers in mice. We find that RNF43 mutations in pancreatic and other cancers and RSPO3 translocations in colorectal cancers, are predictive biomarkers for efficacy of ETC-159. Aberrant Wnt signaling may play a central role in driving proliferation as well as blocking cancer differentiation. We find that inhibition of PORCN leads to a marked remodeling of the transcriptome, with significant decreases in expression of cell cycle, stem cell, and proliferation genes and an increase in expression of differentiation genes.

Conclusion. Inhibition of Wnt signaling by the PORCN inhibitor ETC-159 holds promise as differentiation therapy in genetically defined human cancers.

Conflict of interest: The authors share interest in a patent on ETC-1922159

444 Strategy for synchronous and multiple liver metastases

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Surgical indications for resection of synchronous metastasis from colorectal cancer (CRC) and the optimal timing of hepatectomy are still controversial. Patients: Synchronous and multiple metastatic liver tumors were detected in 57. Our treatment policy has been to perform hepatectomy first, if the resection can be done with no limit on size and number of tumors. However, if curative resection is not, chemotherapy is begun first and timing for the possibility of a radical operation is planned immediately. **Results.** 1) In 37 patients whose tumors were located only in the liver, primary tumor resection was performed first in 16 patients, and after tumor-decreasing by chemotherapy, operation was performed in 7 patients. In 20 patients in whom chemotherapy was performed first, after controlling the distant metastasis, hepatectomy was performed in 3 patients, and staged hepatectomy was performed in 10 patients. 2) Recurrence was detected after hepatectomy in 75.0% of simultaneous resection cases and in 70.0% of staged cases. In the recurrence cases, early detection (within 6 months) after tumor resection occurred in 58.3% of the simultaneous and 14.2% of the staged. 3) No differences in results of pre- and postoperative liver function tests were found between these groups, and duration of hepatectomy and blood loss were also similar. No deaths occurred, and one incidence of bile leakage was detected in each group. 4) Median survival time (MST) and 2-year survival rate were significantly better in the hepatic resection cases than in the non-operated cases. There was no significant difference in MST or 2-year survival rate between simultaneous and staged cases. 5) In 10 staged cases, length of chemotherapy had no effect on pre- or postoperative liver function test results, and survival curves. 6) Repeat operation was performed for recurrence in 75% of the simultaneous and 14.3% of the staged cases. The average time between first and second operation was 13.1±7.7 months, and 2-year survival was 100%. **Conclusion.**Neoadjuvant chemotherapy does not increase the risk of postoperative complications or the surgical difficulties of hepatectomy for colorectal metastases.

No conflict of interest.

447 Biological characterization of nanocomposite hydrogels

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Introduction. The effectiveness of systemically administered antineoplastic or antiangiogenic drugs is limited by difficulties in achieving therapeutic doses within tumors, concurrently to low side effects. At the present, one of the most innovative strategy for targeted drug delivery consists in coupling the drugs to nanomaterials such as nanoparticles (NPs). However, this technique present several problems including the toxicity of NPs per se and the possibility of drug deactivation once it is chemically bound to the NP. To bypass these problems novel hybrid magnetic hydrogels containing magnetic CoFe2O4 or Fe3O4 NPs, as a crosslinker agents of carboxymethylcellulose, have been synthesized and loaded with drugs (Pasqui et al., 2011; Barbucci et al., 2012).

Methods. The viability and functions of various cell types (stromal cells as fibroblasts, or potential target cells as endothelium or tumor cells) were studied in the presence of the hybrid hydrogels, and the in vivo biocompatibility of the hydrogels once subcutaneously implanted in mice was assessed by post mortem tissue histological examination.

Results. Our data document that CMC hydrogel was highly biocompatible, not toxic and once implanted in vivo safe and biodegradable. Conversely, the presence of high amount of NPs combined with CMC affected viability of different types of cells, including stroma, endothelial and tumor cells. Mammary tumor cells were particularly sensible to the toxic effects of high NP concentrations, while endothelial cells acquired an inflammatory phenotype, thus strengthening the scarce biocompatibility of hybrid biomaterials once implanted in vivo

Conclusion. The results here reported demonstrate that CMC hydrogel is highly biocompatible, not toxic and once implanted in vivo safe and biodegradable. Conversely, the presence of high amount of NPs combined with CMC affect viability of different types of cells, including stroma, endothelial and tumor cells.

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Poster Session: Experimental/Molecular Therapeutics, Pharmacogenesis II

448 New insights into drug resistance phenotypes using a novel microdevice for high-throughput in vivo drug sensitivity testing

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We have developed an implantable device that can perform drug sensitivity testing of a large number of anti-cancer drugs inside the living tumor. This device contains reservoirs which release microdoses of single agents or drug combinations into distinct spatial regions of the tumor in local concentrations that are representative of concentrations achieved during systemic drug treatment. The efficacy of each of up to 30 individual drugs or combinations can be assessed from a single specimen without systemic drug exposure through minimally invasive biopsy. This assay takes into consideration physiologic effects that contribute to drug response by allowing drugs to interact with the living tumor in its native microenvironment. Using this technology, we have been able to assess phenotypic drug response for multiple targeted and cytotoxic drug therapies in parallel before, during and after systemic drug treatments to investigate emerging drug resistance at the phenotypic level. The results demonstrate new patterns of escape and adaptive signaling mechanisms, and may offer clues for effective combination therapy.

No conflict of interest.

449 ABCB1 overexpression represents a novel mechanism for acquired resistance to polo-like kinase 1 inhibitors volasertib

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Introduction. The overexpression of the serine/threonine kinase polo-like kinase 1 (Plk1) has been detected in many tumor types. Plk1 has emerged as an important target for cancer treatment due to its involvement in the regulation of mitosis, and the inhibition of Plk1 leads to cell cycle arrest, onset of apoptosis and cell death. Volasertib is a potent inhibitors of Plk1 that is effective against many types of cancer by promoting G2/M cell cycle arrest at nanomolar concentrations. However, the risk of developing drug resistance to volasertib mediated by the overexpression of ATP-binding cassette (ABC) drug transporter ABCB1 can present a significant therapeutic challenge. Here, we investigated the impact of ABCB1 on the cellular efficacy of volasertib.

Material and method. Cytotoxicity assay: CCK-8 and MTT assays were used to determine the general sensitivities of cells to the tested chemicals.

Fluorescent drug accumulation assay: The effect of volasertib and tariquidar on ABCB1-mediated calcein-AM efflux was measured, analyzed and the mean fluorescence intensity was calculated with the histogram stat program in CellQuest software (Becton-Dickinson).

ATPase assay of ABCB1: Vanadate (Vi)-sensitive ABCB1-specific ATPase activities were recorded by using the Pgp-Glo assay system (Promega) according to the manufacturer's instructions. The drug-stimulated ATPase activity of ABCB1 was determined based on endpoint Pi assay.

Immunoblotting: Antibodies C219 (1:1000) and anti-α-tubulin (1:2000) were used to detect ABCB1 and tubulin as positive control for Western blotting, respectively. The secondary antibody used was the Horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000).

Cell cycle analysis: Standard propidium iodide (PI) staining method and analyzed using a FACSort flow cytometer equipped with CellQuest software was used. Cells were treated with indicated regimens for 24 hours before harvested in PBS and fixed in ethanol overnight. Cells were washed once with PBS, then treated with 0.5 % TritonX-100 and 0.05 % RNase in PBS at 37 °C for 1 hr. Cells were washed, propidium iodide added, then incubated at 4 °C for at least 20 min before analysis.

Results and discussion. We revealed that the overexpression of ABCB1 represents a novel mechanism of resistance to volasertib in human cancer cells. We showed that volasertib competitively inhibited the function of ABCB1 and stimulated the ABCB1 ATPase activity without affecting the protein expression of ABCB1. We also showed that the G2/M cell cycle arrest and apoptosis were significantly reduced in ABCB1-overexpressing cancer cells, which can be significantly reversed by inhibiting the function of ABCB1.

Conclusion. In conclusion, in order to achieve a better therapeutic outcome, combination therapy of volasertib with a modulator of ABCB1 should be further investigated as a potential treatment approach for patients.

No conflict of interest.

450 Functional cooperation of MYC and EGR1 elicits apoptosis by controlling NOXA and BIM transcription upon proteasome inhibition by bortezomib

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Deregulated c-MYC (MYC) is well known for driving numerous oncogenic programs. However, depending on the cellular and molecular context, MYC also triggers apoptosis. In this study we observed a signi?cant increase of MYC protein levels upon Bortezomib treatment and found that endogenous MYC is necessary for the induction of apoptosis. This kind of MYC-induced cell death is mediated by enhanced expression of the pro-apoptotic BH3-only members NOXA and BIM. qChIP experiments further revealed binding of MYC to the promoters of NOXA and BIM upon proteasome inhibition, correlating with increased transcription. We demonstrate that recruitment of MYC to low affinity binding sites of the NOXA as well as to the BIM gene promoters depends on MYC's interaction with the zinc ?nger transcription factor EGR1 and an EGR1-specific binding site in both promoters. Furthermore, we provide evidence that in our apoptosis models cell death occurs independently of p53 or ARF. This study uncovers a novel molecular mechanism: For bortezomib-induced cell death the functional cooperation of MYC with EGR1 is required. This observation may be important for novel therapeutic strategies engaging the inherent pro-death function of MYC.

No conflict of interest.

451 Ascochlorin attenuates chemoresistance and regulates the plasticity of doxorubicin induced EMT via modulation of NF-kB pathway in hepatocellular carcinoma

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Introduction. Doxorubicin-based therapy is not effective for the treatment of advanced stage hepatocellular carcinomas (HCCs), which often undergo epithelial–mesenchymal transition (EMT) during tumor progression. Increasing evidence suggests that epithelial cell transformation to mesenchymal phenotype may enhance the ability of stem cells to self-renew and also confer greater resistance to the conventional chemotherapeutic drugs. The purpose of this study was to decipher the molecular mechanisms through which ascochlorin, an isoprenoid antibiotic can chemosensitize HCC cells to doxorubicin therapy.

Material and Methods. The effect of doxorubicin and ascochlorin on HCC cell lines was determined by MTT, western blot, immunofluorescence, PCR and NF-?B DNA binding assay.

Result and discussion. Our results indicate that HCC cells that show a mesenchymal-like phenotype are resistance to the doxorubicin therapy which led to an increased Slug expression. We also observed that activation of NF-?B pathway plays an essential role in doxorubicin induced-chemoresistance and pharmacological inhibition of this pathway with ascochlorin reversed drug-induced invasion/migration and resistance.

Conclusion. Our results suggest that combination treatment of doxorubicin with ascochlorin has the potential to inhibit HCC growth and metastasis. In future work, we aim to further characterize the molecular mechanism.

No conflict of interest.

452 Targeting mesenchymal-like triple negative breast cancers with the combination of FGFR and Src inhibition

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Introduction. Triple negative breast cancer (TNBC) is a highly heterogeneous subgroup of breast cancers with diverse oncogenic dependencies, which makes it challenging to target pharmaceutically. Recently, distinct gene expression subtypes of TNBC have been described, and we have previously demonstrated that mesenchymal TNBC cell lines express autocrine FGF2 ligand and are moderately sensitive to FGFR inhibition in vitro1. Here we examined for therapeutic strategies that would leverage this partial dependence on FGFR signalling to identify effective combination strategies for this subtype of TNBC.

Materials and method. A panel of mesenchymal-like TNBC cell lines (Hs578T, MDA-MB-157, CAL51 and BT549) were treated with the FGFR inhibitor AZD4547, and signalling changes were examined using western blot and phospho-RTK (receptor tyrosine kinase) antibody arrays. In parallel, a drug screen with 80 select targeted therapies at multiple concentrations in 384 well format2 was performed in triplicate to identify novel combination strategies with AZD4547. Results were confirmed in long-term clonogenic assays, and additional TNBC cell lines of various subtypes3 (HCC1143, SUM149PT, CAL120, SUM159PT, MDA-MB-453 and SUM185PE) were analysed for drug combination response.

Results and discussion. RTK phosphorylation changes in response to AZD4547 treatment were found to be highly cell line-specific. Combination drug screen revealed cell line-specific sensitising agents that correlated with known oncogenic dependencies of the cell lines, but suggested that co-targeting of alternative RTKs was not a viable tumour subtype strategy. In the combination drug screens, a common feature was sensitisation to AZD4547 by dasatinib, an inhibitor of Src family kinase. Synergy with dasatinib was confirmed in short-term survival assays, in clonogenic assays, in 3D matrigel culture, and with alternative FGFR and Src family inhibitors. Analysis of additional TNBC cell lines of various subtypes revealed synergistic effect of the drug combination exclusively in cell lines of mesenchymal-like TNBC subtype3. Inhibition of FGFR signalling alone was shown to induce Src phosphorylation, and the combination of dasatinib and AZD4547 demonstrated that drug combinations are more effective in supressing MAP kinase signalling than either drug alone.

Conclusion. FGFR inhibition drives mesenchymal-like TNBC cell dependence on a diverse alternative RTK. The resulting signalling from RTKs is mitigated by combination of FGFR inhibition with dasatinib. Given the immediate need for new therapeutic strategies in treatment of TNBC patients, this novel drug combination may therefore be of clinical benefit in treatment of mesenchymal TNBCs with high levels of autocrine FGF2 expression.

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No conflict of interest.

454 Experimental studies on the selection of effective anticancer drugs in vitro for recurrent breast cancer patients with skeletal metastases

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Introduction. Chemotherapy is an effective treatment for patients with breast cancer. The main goal of chemotherapy - achieving tumor regression by choosing drugs that selectively act on tumor cells, not damaging normal tissue. Therefore, the constant search for new drugs and their combinations is quite understandable.

Materials and Methods. The material of the experimental study were tumor cells obtained by biopsy in breast cancer patients with recurrent disease. The following anticancer drugs were investigated: doxorubicin, cisplatin, methotrexate, fluorouracil, vinorelbine, paclitaxel, cyclophosphamide. Study of the sensitivity of tumor cells to anticancer drugs in vitro was performed according to the protocol: the selection of cells from biopsy material; adding the cell suspension at the concentration of 3-5x104 in alveolus with the nutrient medium onto the 96-alveolar plates containing dilution of chemotherapy; the analyzed chemotherapy drugs were used in concentrations from 6 to 10 and corresponded to the range from 400% to 3%; and then the cells were cultivated for 5 - 7 days at 370 ° C in a humidified atmosphere of 5% CO2. The results were taken into account after the expiry of incubation of the cells with chemotherapy by optical density using a multi-alveolar spectrophotometer.

Results and discussion. When studying the effect of drugs on tumor cells were established the following results: patients with breast cancer metastases in the

bone tumor cells were not sensitive or had low sensitivity (cytotoxic effect only at high doses: 200 - 400%) to the drugs commonly used in chemotherapy schemes: methotrexate and cisplatin. The effect of doxorubicin and paclitaxel has shown average sensitivity of cells (up to 50% of the toxic effect) only at high doses. Cells were highly sensitive to the drug of plant origin - vinorelbine, which is absent in the schemes of chemotherapy of patients with this kind of cancer.

Conclusions. Monitoring of tumor cells sensitivity taken from the clinical material in breast cancer patients with recurrent disease and bone metastases - is necessary, because it allows you to perform the selection of effective anticancer drugs for the further courses of chemotherapy.

No conflict of interest.

455 Overexpression of ABCB1 or ABCG2 in cancer cells reduced the activity of CUDC-101, a multi-targeted inhibitor of HDAC, EGFR and HER2

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Introduction. CUDC-101 is the first small-molecule inhibitor designed to simultaneously inhibit epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2) and histone deacetylase (HDAC) in cancer cells. Recently, in its first in human phase I study, CUDC-101 showed promising single agent activity against advanced solid tumors and favorable pharmacodynamic profile. However, the risk of developing ABC transporter-mediated drug resistance to CUDC-101 can still present a significant therapeutic challenge to clinicians in the future. Here, we have investigated the impact of ABCB1 and ABCG2 on the efficacy of CUDC-101 in human cancer cells.

Material and method. Cytotoxicity assay: CCK-8 and MTT assays were used to determine the general sensitivities of cells to the tested chemicals.

Immunoblotting: Antibodies anti-acetyl-Histone H3, anti-acetylated tubulin, anti-EGFR, anti-phospho-EGFR, anti-HER2, anti-phospho-HER2,C219, BXP-21 and anti-α-tubulin were used to detect class I HDAC, class II HDAC, total EGFR, p-EGFR, total HER2, p-HER2, ABCB1 and ABCG2, respectively. The secondary antibodies used were the Horseradish peroxidase-conjugated goat anti-mouse IgG and anti-rabbit IgG.

Fluorescent drug accumulation assay: ABCB1 and ABCG2-mediated efflux assays were carried out using a FACSort flow cytometer. The effect of CUDC-101, tariquidar or Ko 143 on ABCB1-mediated calcein-AM efflux or ABCG2-mediated efflux of mitoxantrone was calculated with the histogram stat program in CellQuest software (Becton-Dickinson).

Apoptosis assay: For the determination of apoptotic cells, cells were treated with indicated regimens for 48 h before harvested by a series of washing, centrifugation, and resuspended in FACS buffer containing 1.25 mg/mL annexin V–FITC (PharMingen) and 0.1 mg/mL PI and incubated for 15 min at room temperature. The labeled cells were then analyzed by FACScan (BD Biosciences) using the CellQuest software (Becton-Dickinson). Cells in the lower right dot-plot quadrant (PS-positive and PI-negative) were counted as apoptotic and have intact plasma membranes, whereas cells in the upper right dot-plot quadrant (PS-positive and PI-positive) have leaky membranes and can be either necrotic or late apoptotic.

Results and discussion. We showed that CUDC-101 modulated the function of ABCB1 and ABCG2 without affecting their protein expression, and that both ABCB1 and ABCG2 confer significant resistance to CUDC-101 in human cancer cells, reducing the activity of CUDC-101 against HDAC, EGFR and HER2.

Conclusion. In conclusion, this work revealed that although CUDC-101 has potent antiproliferative and proapoptotic activities against most cancer cell lines, the overexpression of ABCB1 or ABCG2 in cancer cells significantly reduced the activity of CUDC-101 against HDAC, EGFR and HER2, as well as its cytotoxicity and proapoptotic activity.

No conflict of interest.

456 Small activating RNAs activate DPYSL3 gene expression in prostate cancer cells

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Introduction. In attempt to explore a novel strategy in suppressing tumor metastasis of prostate cancer, we used small double-strand RNA molecules, termed as small activating RNAs (saRNA) that are complimentary to gene-specific promoter region, to enhance gene transcription. The targeted DPYSL3 gene (Dihydropyrimidinase-like 3, protein name CRMP4) in this study was recently identified as a metastatic suppressor in prostate cancers by our group (PMID: 20543870).

Materials and Methods. We first evaluated the gene expression profiles in multiple prostate cancer cell lines and human prostate cancer specimens. Six common-used human prostate cancer cell lines LAPC-4, LNCaP, C4-2, 22RV1, PC-3 and DU145 plus two benign prostate epithelial cell lines BPH1 and RWPE1 were included in the assessments. Real-time quantitative RT-PCR analysis was utilized for gene expression assessment. Small activating RNAs were chemically synthesized and transfected to cells using RNAiMAX agent. Protein expression was assessed by western blot assays.

Result and discussion. Based on the gene database from UCSD Genome Browser, we noticed two isoforms of DPYSL3 gene that uses distinct promoters, translating into two proteins that differ in N-terminal amino acid sequences of the exon 1 region. These two isoforms are differently expressed in prostate cancer cell lines, of which the isoform-1 is mainly expressed in 22RV1, DU145, LAPC-4 and BPH1 cells whereas the isoform-2 is expressed in C4-2, LNCaP, PC-3, 22RV1 and BPH1 cells. RWPE1 has the lowest level of these two isoforms. Although CRMP4a (DPYSL3 isoform-2) protein expression was seen in all cell lines, CRMP4b (DPYSL3 isoform-1) was only observed in 22RV1 and DU145 cells. CRMP4a protein (DPYSL3-v2) is expressed at a rather low level in BPH1, LAPC4 and LNCaP cells. In human specimens that include individually matched benign compartments and cancer tissues, only isoform-2 (CRMP4a) is detected at a significantly lower level in cancer tissues compared to that in benign compartments, confirming our previous conclusion that CRMP4 expression is down-regulated in prostate cancers. A total of 20 saRNAs were synthesized targeting either isoform-1 or -2, and transiently transfected into prostate cancer cells at 10 nM in the culture media. At day 3 after transfection, our data revealed that isoform-2 mRNA expression and CRMP4a protein expression were strongly enhanced by several saRNAs. In contrast, no significant up-regulation of isoform-1 was noticed in cells tested. These positive saRNAs also largely suppressed the mobility and invasiveness of prostate cancer cells. Further analysis will be focused on testing saRNA-mediated DPYSL3 up-regulation on tumor metastasis in xenograft animal models.

Conclusion. This study demonstrated that small activating RNA is a potent approach to activate tumor suppressor gene as a novel anti-cancer strategy.

No conflict of interest.

458 Apoptotic pathway of zinc on cervical cancer cell line

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Introduction. There is evidence that the mineral zinc is involved in apoptotic cell death of the various carcinoma cells. In this study, we are aim to see whether zinc in the form of CIZAR induce apoptosis on cervical carcinoma cells by increasing intracellular zinc concentration.

Material and method. CaSki, HeLa cervical carcinoma cells and HPV-16 DNA transformed keratinocyte (CRL2404) were treated with different concentrations of CIZAR. Cell viability test and intracellular level of zinc were determined and apoptosis was confirmed by flowcytometry after propidium iodide (PI) staining and fluorescence microscopy undr DAPI staining. Expression of cell cycle regulators were analyzed by Western blot including knock down of p53 and expression of HPV E6 and E7 genes by RT-PCR.

Result and discussion. Intracellular zinc accumulation induced the down-regulation of E6/E7 proteins through targeting for the specific transcriptional factors in the upstream regulatory region. p53 were induced after treatment of CIZAR and p53-dependent apoptosis was not occurred after knock down by p53 siRNA. In cervical carcinoma cells regardless of HPV-infection, CIZAR induces apoptosis by activation of the p53-independent pathways through the up-regulation of p21waf1, the down-regulation of c-Myc, and also decreasing the Bcl-2/ Bax ratio.

Conclusion. CIZAR induces apoptosis not only through the restoration of p53/Rb-dependent pathways on HPV-positive cells but also through the activation of p53/Rb-independent pathways and the mitochondrial death-signal pathway on cervical carcinoma cells regardless of HPV-infection.

No conflict of interest.

459 Blockade of SDF-1/CXCR4 axis is additive/synergistic with radiotherapy and/or temozolomide treatment both in vitro and in vivo in preclinical models of glioblastoma

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Background. Glioblastoma (GBM) is the most malignant and aggressive primary brain tumor with less than 5% 5-year survival of patients. Aggressive standard therapy, radical surgery plus concurrent chemo-radiation treatment provides palliative treatment only.The infiltrative expansion and aggressiveness of GBM is highly correlated with the level of CXCR4 expression, which alsopromotes resistance to chemotherapy and/or radiotherapy in several tumors.

Methods. We determined the CXCR4 expression in 8glioma-derived cell lines (U87, U251, U373, U118, LN229, SW1783, T98G and A172),and the effect of the CXCR4 antagonist Compound B on colony-forming capacity, proliferation and gene expression. The in vivo growth of selected GBM cells in subcutaneous xenografts with or without Compound B treatment, after 5 days treatment with irradiation(3 x 2 Gy) and/or temozolomide (16mg/kg), was examined in tumours derived from glioma cell lines sensitive (U251 and U87) or resistant (T98G) to temozolomide (TMZ), over a period of 50 days.

Results. All cell lines tested expressed CXCR4 and showed increased colony formation in response to CXCL12 stimulation, in a Compound B sensitive manner. Irradiation

of GBM cell cultures enhanced expression of CXCR4 in a dose-dependent manner. Blockade of CXCR4 with Compound Bin vitro reduced the expression of stem cell associated genes and in combination with TMZ abolished neurosphere forming ability in GBM CSCs.After combined treatment of subcutaneous xenografts with irradiation and TMZ, TMZ alone or irradiation alone, subsequent treatment with Compound B significantly ($p<0.001$ for all three cell lines) reduced the number of tumours progressing. After irradiation, Compound B inhibited tumour regrowth by more than 50%, and after TMZ (with or without irradiation) by approximately 90%.

Conclusions. Targeting the CXCL12/CXCR4 axis with Compound B reduced the stem cell expression signature in vitro, and abolished neurosphere forming ability when dosed with TMZ. Compound B significantly delayed tumour regrowth after irradiation and/or TMZ treatment. Anti-tumor activity was associated with significant changes in tumor cell proliferation and apoptosis, and a reduction in the numbers of perivascular cells expressing the stem cell marker nestin.

Conflict of interest: Other substantive relationships: Peter J RICHARDSON, Lee PATIENT and Stephen BURBIDGE are employee of Proximagen Ltd

460 Investigation of the anti-tumor activity of a new synthetic n-tosyl-aza-pterocarpan LQB-223 in breast cancer cells

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Background. Drug resistance is one of the major obstacles for successful treatment in breast cancer patients, thus resulting in relapse and poor survival. It is crucial the identification of novel compounds that might be able to circumvent the mechanisms of resistance in breast cancer and exhibit lower toxicity against normal cells. The n-tosyl-aza-pterocarpan LQB-223 compound has been tested by our group in in vitro models of tumor cells and shown to inhibit cell viability in chronic and acute myeloid leukemia cell lines. The aim of this study was to evaluate the anti-tumor effects of LQB-223 in breast cancer cell lines.

Material and Method. We used the human breast cancer MCF-7 (non-invasive, wild-type p53) and MDA-MB-231 (invasive, mutant p53) cell lines. The MTT, annexin-V/PI and clonogenic assay, morphological analysis, DNA content by flow cytometry, Western blotting and analysis of topoisomerase II alpha activity were performed in this study.

Result and discussion. LQB-223 decreased cell viability in both MCF7 and MDA-MB-231 cell lines, which was followed by changes in cell morphology. Treatment with the compound resulted in decrease in procaspase levels and increase in annexin-V staining, suggesting that it might activate the apoptotic machinary. In addition, LQB-223 inhibited the capacity of colony formation and induced a dramatic G2/M arrest in both cell lines, indicating that it can also impair proliferation. Importantly, the same concentrations of LQB-223 were tested in peripheral blood cells from healthy donors and there was no cytotoxicity, suggesting that the compound presents a selective activity towards cancer cells. The cytotoxic effects of LQB-223 were associated to the induction of p53 and p21, tumour suppressor molecules. Also, we observed downregulation of the expression of FOXM1 oncogenic transcription and inhibition in the activity of the topoisomerase II alpha, which is a downstream target for FOXM1.

Conclusion. The LQB-223 compound can sensitize breast cancer cells and modulate important signalling pathways and point LQB-223 as a promising molecule with anti-tumour activity against breast cancer.

No conflict of interest.

461 Identification of genes involved in triple-negative breast cancer sensitivity and resistance to paclitaxel using an in vivo total genome knockdown screen

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Introduction. Despite advances in therapy, triple-negative breast cancer remains a difficult to treat subtype of breast cancer that affects 10-20% of patients. In addition to surgery and radiation, treatment often involves the use of chemotherapeutics including taxanes such as paclitaxel. Unfortunately, some patients do not respond to this drug. The ability to predict response to treatment prior to administration of paclitaxel would improve treatment efficacy and patient survival. To improve paclitaxel therapy for triple-negative breast cancer patients, we propose to identify the genes which when expressed in a tumor predict sensitivity or resistance to paclitaxel.

Materials and Methods. A genome-wide lentiviral-based shRNA screen was performed with MDA-MB-231 tumor xenografts in female NOD/SCID mice. Groups of six mice received daily intraperitoneal injections of paclitaxel (10 mg/kg) or phosphate buffered saline. Treatment started 24 days post cancer cell injection and continued for eight days. Subsequently, all mice were sacrificed, the tumors harvested, genomic DNA extracted and shRNA sequences retrieved. This allowed the identification of enriched and depleted shRNA sequences that theoretically target paclitaxel sensitivity and resistance genes, respectively.

Results. Completion of 6 replicates of the total genome shRNA screen identified 26 putative paclitaxel sensitivity genes and 14 putative paclitaxel resistance genes for triple-negative breast cancer. Interestingly, some of the genes identified in the screen have been associated with metabolic pathways targeted by paclitaxel. Moreover some of the screen-identified genes have been previously linked to chemotherapy resistance and metastasis in breast cancer and other cancers. However, some of the genes have never been associated with paclitaxel sensitivity or resistance before and potentially represent novel findings.

Conclusions. We have identified several genes with a potential novel role in paclitaxel sensitivity and resistance in triple negative breast cancer. Confirmation experiments will help generate a genetic profile which can be used to identify candidate breast cancer patients who would most benefit from paclitaxel treatment as opposed to treatment with other drugs.

No conflict of interest.

462 The MUC4 oncomucin mediates human pancreatic cancer cell resistance to 5-fluoro-uracil and oxaliplatin chemotherapeutic drugs

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Introduction. Pancreatic adenocarcinoma (PDAC) is one of the most deadly cancers in western countries with an extremely poor prognosis because of a lack of efficient therapeutic tools. Gemcitabine, a fluorinated analog of deoxycytidine, is the main chemotherapy in PDAC, but survival remains poor. FOLFIRINOX, a more aggressive protocol combining 5-fluorouracil (5-FU), irinotecan/SN-38, oxaliplatin and leucovorin, emerged as a new option in patients with metastatic pancreatic cancer. MUC4 is a membrane-bound oncomucin involved in pancreatic cancer (PC) pathogenesis and chemoresistance to gemcitabine. MUC4 is not expressed in healthy pancreas whereas its expression increases constantly during carcinogenetic progression. In this work, we aimed to decipher the involvement of MUC4 and associated cellular mechanisms in chemoresistance to FOLFIRINOX drugs.

Material and methods. MUC4 expression was knocked down (KD) by stable ShRNA in CAPAN-1/-2 PC cells. IC50 and cell viability to FOLFIRINOX drugs were determined by tetrazolium salt (MTT) assays. Expression of genes involved in drug metabolism was assessed by quantitative RT-PCR (qRT-PCR) and Western blot. MiRnomes of Mock and MUC4-KD cells were determined using miRNA array (8x15K Agilent).

Results. MUC4 depletion in PC cells leads to an increased sensitivity to 5-FU and to an increased resistance to oxaliplatin. Combined treatments of 5-FU and oxaliplatin (FOX) lead to an intermediate survival rate suggesting additional effects. This altered chemosensitivity is associated with an altered expression of drug transporters/channels and drug metabolism actors. Notably, an increase of CTR1 and ATP7B transporters and DPYD enzyme was observed in MUC4-KD cells. Furthermore, 9 and 8 miRNAs were respectively differentially up- (i.e. miR-361-3p, miR-584-5p) or down-regulated (i.e. miR-132-3p and miR-96-5p), suggesting a possible link between MUC4 expression, drug resistance and regulation by miRNAs. The involvement of these molecules is currently validated by transient RNA interference and miRNA transfections.

Conclusion. This work led to the identification of proteins and miRNAs specifically involved in priming PC chemoresistance. These factors may represent critical therapeutic targets and also robust prognostic/predictive markers, and thus provide better healthcare and management of PC patients.

No conflict of interest.

466 Organic Cation Transporter-1 (OCT1) dependent and independent mechanisms of sorafenib resistance in human hepatocellular carcinomas

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Introduction. Sorafenib is a FDA approved drug used for the treatment of advanced hepatocellular carcinoma (HCC). This drug inhibits several tyrosine protein kinases therefore blocking the molecular pathway involved in tumor progression and angiogenesis. However, many HCC patients develop resistance to sorafenib. The mechanism of sorafenib resistance is not well understood. Aim: This study was performed to explore whether intrinsic differences in sorafenib sensitivity contributes to the mechanisms of resistance using a panel of HCC cell lines.

Materials and Methods. Multiple HCC cell lines were treated with increasing concentrations of sorafenib. Cellular cytotoxicity and cell proliferation was determined by MTT and cell colony assay. HCC cell lines resistant to sorafenib were isolated and their mechanisms of resistance were investigated.

Results and Discussion. We found that the amount of sorafenib required for reducing 50% cell viability varies significantly among HCC cell lines. We have isolated two HCC cell lines (SR-Huh-7.5, SR-Huh7-Px) that are totally resistant to sorafenib; these two cell lines grow in culture in the presence of sorafenib. The mechanism of resistance in one cell line (SR-Huh-7.5) relates to the impaired sorafenib uptake due to reduced expression of organic cation transporter-1 (OCT1). We showed that overexpression of OCT1 induced cytotoxicity of sorafenib and overcame the resistance. Western blot analysis show that the mechanism of resistance in another HCC cell line (SR-

Huh7-PX) relates to the activation of RAF kinases and PI3K/AKT pathway. **Conclusion.** Our results indicate that there are intrinsic differences among HCC cell clones that affect sorafenib sensitivity. We propose that a detailed understanding of the sorafenib sensitivity and resistance mechanisms should allow for novel treatment options to improve sorafenib chemotherapy response in patients with liver cancer.

No conflict of interest.

467 Metformin inhibits the growth of human osteosarcoma xenografts by inducing cell cycle arrest in Go/G1 phase

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Introduction. Metformin, a biguanide derivative used in the treatment of type II diabetes, is recently drawing attention for its potential antitumor effect. In this study, we evaluated the effect of metformin against human osteosarcoma.

Materials and Methods. Four osteosarcoma cells (KHOS/NP, HOS, MG-63, U2-OS) were treated with metformin. Cell proliferation, cell cycle progression, migration and apoptosis were evaluated using MTT assay, wound-healing and flow cytometric analysis. For in vivo study, 14 female Balb/c-nude mice were grafted into the thigh with 2×106 KHOS/NP cells. When tumors reached a diameter of 50-100 mm, mice were randomized into control (n=7) and metformin-treatment group (n=7). Metformin was dissolved in the drinking water to a final concentration of 2 mg/mL. Mice were allowed to drink the water, and the water was replenished every 4 days. Tumor volume was measured every 3-4 days for a period of 4 weeks.

Results. After treatment with metformin (1.5-2.5 mM), cell cycle was arrested in Go/G1 phase and migration of osteosarcoma cells was interfered. However, metformin did not appear to induce apoptosis of osteosarcoma cells. In mouse xenograft model, metformin significantly inhibited the growth of KHOS/NP tumor. From the 14th day of study entry, tumor volume of the metformin-treated mice was significantly smaller than the control mice ($P < 0.05$).

Conclusion. Metformin showed some efficacy in osteosarcoma cells and mouse xenograft models. Further studies are necessary to explore its therapeutic potential and possibilities of using it as an adjuvant therapy for osteosarcoma.

No conflict of interest.

468 Oncogenic activity of HNF4alpha in gastric cancer: AMPK to WNT signaling in early-stage gastric cancer

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Introduction. Worldwide, gastric cancer is the fourth leading cause of cancer death in the world, having an estimated 989,000 new cases of cancer annually, with 738,000 deaths, and the most common cancer in East Asia. While several risk factors are known (including H. pylori infection, dietary factors, and gastric reflux), development of targeted therapies for this disease has focused on a few known oncogenes and remain largely undeveloped. It is our objective to determine oncogenic mechanisms and novel therapeutic targets specific for gastric cancer by identifying commonly dys-regulated genes from the tumors of both Asian-Pacific and Caucasian patients.

Materials and Methods. We generated transcriptomic profiles of 22 Caucasian gastric cancer tumors and their matched non-cancerous samples, and performed an integrative analysis across different gastric cancer gene expression datasets. We examined the inhibition of commonly overexpressed oncogenes and their constituent signaling pathways by RNAi and/or pharmacologic inhibition.

Results and discussion. We found that HNF4α upregulation was a key signaling event in gastric tumors from both Caucasian and Asian patients, and HNF4α antagonism was antineoplastic. Perturbation experiments in GC tumor cell lines and xenograft models further demonstrated that HNF4α is downregulated by AMPKα signaling and the AMPK agonist metformin; blockade of HNF4α activity resulted in cyclin downregulation, cell cycle arrest, and tumor growth inhibition. HNF4α also regulated WNT signaling through its target gene WNT5A, a potential prognostic marker of diffuse type gastric tumors. Our results indicate that HNF4α is a targetable oncoprotein in gastric cancer, is regulated by AMPK signaling through AMPKα, and resides upstream of WNT signaling. HNF4α may regulate 'metabolic switch' characteristic of a general malignant phenotype and its target WNT5A has potential prognostic values. The AMPKα-HNF4α-WNT5A signaling cascade represents a potentially targetable pathway for drug development.

Conclusion. Development of therapies that specifically target the AMPK/HNF4α/WNT signal cascade would likely represent effective approaches with minimal toxic effects for the management of gastric cancer.

No conflict of interest.

469 A pathway-based approach: Identifying biomarkers of tumor progression to Trastuzumab-resistant breast cancer

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Introduction. Trastuzumab is a successful targeted therapy for breast cancer patients with tumors expressing HER2 (ERBB2). A number of studies have now reported a variety of possible cellular/molecular mechanisms of trastuzumab resistance, largely based on comparisons of sensitive vs. resistant breast cancer cell lines. Of those, one of the most reported contributors to resistance is overactivity of the mitogenic PI3K/Akt pathway. In particular, activating mutations in PI3K and loss of PTEN (a PI3K antagonist) have been identified in both preclinical and clinical studies of trastuzumab-resistant breast cancer. However, it is now known that most patients eventually acquire trastuzumab resistance, of which the mechanism(s) is likely multifactorial.

Methods and materials. Here we identified subpathways differentially expressed between trastuzumab-resistant vs. trastuzumab-sensitive breast cancer cells, in intersection with other preclinical and clinical gene datasets, to rigorously identify overexpressed, resistance-associated genes. Correlation of these upregulated genes with resistance was further validated by qRT-PCR and siRNA knockdown of sensitive (SKBR3) vs. resistant (JIMT-1) breast cancer cells, in addition to in vivo expression analysis of refractory HER2+ patient tumors.

Results and discussion. From this approach, we identified 32 genes reproducibly upregulated in trastuzumab resistance, 24 of which were downregulated in sensitive cells, concomitant with upregulation of all 25 genes in resistant cells (which also downregulated HER2 protein by >80%), in the presence of trastuzumab. Trastuzumab-associated expression patterns of all 25 genes was reversed by siRNA knockdown in both sensitive and resistant cells, and overexpression of 7 of the 25 genes was found in at least one of three refractory breast cancer patient tumors. In summary, our computational approach, followed by experimental validation, significantly expressed ATF4, CHEK2, ENAH, ICOSLG, and RAD51 as potential biomarkers of trastuzumab resistance, relevant to previously hypothesized resistance pathways. These results provide further proof-of-concept of our approach for successfully identifying potential biomarkers and signal pathways involved in tumor progression to drug resistance.

Conclusion. Based on these results, we believe our subpathway and network permutation approach represents a feasible method for identifying biomarker genes and pathways responsible for resistance to trastuzumab and other antineoplastic agents.

No conflict of interest.

470 Cardiac Glycosides induce cell death in drug resistant PTEN deficient liver cancer cells

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Introduction. Hepatocellular carcinoma (HCC) is the second deadly and sixth most common cancer worldwide. The treatment options for HCC are very limited and there is only one FDA approved drug, Sorafenib, for the treatment of aggressive HCC that can only prolong survival up to 3-5 months. Therefore, it is essential to develop new strategies for the treatment of HCC. It has been reported that loss of PTEN, resulting in hyperactivity of PI3K/Act pathway, is observed in various cancers including liver. In this study, we showed that cardiac glycosides induced apoptotic cell death in both PTEN adequate epithelial and deficient mesenchymal liver cancer cells through reactive oxygen species induction resulting in alteration of ERK and Akt pathways

Material and Methods. Cytotoxicity analyses were performed by NCI-SulforhodamineB and Real-time cell electronic sensing assay (ACEA biosystems) on HCC cells. The targeted cellular pathways were investigated by flow cytometry, immunoblot and oxidative stress assays. In vivo xenograft experiments were conducted on nude mice and visualized with Magnetic Resonance Imaging.

Results and Discussion. Cardiac glycosides exhibited high cytotoxic activities in liver cancer cells. Compounds induced apoptosis and G2/M cell cycle arrest. Furthermore, Lanatoside C, treatment resulted in induction of oxidative stress and alteration of ERK and Akt pathways. In both drug resistant Mahlavu and sensitive Huh7 liver cancer cells, Lanatoside C administration resulted in extrinsic apoptotic pathway stimulation through activation of JNK1. On the other hand, in PTEN deficient drug resistant cells, activation of JNK2 inhibited cell survival. Furthermore, in xenografts experiments, Lanatoside C resulted in reduction of tumor size.

Conclusion. Lanatoside C can be envisioned in liver cancer therapeutics due to its ability to induce apoptosis through ERK and Akt pathways by differential activation of JNK1 and JNK2 by GSK3β in PTEN deficient tumors.

No conflict of interest.

Poster Session: Experimental/Molecular Therapeutics, Pharmacogenesis II

472 Proteomic quantification of drug transporters, metabolizing enzymes and cancer target MTH1 in cancer cell lines

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Introduction. Drug transporters (DT) and metabolizing enzymes (DME) play a key role in the delivery of many cancer drugs to the tumor. They influence the intracellular concentration of drugs in the target tissue and thereby the fraction available for intracellular drug targets. Drug targets can also vary in expression level and function in tumor compared to healthy tissue. Therefore it is crucial to quantify levels and functionality of DTs, DMEs, and the drug target of interest to accurately predict target efficacy.

Material and methods. We aimed at quantifying drug target, DTs and DMEs in a set of nine cell lines, with a focus on colorectal cancer. The selected drug target is nucleotide hydrolase MTH1, a new cancer target. The levels of fourteen uptake and efflux drug transporters (ABC and SLC families) and thirteen phase I and II drug metabolizing enzymes (CYP P450 and UGT families) were assessed in whole cell lysates of each cell line. Mass spectrometry-based proteomics was used, with up to fourteen proteins analyzed per run for targeted proteomics and 4h gradients for quantitative label-free global proteomics.

Results and discussion. The global proteomic analysis quantified a total of 9748 proteins, amongst them MTH1, ABCB1 and ABCC3. Other well-recognized DTs and DMEs were not detected due to the short 4h gradient. Cancer cell lines expressed significantly higher levels of MTH1 than non-transformed cell lines (3.2 and 0.1 pmol/mg total protein, respectively).

In targeted proteomic analyses, multidrug resistance related transporters (ABCC2, ABCC3 and SLCO1A2) were detected at high levels, though with variations between cell lines, indicating a possible impact on screening studies. Functionality will be assessed before predictions of drug efficacy can be made. Among DMEs, CYP2C8, 2D6 and 3A5 showed the highest expression, together with UGT1A3.

Conclusions. The global proteomics analysis quantified a large number of proteins, albeit only a limited number of DTs and DMEs were among them. The drug target of interest, MTH1, was detected in all cell lines, with significantly higher levels in cancer cell lines. Longer run times are necessary to detect low abundant proteins such as DTs and DMEs. In contrast, these were easily quantified in targeted proteomic analyses. A varying protein expression pattern was observed, which must be considered when selecting candidate drugs in early drug development. Further studies will be performed on colorectal cancer tissue.

No conflict of interest.

473 The FDA approved antiviral agent cidofovir inhibits metastasis of FGF2-driven, virus-independent tumors

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Introduction. The broad spectrum anti-DNA virus agent cidofovir [(S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine, CDV, Vistide®] has been approved for the treatment of cytomegalovirus-induced retinitis in AIDS patients and is currently being evaluated in phase II/III clinical trials for the treatment of human papillomavirus (HPV)-associated tumors. However, we previously showed that CDV also inhibits the growth of vascular tumors induced by fibroblast growth factor-2 (FGF2)-transformed FGF2-T-MAE cells. Therefore, we wanted to assess the capacity of CDV to inhibit the metastatic growth of virus-independent, FGF2-driven tumors.

Material and Method. The cytostatic/cytotoxic and anti-metastatic activity of CDV was assessed on tumorigenic FGF2-dependent FGF2-T-MAE and B16-F10 melanoma cells. Experimental lung metastases of luciferase-expressing cells was evaluated non-invasively and lungs were analyzed for p53 and Ki67 expression. The anti-metastatic activity of CDV on B16-F10 cells was also assessed in zebrafish embryos.

Results and Discussion. Pre-treatment of luciferase-expressing FGF2-T-MAE cells with 10 µg/ml of CDV reduced single cell survival and anchorage-independent growth in vitro and lung metastasis formation upon intravenous inoculation into SCID mice. Under these experimental conditions, the growth of subconfluent tumor cell cultures in vitro or subcutaneous tumors in mice was not suppressed by CDV. Also, the compound did not affect the homing of FGF2-T-MAE cells to the lungs. Accordingly, CDV protected against lung metastasis when given systemically after tumor cell injection (150 mg/kg, intraperitoneally, once weekly). Notably, CDV prolonged animal survival also when treatment was started 2 days after cell injection. Immunohistochemical staining of lung metastases revealed reduced Ki67 expression and increased nuclear accumulation of p53 in CDV-treated mice, supporting the notion that CDV inhibits metastasis by inhibiting single cell survival properties, possibly by increasing p53 activity. The anti-metastatic potential of CDV could be extended to FGF2-dependent B16-F10 melanoma cells, both in zebrafish and mice.

Conclusion. CDV may have therapeutic potential as an anti-metastatic agent. Further studies are warranted to select those tumor types that are most likely to benefit from CDV therapy.

No conflict of interest.

476 Dual targeting of murine melanoma model; antitumor and antiangiogenic effects of integrin targeted gene therapy

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Introduction. In cancer therapy, not only direct antitumor, but also antiangiogenic strategies are needed for effective antitumor treatment. AMEP (Antiangiogenic MEtargidin Peptide) binds $\alpha5\beta1$ and $\alpha v\beta3$ integrins, overexpressed on melanoma tumors, and thus targeting both therapeutic targets. The aim of the study was to determine the antitumor and antiangiogenic effects of gene therapy with Plasmid AMEP.

Materials and Methods. The effects of intratumoral gene electrotransfer (GET) of Plasmid AMEP were determined in two subtypes of murine melanoma model, B16F1 with low- and B16F10 with high metastatic potential. Our standard protocol for GET was used (8 square wave electric pulses, 600 V/cm, 5 ms, 1 Hz). Antitumor effectiveness was determined by tumor growth delay assay and histological analysis. The expression levels of AMEP mRNA after GET were determined by qPCR. Development of metastases was followed using Perkin Elmer IVIS Spectrum system. Antiangiogenic effect was determined by intravital microscopy, where the formation of tumor vessels was observed in tumors growing in dorsal window chamber.

Results and discussion. The results of the study demonstrated significant antitumor effectiveness after GET of Plasmid AMEP, irrespectively of the tumor model. The growth delays were 9.4±0.8 and 8.7±1.1 in B16F1 and B16F10, respectively. The antitumor effectiveness did not correlate with the expression level of AMEP mRNA in tumors after GET, which was up to 10 fold higher in B16F10 than in B16F1 tumors, but to the quantity of the integrins in the tumors, which was similar in both tumor models. Histological analysis showed high tumor necrosis and reduced tumor cell's proliferation. However, the overall response to gene therapy was more pronounced in B16F10 melanoma, where the proportion of complete responses was higher, most likely due to strong antiangiogenic effect of AMEP. Gene therapy with Plasmid AMEP also has strong antimetastatic potential, resulting in 92% metastases free mice. Furthermore, the antiangiogenic effectiveness demonstrated in dorsal window chamber model showed complete prevention of tumor vessels formation in the treated tumors. Additionally, histological analysis of tumor sections showed reduced number of vessels in treated tumors.

Conclusion. The study demonstrates the effectiveness of local gene therapy with Plasmid AMEP, where the overall effect is a combination of the direct antitumor and antiangiogenic effects of protein AMEP.

No conflict of interest.

477 Down-regulation of PTTG1 contributes to dabrafenib-induced inhibition of melanoma cell proliferation and invasiveness

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Introduction. Pituitary tumor transforming 1 (PTTG1) is an oncogene over-expressed in different tumors, including melanoma. The PTTG1 protein is involved in cell cycle progression, DNA repair, apoptosis, tumor angiogenesis and metastasis. In this study we investigated the role of PTTG1 in melanoma cell response to dabrafenib, a BRAF inhibitor approved for the treatment of unresectable or metastatic melanoma harbouring BRAFV600E.

Materials and Methods. The A375 melanoma cell line, homozygous for BRAFV600E, was cultured with graded concentrations of dabrafenib for 5 days and then assayed for proliferation using the MTT assay. Cell sensitivity to drug treatment was expressed in terms of IC50 (drug concentration producing 50% inhibition of cell growth). A375 cells were also treated with 100 nM dabrafenib for 48 hours and then tested for: a) ability to invade the extracellular matrix (ECM) in vitro, either spontaneously or in response to VEGF-A (50 nM), using Boyden chambers equipped with matrigel-coated filters; b) PTTG1 protein expression by Western blotting. Finally, A375 cells were transiently transfected with 10 nM of a small interfering RNA (siRNA) targeting PTTG1 (siPTTG1) or a nonsilencing negative control siRNA (siCTRL). Twenty-four hours after transfection, the cells were either incubated with dabrafenib and tested for proliferation 5 days later, or treated with 100 nM dabrafenib for 48 hours and then assayed for invasion of ECM.

Results and Discussion. As expected, dabrafenib strongly impaired A375 cell proliferation. The drug also caused a marked reduction of PTTG1 protein levels and significantly inhibited basal and VEGF-A-induced ECM invasion. A375 cells transfected with siPTTG1 (A375/siPTTG1) showed reduced proliferation and invasiveness as compared with the cells transfected with siCTRL (A375/siCTRL). A375/siPTTG1 and A375/siCTRL cells displayed comparable values of dabrafenib IC50 in proliferation assays. Inhibition of ECM invasion caused by PTTG1 silencing alone was lower than

that caused by dabrafenib treatment. Finally, inhibition of ECM invasion detected in dabrafenib-treated A375/siPTTG1 cells was comparable to that detected in dabrafenib-treated A375/siCTRL cells.

Conclusion. Our data suggest that dabrafenib-induced down-regulation of PTTG1 contributes, at least in part, to the effects of the drug on melanoma cell proliferation and invasiveness.

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No conflict of interest.

478 A novel water-soluble nitrobenzoxadiazole is highly effective against vemurafenib-resistant human melanoma cells

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Introduction. We recently designed and synthesized novel analogues of 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX), a potent activator of the MAPK/JNK pathway, which is a major pathway in apoptosis. Among them, MC3181 shows good water solubility, high effectiveness against a panel of human melanoma cell lines in vitro, and remarkable therapeutic activity towards BRAF-V600E-mutated A375 melanoma xenografts after intravenous (i.v.) or oral (per os) administration. Herein we demonstrate that NBDHEX and MC3181 are active against human A375 cells selected for resistance to the BRAF inhibitor vemurafenib.

Material and method. The A375-VR8 cell line was generated by exposing the A375 cells to increasing concentrations of vemurafenib over three months. Drug cytotoxicity was evaluated by both sulphorhodamine B (SRB) and colony formation (CF) assays. Apoptosis was determined by measuring caspase activity and by flow cytometry analysis. The total and phosphorylated levels of JNK1/2, p38, ERK1/2, AKT, CRAF, ATF2, and p53 were evaluated by western blotting. For in vivo studies, A375-VR8 cells were injected i.m. in the hind leg of athymic CD-1 mice. Drug dosage schedules were as follows: MC3181, 8 mg/kg/day i.v. for 3 weeks; temozolomide, 60 mg/kg/day i.p. for 1 week; vemurafenib, 40 mg/kg/day per os for 3-9 weeks.

Results and discussion. A375-VR8 cells were 14- and 31-fold less sensitive to vemurafenib than A375 wild-type cells in SRB and CF assays, respectively. Vemurafenib only partially decreased ERK phosphorylation in A375-VR8 cells, which also showed a significantly higher basal level of phosphorylated AKT, compared to wild-type A375 cells. Conversely, MC3181 and NBDHEX exerted comparable anti-proliferative effects in vemurafenib-sensitive and -resistant cells, being their IC50 values, evaluated by CFA, ~500-fold lower than those of vemurafenib. Moreover, NBDHEX and MC3181 triggered a prolonged activation of different MAPK pathways and induced marked apoptotic effects in A375-VR8 cells. Notably, MC3181 strongly reduced A375-VR8 growth in vivo, whereas vemurafenib was ineffective. Of note, the in vivo therapeutic activity of MC3181 was higher than that of temozolomide, and no signs of toxicity were observed in MC3181-treated mice.

Conclusion. Our data indicate that MC3181 is active towards vemurafenib-resistant A375 cells. Thus, this compound deserves further studies for its therapeutic potential towards BRAF-mutated vemurafenib-resistant tumors.

No conflict of interest.

479 Nitrobenzoxadiazoles exert anti-melanoma activity modulating the MAPK/JNK signaling

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Introduction. Deregulation of MAPK pathways promotes tumor proliferation and metastasis. Therefore, protein kinases' inhibitors are often used in combination with chemotherapeutic agents. However, sustained activation of the MAPK pathway mediated by c-Jun N-terminal kinase (JNK), may trigger phosphorylation of pro-apoptotic proteins and enhance chemotherapy efficacy. In particular, we have shown that 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (NBDHEX) triggers a strong activation of the MAPK/JNK signaling cascade and is highly effective against human melanoma xenografts, when used as a single agent or in combination therapy with temozolomide. Herein, we demonstrate that MC3181, a novel nitrobenzoxadiazole compound, is active against human melanoma sensitive and resistant to the BRAF inhibitor vemurafenib through the modulation of the MAPK/JNK signaling.

Material and method. Interaction of GSTP1-1 with JNK or TNF receptor-associated factor 2 (TRAF2) was evaluated by ELISA. The cytotoxicity of MC3181 or NBDHEX was determined in MALME-3M, G-361, IST-MEL-1, SK23-MEL, A375, and A375-VR8 vemurafenib-resistant cells by an SRB assay. Determination of caspase activity and flow cytometry were used to evaluate drug-induced apoptosis. Proteins' phospho-activation was evaluated by Western blot. For in vivo studies, SK23-MEL, G-361, A375 and A375-VR8 cells were injected s.c. into immunodeficient mice. Drug dosage schedules were as follows: MC3181, 8 mg/kg/day per os or i.v. for 3 weeks; temozolomide, 60 or 100 mg/kg/day i.p. for 1 week; vemurafenib, 40 mg/kg/day per os for 3-9 weeks.

Results and discussion. We recently designed and synthesized various NBDHEX analogues, some of which were endowed with greater water solubility than the parent compound. Among them, MC3181 demonstrated overt improved chemical properties compared to NBDHEX. Like NBDHEX, MC3181 disrupted the protein-protein interactions that inhibit JNK, thus leading to JNK activation and tumor cell death. Both MC3181 and its parent compound showed a broad spectrum of anti-melanoma activity in vitro against both vemurafenib-sensitive, as well as the vemurafenib-resistant A375-VR8 human melanoma cells. Noteworthy, oral or intravenous administration of MC3181 was markedly effective against both vemurafenib-sensitive and -resistant BRAF-V600E-mutant xenografts, at well tolerated doses. Notably, MC3181 was more effective when administered per os than i.v.

Conclusion. There is still an urgent need for novel effective and safe anti-melanoma agents. Our findings support the potential use of the nitrobenzoxadiazole MC3181, a water-soluble MAPK activator, as part of new therapies for melanoma treatment.

No conflict of interest.

480 Therapeutic potential of metformin in refractory osteosarcoma: Metformin inhibits the proliferation of cisplatin-resistant osteosarcoma cells, but does not increase their radiosensitivity

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Introduction. Patients with unresectable, relapsed or refractory osteosarcoma need novel therapeutic agent. In such cases, radiotherapy is administered as a local treatment modality. Metformin is recently drawing attention for its radiosensitizing effect. We aimed to assess its therapeutic potential in this setting.

Materials and Methods. To simulate the clinical setting of refractory osteosarcoma, we established cisplatin-resistant cells by exposing KHOS/NP and U-2OS cells to cisplatin-containing medium (up to 40 μM) and selecting the surviving cells. Combination effects of metformin and radiation were evaluated both in native and cisplatin-resistant cells. Cells were treated 1.25 mM metformin and were exposed to increasing doses of radiation (2, 4 and 8 Gy), and MTT (3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and colony forming assays were performed.

Results. Metformin inhibited the proliferation of both native and cisplatin-resistant KHOS/NP and U-2OS cells. The IC50 values of metformin were higher in cisplatin-resistant cells than in native cells, 4.2-4.5 mM vs. 2.2-2.9 mM. The native KHOS/NP cells were not sensitive to radiation, showing no difference in cell viability according to radiation doses (2, 4, 8 Gy). However, combination of 1.25 mM metformin with 4 Gy radiation decreased cell viability. For cisplatin-resistant KHOS/NP cells, combined treatment with 1.25 mM metformin and 8 Gy radiation slightly decreased the viability. However, combined treatment with metformin and radiation did not appear to influence the cell viability of cisplatin-resistant osteosarcoma cells, when compared to native osteosarcoma cells.

Conclusion. Metformin showed some efficacy in cisplatin-resistant osteosarcoma cells, however, did not appear to enhance the radiosensitivity. Further studies are necessary to explore the possibilities of using metformin as an adjuvant therapy for refractory osteosarcoma.

No conflict of interest.

481 In vitro and in vivo evaluation of electrochemotherapy with trans-platinum analogue trans-[PtCl2(3-Hmpy)2]

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Cisplatin is a chemotherapeutic drug used for treatment of different types of cancer. Side effects that can occur during the treatment and the ability of tumor cells to become resistant to cisplatin, lead to the synthesis and evaluation of new platinum complexes. The aim of our study was to evaluate cytotoxicity in vitro and antitumor effectiveness in vivo of a new platinum complex trans-[PtCl2(3-Hmpy)2] (3-Hmpy = 3-hydroxymethylpyridine) (compound 5) alone or in combination with electroporation (electrochemotherapy, ECT).

In vitro, TBLCl2 ovarian carcinoma sensitive to cisplatin and the resistant subclone TBLCL2/Pt, as well as to cisplatin resistant SA-1 sarcoma cells were used for clonogenic assay. The inhibitory concentrations that reduced survival of cells to 50% (IC50) after treatment with drug (compound 5 or cisplatin) alone or after ECT were determined. Furthermore, the antitumor effectiveness of ECT with compound 5 or cisplatin was evaluated on subcutaneous TBLCl2 ovarian carcinoma and SA-1 sarcoma tumors using tumor growth delay assay. Additionally Pt accumulation and amount of Pt bound to the DNA were determined in SA-1 tumors after ECT using inductively coupled plasma mass spectrometry.

By using electroporation to increase drug uptake into the cells (ECT), improved

cytotoxicity of compound 5 or cisplatin was determined. ECT with compound 5 decreased IC50 value in sensitive ovarian TBLCl2 carcinoma cells for 5-fold, for 2-fold in its resistant subclone and for 6-fold in resistant SA-1 sarcoma cells. Most pronounced effect was observed after ECT with cisplatin in all tested cell lines, up to 16-fold decrease in IC50 value in resistant TBLCl2/Pt cells was determined. The antitumor effectiveness of ECT with compound 5 or cisplatin was also confirmed in vivo in TBLCl2 ovarian and SA-1 sarcoma tumor models. The ECT treatments resulted in prolonged tumor growth delay up to 16 days compared to treatments with drugs only. Similar to in vitro results, growth of subcutaneous tumors after ECT with compound 5 was less delayed compared to ECT with cisplatin. However, the highest improvement of ECT with cisplatin or compound 5 was obtained in TBLCl2 cisplatin sensitive tumor model; the treatment resulted in 67% and 11% of tumor cures respectively. Furthermore, the measurements of Pt uptake in tumors after ECT with compound 5 or cisplatin determined approximately 2-times increased uptake of drug in the tumors compared to the drugs treatment only and this was reflected to the same extent in higher amount of Pt bound on its target of action, to the DNA.

The results of our study demonstrate that ECT increases cytotoxicity of compound 5 in ovarian and sarcoma cells in vitro and antitumor effectiveness in vivo, however, to a lesser extent compared to ECT with cisplatin.

No conflict of interest.

482 Identification of novel functional interactors of BRAFV600E through a genetic screening in yeast

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Introduction. In metastatic melanoma, BRAFV600E-specific inhibitors have been shown to outperform conventional chemotherapeutic drugs. However, they are not immune of limitations, which need to be overcome by using drug cocktails1.

With the ultimate goal of discovering new therapeutic targets that can be coupled with BRAFV600E, our studies are aimed at increasing the knowledge about the pathways in which this protein is involved. To this end, we have decided to take advantage of a genetic screening in the yeast S. Cerevisiae.2 Specifically, we have used a yeast deletion pool (YDP) in order to identify BRAFV600E functional interactors (FI), i.e. those genes that, when deleted, alter the growth of yeast cells that overexpress BRAFV600E.

Material and methods. The YDP consists of a collection of ~4,700 clones deleted in non-essential genes. Each gene is precisely deleted from the start to the stop codon and replaced with a cassette containing the selection marker KanMX4 conferring resistance to G418. The KanMX gene is flanked by two distinct 20-nucleotide sequences that serve as 'molecular bar codes' to uniquely identify each deletion mutant.3

Results and Discussion. BRAFV600E ORF was cloned in the pYES2 plasmid under the control of the galactose (GAL) inducible promoter and its expression in transformed yeast cells was confirmed by western blot. Furthermore, the functionality of human BRAFV600E in yeast cells was checked by performing multiple assays. In particular, we found that hBRAFV600E increases the proliferation of yeast cells in stress conditions, such as hyperosmolarity.

The pYES2-BRAFV600E plasmid was then used to transform the YDP and the individual deleted clones showing an altered growth pattern in GAL-containing medium have been selected for further analyses. In particular, their up and down tags have been sequenced and the missing gene functions have been identified. So far, we have identified more than 20 BRAFV600E FIs. Interestingly, many of them appear to be directly or indirectly related with the Hog1 pathway in yeast and/or the ERK pathway in human, which confirms the validity of our screening approach.

Conclusion. The use of a YDP has allowed us to identify novel BRAFV600E functional interactors that warrant further analyses under many points of view: to establish the molecular nature of their direct or indirect relationship with BRAFV600E; to assess their role in human melanoma cells; to evaluate if they are druggable and suitable for combinatorial therapeutic approaches together with BRAFV600E inhibitors.

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No conflict of interest.

483 The new alkylating histone-deacetylase inhibitor (HDACi) fusion molecule EDO-S101 shows significant MGMT expression independent antitumor activity in preclinical models of human glioblastoma and is synrgistic with radiotherapy

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Background. EDO-S101,a first in class fusion molecule that combines the DNA damaging effect of bendamustine with the pan-histone deacetylase inhibitor (HDACi), vorinostat.

Methods. Eight glioblastoma cell lines and five patient derived glioblastoma stem cell lines were studied in vitro. For in vivo s.c. xenograft experiments,U251 (MGMT negative), U87MG (with partially methylated MGMT gene) and T98G (MGMT-positive) cells were inoculated into the flank of cd1 nu/nu female mice. For experiments with orthotopically growing tumors, luciferase transfected U87MG were injected into the brain of mice. Tumour volumes were measured every two days. Bioluminescence and NRI evaluations were performed weekly to record intra-brain tumor growth. Overall survival was determined.

Results. Bi-functional mechanism of EDO-S101 was confirmed. EDO-S101 induces hyperacetylation of histones, tubulin and p53 that was associated with differentiation of GBM and stem cells demonstrated by increased expression of gliar fibrillary acid protein (GFAP) and N-cadherin, cytoplasmatic translocation of β-catenin, and reduced expression of nestin. These effects translate to cell cycle arrest, inhibition of TORC1 and TORC2 activity and prevent the phosphorylation of both, S6 ribosomal protein and Akt. Cytostatic and pro-apoptotic activity was mediated from CRM1-mediated reduction of survivin and cyclin D1, caspase-3 activation and apoptosis. Antiangiogenic and radiosensitizing effects were also recorded. Human brain endothelial cells showed lowest number of tubules and GBM cells had lowest clonogenic capacity when RT was combined with EDO-S101. In vivo, EDO-S101 (60 mg/kg, day 1, 8, 15,q28 days, iv) effects were compared with RT (5x2Gy q7days), or temozolomide (16 mg/Kg orally on 5 consecutive days q7days), and the combination of both. EDO-S101 significantly increased Time to Tumor progression (TTP) compared with temozolomide, both in MGMT negative and positive cells suggesting that its efficacy was MGMT independent. TTP was significantly longer in EDO-S101 treated animals compared to RT in all cell systems.Similar TTPs were recorded for EDO-S101 single agent compared to the combination of RT + temozolamide in U87MG and U251 cell models, except in the T98G model where EDO-S101 showed a moderately higher TTP. Finally, combination of EDO-S101 with RT showed superior TTPs compared with temozolamide + RT,suggesting better efficacy or higher radio sensitizing effect of EDO-S101 than temozolamide.

In orthotopic U87 model, efficacy of EDO-S101 in terms of overall survival was higher compared to temozolamide and RT alone, but lower than the RT + temozolamide combination. The results suggest that EDO-S101 is effective in treating human glioblastoma and could be an alternative therapy in combination with RT because of the proposed independence from MGMT status. Our data today support to study the activity against glioblastoma in a first in human clinical trial with EDO-S101.

No conflict of interest.

484 PEA-15 protein influences cisplatin resistance by altering ERK localization in ovarian cancer cells

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Introduction. The clinical use of the anti-cancer drug cisplatin is limited due to the rapid development of resistance against this drug. However, the mechanisms of resistance are not completely understood yet. Extracellular signal regulated kinase1/2 (ERK1/2) is activated in response to cisplatin treatment in ovarian cancer cells. Activated ERK1/2 induces the expression of survival genes upon translocation to nucleus (1), which may confer resistance to this drug. This project aims at investigating the influence of PEA-15 protein on cisplatin resistance by altering the ERK nuclear translocation.

Material and method. EFO27 and A2780 ovarian carcinoma cells and their corresponding cisplatin-resistant variants (EFO27rCDDP2000 and A2780cis) were investigated. The cytotoxicity of cisplatin was estimated using the MTT assay. Regulatory sites mutated PEA-15 protein was overexpressed by lipofectamine-mediated transfection. Phosphorylated ERK1/2 translocation to the nucleus was confirmed using the nuclear/cytosolic fractionation kit. Phosphorylation of ERK1 (T202/Y204) and ERK2 (T185/Y187) and transfected protein expression were detected by Western blot.

Results and Discussion. Cytotoxicity of cisplatin was assessed with and without inhibition of ERK1/2 by U0126. Whereas U0126 decreased cisplatin sensitivity in A2780 and A2780cis cells, no significant effect was found of EFO27 and EFO27rCDDP2000

cells. Translocation of ERK1/2 to the nucleus in response to cisplatin was detected in both EFO27 cells and in A2780 but not in A2780cis cells. Further, PEA-15, a small protein which inhibits ERK1/2 translocation to the nucleus was used to investigate the relevance of ERK1/2 cytoplasmic sequestration for cisplatin resistance. A mutated form of PEA-15 was overexpressed in A2780cis and EFO27rCDDP2000 and the cytotoxicity of cisplatin in transfected cells was measured. The pEC50 values obtained were 5.019 ± 0.05, 5.09 ± 0.14, 4.87 ± 0.02 in A2780cis, A2780cis-HA (empty vector), and A2780cis-PEA-15AA (n= 4-6), respectively. The pEC50 values obtained for EFO27rCDDP2000, EFO27rCDDP2000-HA and EFO27rCDDP2000-PEA-15AA were 4.54 ± 0.04, 4.54 ± 0.11, 4.85 ± 0.02, respectively.

Conclusion. Mutated PEA-15 protein increases sensitivity of EFO27rCDDP2000 cells to cisplatin, but decreases cisplatin sensitivity in A2780cis cells. Further ovarian cancer cell lines will be investigated to elucidate the influence of PEA-15 on cisplatin resistance.

No conflict of interest.

485 Tumor heterogeneity in EGFR-mutant non-small-cell lung cancer

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Introduction. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) represent the best therapeutic option for first-line treatment of non-small cell lung cancer (NSCLC) patients with activating EGFR mutations, although response rate varied between 58% and 83% in clinical trials. Recent studies suggested that some EGFR mutant tumors might have intra-tumor heterogeneity as demonstrated by variable proportion of EGFR mutant cells in EGFR mutant lung tumors; different EGFR mutational status between primary tumor and metastases; and presence of clones carrying different EGFR mutations in the same tumor. We performed next generation sequencing (NGS)-based analysis of a large cohort of EGFR mutant NSCLC to assess the level of intra-tumor heterogeneity.

Material and methods. Genomic DNA from EGFR mutant NSCLC as assessed with routine diagnostic methods (N. 129; 93 tissue specimens and 36 cytology samples), was retrospectively analyzed with the Ion AmpliSeq Colon and Lung Cancer Panel using Ion Torrent semiconductor sequencing, with a sensitivity of 2% on hotspot mutations. Mutations with allelic frequency >2% and <5% were confirmed by droplet digital PCR, if material was available.

Results and discussion. Analysis of EGFR mutant samples with NGS revealed the presence of two different hotspot EGFR mutations in 9/129 cases (7%). In 6 cases a sensitizing mutation and the p.T90M resistance mutation were detected; 3 tumors carried two different EGFR activating mutations at different allelic frequency. The correlation between the frequency of the EGFR mutant alleles and the neoplastic cell content was analyzed in 82 cases for which assessment of the fraction of neoplastic cells by two different investigators was possible. Ten cases (12.2%) showed a fraction ≤50% of neoplastic cells carrying an EGFR mutant allele. Finally, 30 samples (23.3%) were found to carry hotspot mutations in genes other than the EGFR, such as KRAS, NRAS, BRAF, ERBB2, PIK3CA or MET, which might cause primary resistance to EGFR targeting drugs. Of these, 19 samples had mutations at an allelic frequency >5%, and 11 at a frequency >2% and <5%.

Conclusion. These preliminary data suggest that a subgroup of EGFR mutant tumors have intra-tumor heterogeneity and are likely to carry tumor clones with different molecular profile. Follow-up data are being collected to assess whether this phenomenon might affect the activity of first -line EGFR TKIs.

No conflict of interest.

486 Selection of a novel berberine derivative with antitumour efficacy on HER2 positive murine breast cancer

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Introduction. Breast cancer (BC) is the most common cause of cancer affecting women. Aberrant up-regulation of Her2 gene is found in 30% of BC and it is associated with worse prognosis.

Berberine is a quaternary alkaloid used in the Chinese medicine, with a broad range of therapeutic effects including activity on mammary tumour and down-regulation of the HER2/PI3K/Akt signaling pathway. We have previously demonstrated that novel berberine derivatives NAX012 and NAX014 show remarkable antiproliferative effects on HER2+ BC cells, by inducing apoptosis and unique ability to modulate Her2/neu expression, differently from currently used drugs for the treatment of HER2+ BC. Aiming at investigating the efficacy of berberine derivatives on the Her2/neu transgenic mice, we tested NAX compounds in terms of: a) definition of the maximum tolerate dose (MTD) and tolerability in mice; b) evaluation of the antitumour and antimetastatic efficacy on Her2/neu murine BC model.

Materials & methods. Drug toxicity and MTD were assessed in healthy mice after repeated (2 times/week/4weeks) intraperitoneal (ip) doses. FVB/N Her2/neu mice were used in vivo experiments to test the antitumour efficacy. NAX014, NAX012 and berberine were tested at 2.5 mg/kg ip 2 times/week/12weeks. NAX014 was further tested by oral administration (po) at 20mg/kg, 2 times/week/8weeks. All administrations started at 18 weeks of age. All mice were checked for detection of tumours twice a week. Lung metastases were counted by using China ink.

Results. NAX014 is the most effective compound, delaying significantly onset and progression of Her2 murine BC with a high number of tumour-free mice at the end of experimentation. NAX014 significantly inhibited tumour growth both after ip and po administrations (71% and 75% inhibition, respectively), and reduced the number of mice with metastases (12.5% and 55.5% in treated vs untreated mice, respectively). In treated mice, size and number of metastases/mouse were also lowered, and tumour vessels were significantly lower than in control mice at the end of experimentation.

Conclusions. NAX014 showed an outstanding antitumour and antimetastatic efficacy on Her2 expressing BC in a rodent transgenic model, and tolerability at the effective doses following oral administration. Based on in vitro data, the compound has a unique mechanism of HER2 modulation which might be related to the down-regulation of the Her2/neu expression.

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No conflict of interest.

487 Studies on the co-expression of growth factor receptors in human breast tumour cells and their responses to treatment with various tyrosine kinase inhibitors

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Introduction. Despite recent advances in both its diagnosis and treatment, breast cancer remains one of the leading causes of mortality worldwide, with acquired resistance being the major cause of treatment failure. It is therefore important to develop a greater understanding of the underlying mechanisms of resistance to treatment with various targeted therapeutics.

Material & method. In this study, we investigated the sensitivity of a panel of breast cancer cell lines to treatment with various small molecule tyrosine kinase inhibitors (including reversible and irreversible HER-family inhibitors, an IGFR inhibitor, and BCR-ABL inhibitors) using the sulforhodamine B colorimetric assay. We also investigated whether there was any correlation between the expression of the target antigens and response to therapy with such agents.

Results & discussion. Of the HER family targeting TKIs, the irreversible HER inhibitors (afatinib, neratinib, and canertinib) were generally more effective than the reversible drugs at inhibiting the growth of these breast cancer cell lines. Afatinib inhibited the growth of breast cancer cells with IC50 values ranging from 0.3 nM (SKBr3) to 0.8 μM (T47D), neratinib IC50 values ranged from 3 nM (SKBr3 and BT474) to 0.9 μM (T47D), and canertinib IC50 values ranged from 3 nM (SKBr3) to 1.4 μM (T47D) respectively. In contrast, gefitinib was less effective and inhibited the growth of breast cancer cells with IC50 values ranging from 0.47μM (BT474) to >10μM (MDA-MB231, MCF-7). MCF7, which had the highest level of IGF-IR among our panel, was the most sensitive breast cancer cell to treatment with the IGFR-inhibitor NVP-AEW (IC50=14 nM). Interestingly, while three of the breast cancer cell lines (MDA-MB231, MDA-MB468, T47D) were highly sensitive to treatment with dasatinib (v-abl/src/C-kit inhibitor), they were relatively resistant to treatment with imatinib. A significant association ($p < 0.05$) was observed between sensitivity to afatinib and co-expression of EGFR and HER-2; and sensitivity to gefitinib and co-expression of EGFR, HER-2 and HER-3.

Conclusions. We conclude the irreversible inhibitors of pan HER family members are superior in inhibiting the growth of breast cancer cells. Our results support the need for further investigation into the therapeutic potential of dasatinib in combination with inhibitors of the HER family members or IGF-IR in breast cancer.

No conflict of interest.

488 Selectively targeting the DNA-binding domain of the androgen receptor as a prospective therapy for prostate cancer

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Introduction. The androgen receptor (AR) is a hormone-activated transcription factor implicated in the development and progression of prostate cancer. The AR contains an N-terminal domain (NTD), followed by DNA binding (DBD) and ligand-binding (LBD) domains. Current therapies for prostate cancer include small molecules, such as Enzalutamide, that compete with androgens for binding to the LBD. Resistance to conventional treatments has been attributed to LBD mutations that render such drugs ineffective, prompting translational research to develop small molecules with a different binding location on the AR and novel mechanism of action. Recently, we

identified a pocket on the AR-DBD protein surface with potential as an alternative drug-target site. Here, we present preclinical data on DBD-specific compounds and their inhibitory effects across multiple prostate cancer cell lines in vitro and in vivo.

Materials and methods. The effect of DBD-specific compounds on AR transcriptional activity, or related nuclear receptors, was assessed by luciferase reporter assay. The effect on expression of AR target-genes was determined by RT-PCR or micro-array analysis in cell-lines producing full-length or splice variant AR forms. Compound interference with DNA binding was assessed by chromatin-immunoprecipitation (ChIP) or with purified DBD protein and subsequent biophysical analysis. In castrated mice, change in volume of tumour xenografts or expression of AR target-genes was determined after compound treatment over 4 weeks.

Results and discussion. The compounds blocked transcriptional activity of full-length and constitutively active splice variant AR forms without cross-reactivity against the closely related GR and PR receptors. Gene expression analysis in LNCaP and other cell-lines reveal the exquisite specificity of the compounds to down-regulate AR target-genes and without any obvious signature associated with toxicity. Biophysical techniques and mutagenesis further clarified the mode and location of compound binding to the AR-DBD, whereas ChIP experiments demonstrated the effect of compounds to prevent interactions with DNA sequences in the enhancer regions of target-genes. Finally, the lead compound suppressed the growth of LNCaP and other tumor xenografts in mice.

Conclusion. The developed compounds may function by interfering with DNA binding by the AR, potentially translating into effective non-cross resistant treatment of castration-resistant prostate cancers.

No conflict of interest.

489 TERT inhibition leads to cell cycle alterations and increases the apoptotic effects of chemotherapeutic agents in EBV-immortalized B lymphocytes

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Introduction. Besides its canonical role in stabilizing telomeres, Telomerase Reverse Transcriptase (TERT) may promote tumorigenesis through extra-telomeric functions. Lymphoblastoid cell lines (LCLs), generated by EBV infection of B lymphocytes, are a useful in vitro model to investigate post-transplant lymphoproliferative disorders and B-cell lymphomas. BIBR1532, a powerful TERT inhibitor, has been evaluated in several cell lines, but no data are available in EBV-driven B-cell malignancies. Our aim was to investigate the effects of BIBR1532 on LCLs alone or in combination with Fludarabine (FLU) or Cyclophosphamide (CY), chemotherapeutic agents employed in the treatment of lymphoproliferative disorders and B-cell lymphomas.

Material and method. We employed LCLs at early and late passages of culture after EBV infection, with low and high levels of TERT expression respectively. Cells expressing ectopic TERT were obtained by infecting TERT-negative LCL with a retroviral vector expressing TERT. TERT levels and activity were assayed by real-time PCR and TRAP assay. Apoptosis and cell cycle profiles were evaluated by flow cytometry and analysis software. Proteins of the cell cycle S phase were studied by western blot. Additional experiments were conducted in vivo on zebrafish.

Results and discussion. BIBR1532 selectively inhibited telomerase activity in TERT-positive LCLs. TERT inhibition led to a decrease (57% ± 3%) of cell proliferation and an increase (23% ± 2%) of apoptosis, compared to untreated cells. In addition, BIBR1532 modified the cell cycle profile with an accumulation of cells in the S phase. Experiments on zebrafish confirmed the results obtained in vitro; BIBR1532 led to accumulation of cells in the S phase and higher apoptosis (227% ± 3%) compared to untreated embryos. TERT-positive LCLs treated with BIBR1532+FLU or BIBR1532+CY showed important alterations of the cell cycle and a significant increase of apoptotic cells, compared to cell treated with chemotherapeutic agents alone (34% vs 15% p=0.0051 and 68% vs 46% p=0.0062, respectively).

Conclusion. TERT inhibition affects the progression of the cell cycle and increases cell susceptibility to FLU and CY. The results suggest new therapeutic applications of TERT inhibitors in EBV-related malignancies.

No conflict of interest.

490 Ranolazine inhibits NaV1.5-mediated breast cancer cell invasiveness and lung colonisation

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Introduction. NaV1.5 voltage-gated sodium channels are abnormally expressed in breast tumours and their expression level is associated with metastatic occurrence and patients' death. In breast cancer cells, NaV1.5 activity promotes the activity of Na+/H+ exchanger type 1 (NHE1) leading to an increase in the efflux of protons. This potentiates the proteolytic degradation of the extracellular matrix by acidic cathepsin proteases and enhances cancer cell invasiveness. Ranolazine is an antiarrhytmic drug characterized for selective inhibition of late sodium current. The aim of this study was to investigate how NaV1.5 expression in human breast cancer cells affect metastatic colonisation of organs in immunodepressed mice and whether its pharmacological inhibition by ranolazine reduced cancer cell invasiveness both in vitro and in vivo.

Materials and methods. Effect of Ranolazine (50 µM) on NaV1.5 current was assessed using whole-cell patch clamp technique. Invasion experiments were performed using MatrigelTM-coated filters. MDA-MB-231 breast cancer cells expressing luciferase were transduced with shCTL or shNaV1.5 and injected in tail vein of NMRI nude mice to assess organ colonisation. Mice injected with shCTL cells were subdivided in two groups treated or not with ranolazine (50 mg/kg/day) and bioluminescence data were collected each week during 8 weeks.

Results. In this study, we showed that the extinction of NaV1.5 expression in human breast cancer cells almost completely abrogated lung colonisation in immunodepressed mice. Furthermore, we demonstrated that ranolazine inhibited NaV1.5 currents in breast cancer cells and reduced NaV1.5-related cancer cell invasiveness in vitro. In vivo, the injection of ranolazine significantly reduced lung colonisation by NaV1.5-expressing human breast cancer cells.

Conclusion. Taken together, our results demonstrate the importance of NaV1.5 in the metastatic colonisation of organs by breast cancer cells and indicate that small molecules interfering with NaV activity, such as ranolazine, may represent powerful pharmacological tools to inhibit metastatic development and improve cancer treatments.

No conflict of interest.

491 Improvement of specificity and safety of skin gene electrotransfer for plasmid DNA under the control of tissue-specific promoter

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Introduction. In order to ensure safe, efficient and controlled gene delivery to skin the improvement of delivery methods together with proper design of DNA is required. Non-viral delivery methods, such as gene electrotransfer, and the design of tissue-specific promoters are promising tools to improve the delivery of therapeutic genes for vaccination and to ensure the safety of anticancer skin gene therapy. In the scope of our study, a novel skin-specific plasmid DNA with collagen (COL) promoter was constructed. Its specificity and efficiency were evaluated in vitro as well as in vivo after gene electrotransfer in the mouse skin.

Materials and Methods. The plasmid DNA with skin specific promoter COL was constructed by standard molecular cloning methods of restriction and ligation. Its cell specificity and efficiency were first evaluated in vitro, using GET do deliver the plasmids to fibroblasts and endothelial cell lines. To determine the gene expression level under the control of tissue specific promoter, flow cytometry and ELISA assay were performed. Furthermore, in vivo by gene electrotransfer followed after intradermal injection of plasmid DNA to the mouse skin, the specificity and the level and duration of its expression was determined by fluorescent stereomicroscopy and quantified by ELISA assay.

Results and discussion. In vitro, the plasmid with tissue-specific promoter was predominantly expressed in fibroblasts, which are prone to activate the collagen promoters. In vivo, the plasmid with COL promoter was efficiently transfected to mouse skin. Skin gene electrotransfer proved to be effective for both the plasmid DNA with ubiquitous and tissue-specific promoters, for the reporter gene and also therapeutic IL-12 gene. It resulted in notable, localized and controlled manner, however in lower and shorter expression compared to that obtained with ubiquitous promoter. The lower expression of the plasmid DNA with the tissue-specific promoter was localized to the skin with paracrine action and no systemic shedding of IL-12.

Conclusion. In conclusion, we obtained high skin specificity of plasmid with collagen promoter, both in vitro and in vivo. Furthermore, this study indicates on more controlled and localized expression of the plasmid with tissue-specific promoter. Such controlled transfection is important for safe and effective translation of cutaneous gene therapy, anticancer gene therapy and vaccination into the clinic.

No conflict of interest.

492 Anti-cancer drug discovery based on modulation of alternative splicing

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Introduction. Alternative splicing (AS) is one of the main determinants of the diversity of proteins, with more than 90% of multi-exon genes being estimated to be alternatively spliced in humans. AS is de-regulated in many disease processes and there are many splicing isoforms described to be associated with cancer progression. Understanding the de-regulation of splicing in cancer and finding solutions to reverse it may pave the way to novel anti-cancer therapeutics based on splicing. The main goal of this work is to define key regulators (splicing factors, splice factors kinases, signaling pathways) that influence AS in each (or several) of the 'hallmarks of cancer' areas [see review Oltean S and Bates DO (2014) Oncogene].

Materials and Methods. We have focused so far on the study of AS events associated with two cancer hallmarks: angiogenesis and invasion-metastasis. Specific splicing

events may be studied using splicing-sensitive fluorescent reporters (SSFRs) through which splicing outcome is monitored by the fluorescence output both in vitro and in vivo. We have constructed two bichromatic SSFR that mimic FGFR2 exon IIIc inclusion/exclusion (associated with epithelial-mesenchymal transitions) and VEGF terminal exon splicing (associated with pro- and antiangiogenic VEGF activity) which are being used as sensors for splicing regulation. Stable cell lines expressing the SSFRs have been created and used in pilot screens. The alternative splicing pattern of the reporter was evaluated using flow cytometry and/or a plate reader. Reporter expressing cells were treated with growth factors and various small molecule inhibitors in both low- and high-throughput screens.

Results and discussion. We have found several molecules that affect splicing in our screens. In vitro and in vivo assays confirmed that several of them are able to display anti-angiogenic effects or induce mesenchymal-epithelial transitions.

Conclusion. Targeting alternative splicing is a novel, underexplored and feasible strategy for development of anticancer drugs.

Conflict of interest

Advisory board: Exonate, Ltd

493 Development of dual-targeted inhibitors for the treatment of cancer

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Introduction. Sphingosine Kinase 1 (SphK1) is an oncogenic lipid kinase linked to the development/progression of multiple types of cancer. With this in mind, we have initiated a project with the goal of developing selective SphK1 inhibitors (SKIs) as effective anti-cancer therapeutic agents.

Material and Method. By employing a small molecule library screening approach, we identified a lead compound, SKI-178, as a selective inhibitor of SphK1 that is cytotoxic (apoptotic) to a broad range of cancer cells, including multi-drug resistant (MDR) types. Subsequent to the development of SKI-178, other SphK1-selective inhibitors with nanomolar potency in vitro have been reported, and yet they fail to induce apoptosis in cancer cells. To better understand why SKI-178 is cytotoxic while others are not, we investigated the apoptotic mechanism-of-action (MOA) by which SKI-178 induced apoptosis in multiple cancer cell types.

Results and Discussion. Detailed analysis revealed that SKI-178 is a dual-targeted inhibitor (renamed DTI-1) blocking both 1) SphK1 activity and 2) microtubule polymerization. Mechanistically, our evidence showed that DTI-1 induce prolonged-mitosis follow by apoptotic cell death through the intrinsic apoptotic pathway. Further examination of the MOA implicated the sustained activity of cyclin-dependent protein kinase 1 (CDK1) as a critical factor required for DTI-1-induced cancer cell apoptosis. We also determined that DTI-1 is not a substrate of the drug efflux pump MDR-1 (P-glycoprotein). Importantly, when we examined the effectiveness of DTI-1 in a very aggressive MLL-AF9 mouse model of acute myeloid leukemia (AML), we observed that DTI-1 treatment completely protected MLL-AF9 AML mice whereas vehicle treated mice died within 2 weeks. Remarkably, the DTI-1 treated mice had no traces of MLL/AF9 AML cells in their bone marrow or spleen suggesting the attainment of complete remission. Consistent with these findings, we have determined that SphK1 is required for development/progression of MLL/AF9 AML.

Conclusion. Current literature and our findings indicate that the combinatory targeting of sphingolipid metabolism and microtubule dynamics could represent a novel therapeutic strategy for the treatment of a broad range of cancers. Given the established synergism between these two pathways, we believe that DTI-1, as a single dual-targeted agent, can be translated to clinical development for the treatment of cancers, including MDR types.

No conflict of interest.

494 Cellular ascorbate regulates DNA damage responses and cytotoxicity of bleomycin

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Introduction. Bleomycin (BL) is a long used cancer drug that exerts its antineoplastic activity via oxidative mechanisms. Many BL-treated patients also experience significant side effects, which are particularly severe in the lung. It is not well understood what metabolic or dietary factors impact chemotherapeutic effectiveness of BL and the development of its life-threatening lung toxicity.

Methods. BL-chelated iron is responsible for the oxidation-mediated generation of the primary cytotoxic lesions, which are DNA breaks. Ascorbate is known to promote redox cycling of iron by reducing Fe(III) to the radical-producing Fe(II). Standard cultures of human cells are severely deficient in vitamin C, raising concerns that in vitro models provide inaccurate guidance for animal and clinical testing. Here, we examined the impact of cellular ascorbate on cell death, DNA breakage and DNA damage signaling by BL.

Results and Discussion. We found that cellular ascorbate increased cytotoxicity and clonogenic lethality of BL in human lung carcinoma cells. Cellular ascorbate altered the production of DNA double-strand breaks by BL and activation of the major breaks-responsive signaling and repair pathways. High- and low-ascorbate cells also showed a different sensitivity to inhibition of DNA repair processes.

Conclusions. Our results indicate that cellular ascorbate increases sensitivity of cancer cells to killing by BL and alters their dependence on specific survival mechanisms. Low ascorbate levels in hypoxic tumors can be a significant factor in their resistance to BL-based therapy.

No conflict of interest.

495 Nanoparticulated docetaxel exerts enhanced anti-cancer efficacy and overcomes existing limitations of commercial products

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Introduction. Nanoparticulation of insoluble drugs improves the dissolution rate, resulting in increased bioavailability that brings increased stability, better efficacy, and reduced toxicity of drugs. Docetaxel (DTX), under the trade name TaxotereTM, is one of the representative anti-cancer chemotherapeutic of this era. However, this highly lipophilic and insoluble drug brings lots of adverse effects. Our novel and widely-applicable NUFSTM (Nanoparticulation Using Fat and Supercritical fluid) technology allowed for the successful nano-scale particulation of docetaxel (Nufs-DTX).

Materials and Methods. The physical stability of the Nufs-DTX dispersion was confirmed in water. The prepared Nufs-DTX dispersion was kept at room temperature until it was used in other experiments. The Z-average particle size and poly-dispersity index were determined by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments, UK). The cytotoxicity was evaluated with tubulin or ki67 staining and clonogenic assay in A549 cell culture. In vivo biodistribution and tumor growth delay was accessed in A549 xenograft mouse model and hematological and physical toxicity assays were performed.

Results and Discussion. Nufs-DTX was formulated with reduced ratio of composition of excipient (docetaxel : excipient = 1 : 0.95) compared to TaxotereTM. Nufs-DTX showed enhanced solubility and increased aqueous stability. After confirming the preserved mechanism of action of docetaxel, Nufs-DTX showed similar effects in proliferation and clonogenic assays as TaxotereTM. Nufs-DTX had a greater effect on in vivo tumor growth delay, in parallel with improved tumor accumulation in the biodistribution result. Although both showed no toxicity effect in hematologic toxicity under our administration dose, Nufs-DTX showed much less toxicity than TaxotereTM in edema, paralysis, and paw withdrawal latency on a hot plate that are regarded as fluid retention, peripheral neuropathy, and thermal threshold indicators as toxicological tests.

Conclusions. Compared to TaxotereTM, Nufs-DTX that was generated by our new platform technology using lipid and supercritical fluid, carbon dioxide (CO_2), maintained its biochemical properties as a cytotoxic agent and had better tumor targeting distribution, better in vivo therapeutic effect and less toxicity, thereby overcoming the current hurdles of commercial drugs.

No conflict of interest.

496 CD46 is overexpressed in colorectal cancers and mediates enhanced tumor transduction efficacy of Ad5/35 chimeric adenovirus

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Introduction. The CD46 is a complement inhibitor membrane cofactor protein and also acts as a receptor for certain pathogenic microbes, including group B adenovirus (Ad). Whereas many Ads infect cells through coxsackie-adenovirus receptor (CAR), CAR expression is downregulated in many cancers preventing effective therapeutics of Ad-based therapies. Hence group B Ad is an attractive gene therapy vectors since they can overcome limitations in tumor transduction efficacy through utilizing more ubiquitously expressed CD46.

Materials and Methods. Immunohistochemical staining was performed in 154 selected colorectal tumors with adjacent normal tissues provided from Chonnam National University Hospital in Korea. The CD46 expression in cells was verified by Western blot and/or FACScan analyses. To facilitate Ad infection study, chimeric Ad5/35-GFP, encoding the Ad5 capsid and the Ad35 (group B Ad) fiber knob domain, was applied to the cells followed by FACS. BHK baby hamster kidney cells were stably transfected with either CAR or CD46 to verify transduction efficacy of Ad5/35-GFP and therapeutic efficacy of Ad5/35-TK/GCV suicide therapy in vitro and in vivo.

Results. CD46 is highly overexpressed in most colorectal tumors compared to normal matching tissues. The level of endogenous CD46 expression was also high but variable in most colorectal cancer cells. Using Ad5/35-GFP, CD46 expression was positively correlated with GFP fluorescence in all colorectal cancer cells. Compared to mock-BHK and CAR-overexpressing BHK cells, CD46-ovrexpressing BHK cells showed significantly higher response not only to Ad5/35-GFP but to Ad5/35-TK/GCV suicide therapy. Furthermore, injection of Ad5/35-TK/GCV caused higher anti-cancer effects in CD46-BHK bearing mice compared to CAR-BHK bearing mice.

Conclusion. Our study demonstrated that CD46 is generally overexpressed in colorectal cancers in which group B-based adenoviral gene therapy is better suited than other adenoviral therapies.

No conflict of interest.

497 De novo expression of integrin alpha v beta 3 in Cal27 cells results in pSrc (Y418) inhibition and resistance to antitumor drugs

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Introduction. Integrins are cell-surface adhesion molecules that connect cells to components of the extracellular matrix. They play key roles in the regulation of tumor cell adhesion, migration, invasion and survival to antitumor drugs. In the present study we investigate the susceptibility of tongue squamous carcinoma cells Cal27 with de novo expression of integrin αvβ3 to antitumor drugs.

Materials and Methods. Mock transfected cell clone Cal27-Φ or cell clones Cal27-2B1 and Cal27-2B3 were obtained by stable transfection of Cal27 cells with empty plasmid pcDNA3 or a plasmid containing integrin β3 subunit gene, respectively. The sensitivity of cells to antitumor drugs was determined using MTT assay. Flow cytometry analysis was used to determine the expression of integrins αvβ3 and αvβ5 on the cell surface. Knockdown of integrin subunits αv and β5, Src and ILK was performed by transfection of specific siRNAs. The activity of Src kinase (pSrc(Y418)) was inhibited using dasatinib. The expressions of Src/pSrc(Y418) and ILK were measured by Western blot.

Results and Discussion. Cell clones Cal27-2B1 and Cal27-2B3 as compared to parental cell line Cal27 and control clone Cal27-Φ demonstrate: (i) de novo expression of integrin αvβ3, (ii) increased expression of integrin αvβ5, (iii) increased adhesion properties to fibronectin and vitronectin; (iv) resistance to cisplatin, doxorubicin, mitomycin C and 5-fluorouracil; (v) increased amount of ILK and (vi) decreased amount of Src and pSrc (Y418). ILK knockdown in Cal27-2B1 and Cal27-2B3 had different effects on the cell survival upon treatment with the aforementioned antitumor drugs ruling out its involvement in the resistance to these agents. The Src knockdown in Cal27 cells, as well as concomitant treatment with dasatinib, increased resistance to the four antitumor drugs to the level observed in cell clones Cal27-2B1 and Cal27-2B3.

Conclusion. Since dasatinib treatment in Cal27 mimics the effect of a decreased amount of pSrc (Y418) observed in integrin αvβ3-expressing cell clones Cal27-2B1 and Cal27-2B3, we conclude that integrin αvβ3-induced downregulation of pSrc (Y418) is responsible for drug resistance. Given the strong attention that dasatinib receives as a potential drug for cancer therapy in combination with conventional chemotherapy, it is intriguing that decreased Src (Y418) activity confers a resistance to various antitumor drugs.

No conflict of interest.

498 LY294002@PLGA nanodrug overcomes resistance of PI3K inhibitor in lung cancer cells

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Introduction. LY294002 (LY) is a potent inhibitor of phosphatidylinositol 3-kinases (PI3Ks); however, bio-applications of LY are limited by its poor pharmacokinetics and undesirable toxicity in vivo. We have developed LY-loaded surfactant-free poly lactic-co-glycolic acid nanoparticles (LY294002@PLGA) synthesized by two emulsion method to improve therapeutic effects. LY294002@PLGA unexpectedly increases cancer cell death with its profound endoplasmic reticulum (ER) stress. We compared cellular responses between ER stress-related inducers (tunicamycin, thapsigargin, brefeldin A, and DBeQ) and LY294002@PLGA to explore the underlying mechanisms.

Materials and Methods. Characterizations of LY294002@PLGA were analyzed using dynamic light scattering (DLS) and high-performance liquid chromatography (HPLC). Cellular viability was measured by MTT assay; the expressions of ER-stress-associated proteins were determined using western blotting in lung cancer cell lines (H460 and H157).

Results and Discussion. The mean diameter and encapsulation efficiency of LY294002@PLGA was 174.5±3.29 nm and 54.66%±3.81%, respectively. LY294002@PLGA induced significant more cell death at lower concentration, compared to free form LY294002. The tunicamycin and thapsigargin treatment activated all UPR pathways and induced modest cell death only. The brefeldin A treatment activated PERK and IRE1 pathways and caused more cell death than tunicamycin and thapsigargin. Both DBeQ, a valosin-containing protein (VCP) inhibitor, and LY294002@PLGA treatment induced profound ER stress, primarily through PERK-ATF4-CHOP pathway, and cell death, suggesting VCP may be an important target for LY294002@PLGA. ERK pathway was activated by treatment with free form LY294002 and the aforementioned ER stress-related inducers; whereas, LY294002@PLGA treatment suppressed PI3K/Akt pathway and MEK/ERK pathway simultaneously.

Conclusion.LY294002@PLGA nanodrug possesses dual-targeting activity, therefore, may have great potential to become effective anti-cancer drugs.

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No conflict of interest.

499 Hedgehog signaling inhibition potentiates the effectiveness of cytostatic agents in Ewing sarcoma

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Introduction. Ewing sarcoma are rare mesenchymal tumors, usually diagnosed in children and adolescents. Abnormal activation of the Hedgehog (Hh)-pathway is associated with several cancers including Ewing sarcoma. We found that inhibition of the Glioma-associated oncogene (GLI) transcription factors, downstream effectors of the Hh-pathway, both inhibited viability and induced apoptosis in Ewing sarcoma cell lines. As the ABC transporters PGP, MRP1 and BCRP, involved in multidrug resistance, are targets of the Hh-pathway, we tested potential additive effects of the GLI inhibitor arsenic trioxide (ATO) combined with the cytostatic drugs etoposide and doxorubicin.

Material and methods. Three Ewing sarcoma cell lines as well as mesenchymal stem cells (MSC) for control were treated with increasing concentrations of ATO, etoposide and doxorubicin in single and combined application. Viability and proliferation (MTS-assay, spheroid assay, clonogenic assay) as well as apoptosis induction (western blot, flow cytometry) were analysed.

Results and discussion. In the MTS viability assay ATO treatment reduced the metabolic activity of Ewing sarcoma cell lines significantly (IC50 ATO: A673 0.23 µM, RD-ES 1.91 µM, SK-N-MC 4.42 µM). Moreover, Ewing sarcoma cell lines were sensitive to etoposide (IC50 etoposide: A673 0.88 µM, RD-ES 1.06 µM, SK-N-MC 1.11 µM) and doxorubicin (IC50 doxorubicin: A673 27.18 nM, SK-N-MC 75.15 nM) as well, whereas MSCs remained unaffected by the concentrations used. Combination of drugs potentiated the reduction of viability as well as the inhibitory effect on clonal outgrowth. Moreover apoptosis was induced by ATO, etoposide and doxorubicin in single and combined treatment exclusively in Ewing sarcoma cell lines while MSCs were not compromised.

Conclusion. The experiments show that Ewing sarcoma cell lines are sensitive to GLI inhibition using ATO. Standard therapy for Ewing sarcoma patients is a combination of different cytostatic drugs including etoposide and doxorubicin. During progression drug resistance emerges which usually can not be overcome by classical treatment. ATO, therapeutically used for treatment of acute promyelocytic leukemia (APL), may be a therapeutic option for patients with advanced Ewing sarcoma. Additionally to an increase of effectiveness of cytostatic drugs and prevention of drug resistance, this approach may also reduce adverse effects, because individual doses can be reduced.

No conflict of interest.

502 Activation of RAS family members confers resistance to ROS1 targeting drugs

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Introduction. The identification of genetic alterations that activate signaling pathways and the advent of novel therapeutics that specifically target them have profoundly changed treatment of lung cancer patients. Among these genetic events are those involving the ROS1 proto-oncogene, a receptor tyrosine kinase whose ligand is unknown. Treatment of lung cancer patients bearing rearranged ROS1 tumors (ROS1+) with the kinase inhibitor crizotinib resulted in high response rates and disease control. However, ROS1+ patients treated with crizotinib, invariably undergo relapse. We aimed to identify mechanisms of resistance to ROS1 inhibitors.

Materials and Methods. Cells resistant to ROS1 inhibitors were generated from HCC78 lung cancer cells bearing the SLC34A2-ROS1 rearrangement. WT and activated mutant forms of RAS proteins were expressed in HCC78 cells and their sensitivity to ROS1 inhibitors was determined. Human lung tumor biopsies from ROS1+ patients who relapsed during treatment with crizotinib were analyzed for the presence of specific mutations and genomic alterations.

Results and Discussion. We found that the activation of the RAS pathway in the HCC78 cell line model, due to either KRAS or NRAS mutations or to KRAS amplification, rendered the cells resistant to ROS1 inhibition. These cells were cross resistant to two different ROS1 inhibitors, but sensitive to inhibitors of the RAS signaling pathway. Interestingly, we identified focal KRAS amplification in a biopsy of a tumor from a patient that became resistant following crizotinib treatment. Altogether our data suggest that the activation of members of the RAS family can sustain resistance to ROS1 inhibitors.

Conclusions. The data shown have clinical implications as: (i) RAS genetic alterations in ROS1+ primary tumors are likely negative predictors of efficacy for targeted drugs and (ii) this kind of resistance is unlikely to be overcome by the use of more specific or more potent ROS1 targeting drugs.

No conflict of interest.

503 Glutathione-dependent antioxidant response is crucially involved in neuroblastoma multi-drug resistance

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Introduction. Neuroblastoma (NB), a most common paediatric malignant tumor, is initially sensitive to treatment with etoposide, a drug clinically used, but, subsequently, many patients develop chemoresistance. Cancer cells become able to counteract endogenous and drug-induced ROS overproduction enhancing antioxidant defense and developing chemoresistance. In order to investigate the redox-modulated mechanisms responsible for chemoresistance, a human NB cell line resistant to etoposide was selected and then characterized.

Materials and Methods. HTLA-230 cells, a high-risk human NB cell line, were treated for 6 months with increasing concentrations of etoposide up to 1.25 mM, the dose comparable to that clinically used. In order to characterize the gene expression profile of the etoposide-resistant cells (Etopo-R), a gene microarray was performed. Etopo-R cells were treated with higher concentrations of etoposide, or with other pro-oxidant compounds such as doxorubicin, H2O2 or pre-treated with buthionine sulfoximine (BSO, a glutathione depleting agent). Cells were then characterized in terms of cell viability (MTT analysis) and tumorigenicity (clonogenic assay). Cell oxidative status was evaluated by measuring glutathione (GSH) levels (fluorescence analysis), analyzing the expression of GSH-related enzymes (PCR) and determining reactive oxygen species (ROS) production (cytofluorimetric analyses).

Results. The selected Etopo-R cells were more resistant, than parental cells, to high concentrations of etoposide, to doxorubicin and to H2O2. In particular, Etopo-R cells, in comparison to parental cells, displayed higher GSH levels, overexpressed g-glutamyl-cysteynil ligase, a crucial enzyme in GSH biosynthesis and produced lower amounts of ROS when treated with etoposide, doxorubicin and BSO. In addition, Etopo-R cells were more tumorigenic than etoposide-treated parental cells and the pre-treatment with BSO totally blocked the tumorigenicity of parental cells and reduced by 50% the tumorigenic potential of Etopo-R cells.

Conclusions. Collectively, our results suggest that GSH-dependent antioxidant response in Etopo-R cells is crucially involved in the development of Multi Drug Resistance. For this reason, we believe that the identification of redox-modulated targets might be important to overcome chemoresistance (Grants from CARIGE Foundation 2013, MIUR PRIN20125S38FA and Genoa University).

No conflict of interest.

504 The inhibitory effect of 10-hydroxy-2-decenoic acid (10-HDA) on breast cancer cell proliferation, migration, adhesion and invasion

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Introduction. Breast cancer is presently reported as the most cancer diagnosed in women worldwide. Although, new therapeutic methods are developed and seem to be more effective than in the past, but metastasis is still remained the problem for treatment. New anticancer drugs that react specifically to this process are then needed to develop in order to increase patient survival. As the literature reported that 10-hydroxy-2-decenoic acid (10-HDA), a natural compound extracted from royal jelly, has the inhibitory activity of cell proliferation, cell migration and cellular angiogenesis demonstrated in endothelial HUVEC cells. Hence, 10-HAD may be a potential compound that target to cancer cell metastasis.

Material and Method. Breast cancer cell line (MDA-MB-231) was used as a model of studying. MTT assay was performed to evaluate cancer cell cytotoxicity, while the effect to migration was tested by wound healing assay. Adhesion assay was further investigated using several types of extracellular matrix proteins including collagen type I, II, IV, fibronectin, laminin, tenascin and vitromentin. The transwell coated with matrigel basement membrane matrix was also tested in order to observe the effect to cancer cell invasion.

Results and Discussion. The result of MTT showing that 10-HDA was cytotoxic to breast cancer cells with IC50 at 12 mM. In addition,10-HDA could inhibit migration, adhesion and invasion with dose-dependent manner (0, 0.3,0.6 and 12 mM). Hence, from this results it is supported that 10-HDA is a potential agent that target breast cancer cell metastasis.

Conclusion. This work firstly demonstrated that 10-HDA, a compound extracted from royal jelly, has the anti-metastatic property by inhibit breast cancer cell proliferation, migration, adhesion and invasion. To develop this compound as anticancer agent, further studies involved with other metastatic mediators and signaling used by this compound should be investigated.

No conflict of interest.

505 Development of cyclic peptide CXCR4 antagonist –Peptide R derivatives with higher stability and efficacy. Implication for clinical translation

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Introduction. In cancer CXCR4-CXCL12 signaling favors metastasis in experimental settings, providing the rationale for chemokine receptor antagonists to reduce human cancer metastasis. Previously, we described a new family of CXCR4-ligand based cyclic antagonistic peptides. Among three peptides selected as CXCR4 antagonists, peptide R, S and I, peptide R revealed as the most powerful in vitro and in vivo experiments. However, the therapeutic use of peptides is limited due to the rapid degradation into biological fluids as well as the aspecific distribution into the body and plasmatic protein binding. To overcome these limits a series of peptide R derivatives were synthesized. N-Terminal acetylation of peptide R and D-amino acid scan led to the development of a new series of compounds, Ac-R , including R-29 (Ac-Arg-Ala-[DCys-Arg-Phe-Phe-Cys]-COOH) and R-38 (Ac-Arg-Ala-[DCys-Arg-Phe-Phe-Pen]-COOH) that were evaluated for stability and efficacy.

Materials and Methods. Peptide stability was evaluated in vitro in human serum and in vivo in mice serum through liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). As CXCR4 functional assays CXCR4 Peptide binding (from 1nM to 10μM) was assessed by 12G5anti-CXCR4 PE-antibody competition on CCRF-CEM and Jurkat cells. cAMP synthesis was evaluated in CEM cells in the presence of Peptides (from 10nM to 10μM) and the adenylate cyclase activator, Forskolin to induce cAMP production physiologically reduced by CXCL12, CXCR4 ligand. Migration assay was conducted in Transwell migration chambers with CEM cells in the presence of Peptides.

Results and discussion. As compared to first generation Peptide R, Ac-R peptides showed in vitro greater stability (no degradation at 240' for Ac-R compared to degradation from 7 to 5 aminoacids for Peptide R at 5'). Moreover, R-29 and R-38 significantly competed with 12G5-CXCR4 antibody in CEM and Jurkat cells with an IC50 value ranging between 0.01-0.1uM, 10 fold more powerful than Peptide R. Peptides R-29 and R-38 CEM migration toward CXCL12 was inhibited with an IC50 of 1nM versus 10uM for Peptide R. CXCR4 binds CXCL12 and activates the Gαi reducing cAMP levels within cells. In CCRF-CEM peptide R, R-29 and R-38 almost comparably increased cAMP production.

Conclusions. Peptide R-29 and R-38 showed a longer half-life compared to peptide R. Moreover CXCR4 antagonistic activity improved between 10-100 fold. Currently evaluation of in vivo stability and efficacy is ongoing.

No conflict of interest.

507 Molecular mechanisms of sensitivity and resistance to the atypical retinoid ST1926 in colon cancer cells

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Introduction. Globally, colon cancer ranks third in both incidence and fatality. Despite current aggressive treatment modalities, the cure index is still low. Retinoids regulate crucial biological processes such as cellular proliferation, apoptosis, and differentiation, and have been used therapeutically in some cancers. However, the clinical usage of natural retinoids is limited by side effects and acquired resistance. Synthetic retinoids were developed, such as ST1926, which couple increased specificity and reduced normal cells' toxicity. We investigated the effects of ST1926 on colon cancer cells and elucidated the mechanisms implicated in drug sensitivity and resistance.

Material and Method. Human colon cancer cell lines were used: HCT116, HCT116p53-/-, HCT116p21-/-, and HT29. ST1926-resistant 'HCT116-R' cells were generated by culturing the parental cell line with increasing concentrations (10-9 - 5x10-6 M) of ST1926 for eight months. Cell proliferation and viability were assessed by MTT and trypan blue exclusion assays. Cell cycle phases and apoptosis were evaluated by flow cytometry and TUNEL assay. Cell death and DNA damage were detected by Western blot analysis and the Comet assay. Spheres formation assay was used to evaluate the self-renewal ability of control and treated colon cells.

Results and Discussion. Pharmacologically achievable micromolar concentrations of ST1926 inhibited the proliferation of all tested colon cancer cell lines, while ten-fold higher concentrations had no effect on 'normal-like' colon NCM-460 and HCT116-R cells. ST1926 treatment induced S-phase cell cycle arrest, PARP cleavage, apoptosis, and up-regulated p53 and p21 protein levels. Moreover, ST1926 decreased the self-renewal capacity of tumor cells by reducing the number and size of spheres formed in matrigel. ST1926 caused extensive early DNA damage in all tested colon cancer cells. However, ST1926-treated HCT116-R cells sustained less DNA damage, and hence, cell death was attenuated.

Conclusion. Our results highlight the potential therapeutic use of ST1926 in colon cancer, and suggest that cell resistance to ST1926 is mainly due to the loss of DNA damage. Studies are underway to investigate the therapeutic activities of ST1926, alone or in combination treatments, in colon cancer mouse models.

No conflict of interest.

509 MicroRNA-21 inhibition enhances chemosensitivity of doxorubicin-resistant glioblastoma cells

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Introduction. MicroRNAs (miRNAs) are short non-coding regulatory RNAs that function as negative gene regulators at the post-transcriptional level. Computational analyzes indicate that a unique miRNA can bind to approximately hundred of mRNA targets, thereby playing an important role in many biological processes, including drug resistance. In particular, miR-21 has been considered as a star miRNA in the field of Multidrug Resistance (MDR) research.

Material and method. To recognize miRNAs that play critical roles in MDR mechanism, we have studied the expression profile of 5 miRNAs (miR-21, miR-7, miR-124, miR-128 and miR-137 that are specifically over- or under-expressed in high grade glioma cells) in glioblastoma multiforme (GBM) specimens and in GBM cell lines (A172, U87MG and T98G) after Doxorubicin (Dox) treatment. Dox is an anthracyline drug routinely used in clinical practice of oncology. Data on in vitro and in vivo malignant glioma models suggest that Dox can be effective for these tumors. We focalized our attention on miR-21, one of the most important chemoresistance-related miRNAs, and we studied its role in the progress of drug resistance by using T98G cells that we previously found to be a Dox-resistant cell line. To investigate whether miR-21 regulation influences the drug resistant phenotype, we transfected anti-miR-21 into the cells.

Results and Discussion. The data showed a lower miR-21 expression level and a higher apoptotic rate in T98G transfected cells than in the parental control cells, suggesting that miR-21 plays a significant role in anthracyclines response of GBM cells.

Conclusion. Our findings indicate that blocking the action of miR-21 with inhibitors can re-sensitize the resistant glioblastoma cells to the therapeutic activities of Dox, by enhancing apoptosis. Further studies are needed to expand our preliminary results and to understand whether miR-21 inhibition might be explored as a potential chemotherapy adjunct in the treatment of resistant brain tumors. It could pave the way to new effective approaches for reversing drug resistance in cancer cells.

No conflict of interest.

511 In vitro cytotoxic activities of the oral platinum(IV) prodrug oxoplatin and HSP90 inhibitor ganetespib against a panel of gastric cancer cell lines

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Introduction. Gastric cancer exhibits a poor prognosis and is the second most common cause of cancer death worldwide. Chemotherapy of metastatic gastric cancer is based on combinations of platinum drugs and fluoropyrimidines, with added agents. Oxoplatin is a stable oral platinum(IV) prodrug which is converted to a highly active tetrachlorido(IV) complex under acidic conditions. In the present work we studied the cytotoxic effects of oxoplatin against a panel of four gastric cancer cell lines in vitro. Furthermore, the role of HSP90 in chemoresistance of these lines was investigated using the specific inhibitor ganetespib.

Material and method. The KATO-III, MKN-1, MKN-28, MKN-45 lines were used in MTT chemosensitivity, cell cycle and apoptosis assessment tests. KATO-III is a signet ring diffuse cell type, MKN-1 an adenosquamous primary, MKN-28 a well-differentiated intestinal type and the MKN-45 a poorly differentiated, diffuse type gastric carcinoma line. Markers of apoptotic cell death/stress were investigated with proteome profiler arrays and M30/M65 cytokeratin fragments ELISA kits. Interactions of platinum drugs and ganetespib were calculated with help of the Chou-Talalay method.

Results and discussion. The prodrug oxoplatin revealed low activity against the four gastric cancer cell lines, whereas the platinum tetrachlorido(IV) complex and cisplatin gave IC50 values of 1 -3 µg/ml with increasing chemoresistance observed in the order of MKN-1, KATO-III, MKN-28 to MKN-45. Release of the M30 caspase-cleaved cytokeratin fragment and increased expression of cleaved caspase3 and claspin in response to drug treatment are markers of apoptosis and cell cycle arrest, respectively. With exception of KATO-III and MKN-28/oxoplatin, all other cell lines featured marked synergistic toxicity with clinically achievable concentrations of ganetespib.

Conclusion. Chemotherapy of advanced gastric cancer patients relies on intravenous application of a platinum drug in combination with oral fluoropyrimidine prodrugs. Oxoplatin seems to constitute an oral platinum prodrug whose metabolite exhibited activity comparable to cisplatin. Oral administration of a platinum agent would be of great value for patients and care providers alike. All platinum complexes used here synergize significantly with the HSP90 inhibitor ganetespib. These results suggest that the oncogene-stabilizing HSP90 chaperone represents an important mediator of chemoresistance in gastric cancer.

No conflict of interest.

512 Repurposing valproic acid and simvastatin to enhance the antitumor effect of docetaxel in prostate cancer cells

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Introduction. Although docetaxel remains a standard of care for advanced prostate cancer (PCa), limited long-term responses, side effects and resistant disease suggested the need of novel therapeutical strategies. The mevalonate pathway (MVP) plays a critical role in PCa development and progression. In this study, we analyzed the antitumor effect in PCa cells of docetaxel in combination with the anticonvulsant valproic acid (VPA), an histone deacetylase-inhibitor and the cholesterol-lowering drug simvastatin, an inhibitor of HMG-CoA reductase, a rate-limiting enzyme in mevalonate pathway. Material and Method: Anticancer effects were assessed on androgen-dependent 22RV1 and LNCAP and androgen-independent PC3 and DU145 cells as well as on the highly aggressive SIM-resistant DU145R80 subline developed in our laboratory from DU145 cells (Milone et al. Cell Death Dis. 2013, 4, e641; Milone et al. Oncotarget 2014 Nov 24), by calculating combination index (CI) according to the method of Chou and Talalay. Apoptosis was measured by Annexin-V staining and FACS analysis. Self assembled tumor spheroids were obtained by hanging-drop 3D coltures.

Results and Discussion. We first showed a potent synergistic antiproliferative effect of VPA/SIM combination on all cell lines, including SIM-resistant cells, whatever schedule of administration (simultaneous vs sequential) we used, confirming our previous data on the combination between the HDACi panobinostat and zoledronic acid that by inhibiting farnesyl pyrophosphate synthase also targets the mevalonate pathway (Bruzzese F. et al, Cell Death and Disease (2013) 4, e878). Notably, exposure to triple combinations (VPA/SIM/docetaxel), resulted in a further strong synergistic anti-proliferative effect, as demonstrated by combination indexes (CI) and dose redaction indexes (DRI), with sequential exposure with 24 h delay between VPA/SIM and docetaxel as the best schedule. We confirmed synergistic interaction of VPA/SIM and docetaxel combination by apoptotic assay, soft agar clonogenic assay and on self-assembled PCa spheroids. Ongoing experiments are evaluating the combinatory approach on docetaxel-resistant PCa models recently developed in our laboratory.

Conclusion. All together these findings suggested that the combination of two safe generic drugs such as VPA and SIM can improve docetaxel efficacy, representing a novel therapeutic approach that warrant clinical investigation in advanced PCa patients.

No conflict of interest.

513 Anti -mitochondrial therapy in pancreatic adenocarcinoma

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Introduction. CPI-613 is an Altered Energy Metabolism Directed (AEMD) drug candidate, designed to disrupt the altered energy-production pathways in cancer cells by targeting mitochondrial metabolism. CPI-613 has been shown in-vitro to be highly selective in inducing the simultaneous inhibition of two key mitochondrial enzymes involved in cancer cell metabolism: pyruvate dehydrogenase (PDH) and alpha ketoglutarate dehydrogenase (KGDH). Disruption of PDH and KGDH function cuts off the tumor's mitochondrial energy supply culminating in cell death. CPI-613 is currently investigated in several human clinical trials in solid tumors and hematological malignancies with encouraging results and low toxicity. Patients with stage IV pancreatic cancer have a dismal prognosis and active investigation for more treatment options is needed.

Material and Methods. A Phase I open-label dose-escalation clinical trial of CPI-613 in Combination with FOLFIRINOX (Fluorouracil, Oxaliplatin, Irinotecan) in patients with metastatic pancreatic cancer and good performance status (NCT01835041). The trial is in progress with maximum tolerated dose (MTD) established.

Results and Discussion. a total of 8 patients were treated with combination chemotherapy + CPI 613. The treatment is well tolerated and patients have clinical, radiologic and biologic responses. To date 1 patient had near complete response after 4 months of treatment and 4 other patients had a partial response at 2 months of treatment. These patients are currently still on treatment and tolerating it well. At 500mg/m2 CPI 613 appears to have a synergistic effect in combination with chemotherapy without adding clinically relevant additional toxicity.

Conclusion. CPI-613, when given in combination with FOLFIRINOX, induced dose-related plasma levels at the dose levels tested, with a mean peak plasma level of 8.1 µM with the 500 mg/m2 dose level (n=6) and 56.9 mg/m2 with the 1,000 mg/m2 dose level (n=2). These plasma concentrations exhibited anti-cancer activities against several cancer cell types according to in vitro studies.

The safety profile of CPI-613, which shows the drug to be remarkably well tolerated even in combination with moderately toxic chemotherapy, provides further support for evaluation of CPI-613 in combination with other drugs to maximize benefit. In addition, our data suggests that FOLFIRINOX can be used as a backbone for fit patients in combinations with novel agents with a low toxicity profile.

No conflict of interest.

514 Search for therapeutic response molecular markers in malignant melanoma through NGS: On the way to an anti-BRAF combination therapy

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Introduction. Recent development of new targeted agents is giving viable treatment options to melanoma therapies. Vemurafenib ® specifically targets BRAF-V600E mutation, an highly common alteration found in malignant melanomas (MM). This targeted therapy is very effective on BRAF mutated MM patients but, most of them quickly develops an acquired drug resistance, limiting the therapeutic efficacy. We previously characterized the potential antitumor activity of several compounds on MM cells using a series of cell lines derived from MM patients showing different histopathological features (primary and/or metastatic, different tumor stage and site of onset). Using NGS approaches, we started the molecular characterization of Vemurafenib-resistant BRAF-mutated MM cell lines in comparison with their drug-sensitive counterparts, in order to identify genetic variants associated to the resistance. The evaluation of the antiproliferative activity of some new compounds - like the curcumin-related D6 molecule - on Vemurafenib-resistant MM cell lines is in progress.

Materials and Methods. The NGS testing was performed on cell lines using the Ion-Torrent ® technology. The validation of new markers will be carried out in vitro (same cell lines) and in vivo (MM paraffinated tissue samples) by Sanger sequencing. BRAF-mutated MM cell lines were treated with Vemurafenib (20μM to 5μM) to isolate resistant clones. These were treated with a combination of Vemurafenib and the curcumin-related D6 compound, before performing MTT proliferation assays.

Results and Discussion. A genetic heterogeneity was observed after characterization of several primitive and metastatic MM cell lines by cancer gene mutation profiling. Among them, four paired BRAF-mutated primitive and metastatic MM cell lines were selected to isolate Vemurafenib-resistant clones. These showed to be sensitive to D6 treatments, thus suggesting a combination therapy using Vemurafenib and D6. NGS results are being evaluated in order to identify alterations acting as markers for predicting the antiproliferative response following this treatment. Moreover, they will allow a better understanding of the molecular mechanisms underlying the development of resistance to Vemurafenib in MM.

Conclusion. Our preliminary results suggest the feasibility of Vemurafenib-D6 combination treatment in drug-resistant MM. Our aim is to identify and validate new molecular markers associated to therapeutic response in order to obtain a better classification of the cases to be addressed to the treatment.

No conflict of interest.

515 Synergistic anti-cancer effect of photodynamically-active hypericin and natural antibiotic Manumycin A on oxaliplatin-resistant colon adenocarcinoma cells

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Introduction. Colorectal cancer is one of the most common human malignancies. Oxaliplatin is a chemotherapeutic drug standardly used in the treatment of colon adenocarcinoma, often in combination with other drugs. One of the main problems of anticancer therapy with oxaliplatin is aquisition of chemoresistance of cancer cells resulting in ineffective treatment. Induction of apoptosis with natural agents is one of the possible tools to overcome resistance of cancer cells. In this study, the combined effect of photodynamically-active hypericin (a pigment from Hypericum perforatum) and Manumycin A (a natural antibiotic produced byStreptomyces parvulus) on oxaliplatin-resistant colon adenocarcinoma cell line (HT-29-OxR) was examined.

Material and methods. Oxaliplatin-resistant colon adenocarcinoma cell line was used to investigate the anticancer effect of combination of photodynamically-active hypericin and Manumycin A. Eosin vital staining was used to examine viability, total cell number and number of floating cells. CCK-8 assay was performed to evaluate cytotoxicity and inhibitory concentrations of drugs. CalcuSyn software was used to calculate the combination index and identify the type of interaction between the two agents. BrdU incorporation assay and colony forming assay were performed to study the proliferation of cells. To evaluate the ability of the drug combination to induce apoptosis, PARP p85 fragment was detected using ELISA method. Statistical analyses were determined using one-way ANOVA followed by post hoc Tukey multiple comparison test.

Results and discussion. Treatment of oxaliplatin-resistant colon adenocarcinoma cells with combination of noninhibitory concentrations of photodynamically-active hypericin and Manumycin A caused significant decrease in cell viability and total cell number. Evaluation of combination index revealed a synergistic effect in growth inhibition. Moreover, the combination treatment led to short-term (BrdU incorporation) and long-term (colony formation) inhibition of cell proliferation and also induced an increased number of floating cells and apoptotic PARP cleavage.

Conclusion. These findings show the in vitro efficacy and synergistic activity of combination of photodynamically-active hypericin and Manumycin A in oxaliplatin-resistant colon adenocarcinoma cells.

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No conflict of interest.

516 Cannabinoids-based therapies for the treatment of HER2-positive breast cancer

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Introduction. Although HER2+ breast cancer patients have greatly improve their prognosis due to the development of targeted therapies such as trastuzumab or lapatinib, a significant percentage of them are innately resistant to these treatments and a fraction of the initially responders end up developing resistance as well. Therefore, additional effective and non-toxic therapies are urgently required for this population of patients. It has been extensively reported that cannabinoids exert anti-tumoural actions in different models of cancer. Nevertheless, little is known about the combination effect of these compounds with standard therapies. Hence, the aim of this study was to analyze the anti-tumoural action of cannabinoids alone or in combination with specific therapies targeting HER2.

Material and method. Human breast cancer cells ectopically (MDA-MB-231-HER2) or endogenously (BT474) overexpressing HER2 were challenged with cannabinoids (Δ9-tetrahydrocannabinol, THC, or cannabidiol, CBD) alone or in combination with trastuzumab or lapatinib. Cell viability was determined by the colorimetric MTT test. Ectopic xenografts were generated in immunedeficient mice by subcutaneous injection of BT474 cells. Tumour growth was determined with external caliper. The expression of potential molecular players responsible for the observed anti-tumour responses was analyzed by Western blot.

Results and discussion. Our results show that cannabinoids reduce the viability of human HER2+ breast cancer cells in culture and tumour growth in a xenograft model of this pathology. Importantly, these effects were produced not only by THC but also by CBD, which produces no psychoactive effects. Moreover, the combination of cannabinoids with standard HER2-targeted therapies results in an increased anti-tumoural action in vitro and in vivo. Our mechanistic studies point to the involvement of the non-receptor tyrosine kinase SRC as a pivotal modulator of these anti-tumoural responses.

Conclusion. Taken together, these results provide preclinical evidence for the use of cannabinoids, alone or in combination with anti-HER2 treatments, for the management of HER2+ breast cancer.

No conflict of interest.

517 Nrf2-dependent antioxidant response in cancer cell resistance to bortezomib

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Introduction. The availability of antioxidants is recognized as one of the critical mechanisms able to provide cancer cells with resistance to anticancer therapies and the activation of Nrf2-dependent pro-surviving pathways has been demonstrated to play a crucial role. The use of proteasome inhibitor bortezomib (BTZ) has been proposed as chemosensitizing therapy but its efficacy could be limited by the induction of Nrf2-dependent antioxidant genes.

Methods. The highly aggressive neuroblastoma (NB) cell line HTLA-230 was treated with a low dose of bortezomib, BTZ (2.5 nM) and proteasome activity was detected by using the fluorogenic peptide TAT-EDANSDABCYL (TED). Cell viability was measured by trypan blue exclusion dye. The evaluation of Nrf2 binding activity to the promoters of heme oxygenase 1 (HO-1), the modulatory subunit of glutamyl-cysteine ligase (GCLM) and the transporter for cysteine (x-CT) was performed by Chromatin Immunoprecipitation. Down-regulation of HO-1 was performed by siRNA. Glutathione (GSH) content was detected by HPLC.

Results. BTZ treatment exerted a strong reduction in proteasome activity (-80 % vs untreated cells) but failed in reducing cell viability. In the same experimental condition the binding of Nrf2 to the ARE sequences in the promoter regions of HO-1, GCLM and x-CT was enhanced. Moreover, GSH level increased of about 50% after BTZ treatment. HO-1 silencing and GSH synthesis inhibition obtained by using 1 mM buthionine sulfoximine (BSO) synergistically decreased BTZ-treated cell viability (-80% vs untreated cells). Treatment with all-trans-Retinoic acid (ATRA, 3 μM) partially prevented the binding of Nrf2 to ARE in the promoters of HO-1, GCLM and x-CT, reducing BTZ-treated cell viability by 40%.

Conclusion. These data highlight the key importance of Nrf2-dependent antioxidant pathways as molecular targets to sensitize aggressive cancers, such as high-risk neuroblastoma, to proteasome inhibition by using low doses of bortezomib, increasing the chance of clinical application.

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518 Development of new monoclonal antibodies as target therapy for the treatment of colorectal cancer

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Colorectal cancer (CRC) is one of the most common malignancies in industrialized countries. Despite continuous efforts to improve prevention and therapy, CRC represents one of the most important causes of cancer-associated deaths worldwide. In the last decade monoclonal antibodies (mAbs), a new class of drugs, were developed to improve the treatment of cancer. In contrast to standard therapy, mAbs selectively bind tumours and affect malignant cells by inhibiting survival pathways. Here we describe the generation and characterization of mAbs raised against epitopes expressed on colon cancer stem like cells (CSCs). We immunized mice with primary tumour-derived colon CSCs that are able to maintain the characteristics of tumour-initiating cells. By high throughput FACS analyses we screened more than 20000 hybridoma supernatants for their ability to recognize surface molecules exposed by different colon CSCs, but not PBMCs. We selected a total of 75 positive clones. Later analyses were concentrated on antibodies displaying tumour-tissue specific binding and/or an alteration in biological function of the tumour cells. We identified three candidate mAbs specifically binding colon carcinoma cells, but not normal colon mucosa as detected by IHC analyses of frozen and paraffin embedded tissue sections and FACS analysis of freshly dissociated patient samples. Interestingly, one of these mAbs reacts preferentially with CRC lines derived from metastasis while not binding to CRC lines generated from the tumour bulk. It might recognize a subpopulation of CRC lines with high metastatic capability.

In the second branch of our screening we identified antibodies influencing tumour cells growth and morphology. We concentrated our efforts on one candidate mAb that significantly decreased the growth of commercially CRC lines and colon CSCs in vitro and in vivo. It also impaired adhesion, migration and invasion of CRC lines, indicating a possible therapeutic value of this mAb by acting directly on the primary tumour and also by inhibiting metastasis formation.

Taken together the tumour-specific antibodies identified in this project might represent good candidates for colon cancer diagnosis, prognosis and vectors for ADCs, while the antibodies interfering with cell growth and invasion might represent a valid basis for the development of future targeted anti-tumour therapy.

No conflict of interest.

519 Fibroblast Growth Factor Receptor (FGFR)3 promotes acquired resistance to trastuzumab in gastric cancer

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Introduction. Trastuzumab has been recently demonstrated as valuable treatment in HER2+ gastric cancer (GC). However the majority of patients who achieve an initial response to trastuzumab-based regimens develop resistance within 1 year of treatment. This study was aimed at identifying the molecular mechanisms responsible for this resistance.

Material and Method. A GFP+/luciferase+, HER-2 positive, trastuzumab sensitive NCI-N87 GC orthotopic nude mouse model was used to select resistant models to this agent. Differentially expressed transcripts between trastuzumab-resistant and sensitive GC cells were measured by Illumina whole-genome microarray, and tested for network and functional interrelatedness by IPA software.

Results and Discussion. NCI-N87 orthotopic tumor bearing mice were kept under treatment until the tumors suddenly recurred while on continuous therapy with trastuzumab. Four NCI-N87 trastuzumab resistant (N87-TR) cell lines were established from different excised tumors by repeated GFP flow cytometry sorting and their effective resistance was verified in vitro and in vivo. Microarray analysis showed the dowregulation of HER2, the induction of epithelial-to-mesenchymal transition, and indicated FGFR3 as one of the top 10 upregulated genes in N87-TR cell lines. We found a significant and consistent association of N87-TR gene expression profiles with the activation of the mTOR signaling. Accordingly, N87-TR cell lines showed significantly lower expression of all HER family members, E-cadherin and phosphorylated (p)ERK1/2, and higher levels of FGFR3, vimentin and pAKT than did sensitive control. In vitro, N87-TR cell lines demonstrated a higher sensitivity to the FGFR3 inhibitor dovitinib than did trastuzumab sensitive control. Treatment with dovitinib reduced pAKT and migration in N87-TR cell lines. Oral dovitinib significantly reduced tumor burden and prolonged mice survival duration in N87-TR model, whereas it was ineffective on trastuzumab-sensitive GC tumors. Validation of the described mechanistic model in pre vs. post treatment biopsies from GC patients receiving trastuzumab-based therapies is currently ongoing and will be presented at time of the meeting.

Conclusion. This study identified an FGFR3/AKT/mTOR signaling pathway as responsible for trastuzumab resistance in GC, thus candidating dovitinib as potential agent to modulate this resistance.

No conflict of interest.

520 In vitro cell growth inhibitory effects of HYDAMTIQ, a novel PARP inhibitor, on human tumor cell lines with defective DNA damage response pathways

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Introduction. The poly(ADP-ribose) polymerase (PARP) enzymes play key roles in the regulation of cellular processes (e.g. DNA damage repair, genomic stability). It has been shown that PARP inhibitors (PARPIs) are selectively cytotoxic against cells with dysfunctions in genes involved in DNA repair mechanisms (synthetic lethality).

Preclinical and clinical studies have shown activity of PARPIs as single agents or in combination with anticancer drugs in BRCA1/2 mutated cancers that are unable to repair DNA by 'homologous recombination' (HR) and in colorectal cancer (CRC) with microsatellite instability associated with MRE11 mutations. Drug induced PARP inhibition may also potentiate the activity of anticancer drugs such as 5-fluorouracil (5-FU) by enhancing DNA damage, whose repair involves PARP1 activity.

Aims: To evaluate the effect of a novel PARPI, HYDAMTIQ, on human tumor cell lines, characterized by a different BRCA1/2 gene mutation or microsatellite stability status, or expression of ATM which mediates DNA damage responses.

Methods. A BRCA2-mutated pancreatic cancer cell line (CAPAN-1), its BRCA2 wild-type (wt) clones (C2-6, C2-12, C2-14), a BRCA1/2 wt breast cancer cell line (MCF-7), a BRCA2 mutated CRC cell line (HCT-116), CRC cells with (HCT-8) or without (HT29) microsatellite instability, CRC cells with low (SW620) or high (H630) ATM protein expression levels were used. Cell viability was assessed by the SRB assay after drug exposures of 72-240 hrs; the analysis of ATM protein levels was assessed by immunofluorescence.

Results. HYDAMTIQ showed more potent cell growth inhibitory activity in BRCA2 mutant cell lines (CAPAN-1, HCT-116) compared with wt cells (C2-6, C2-12, C2-14 clones and MCF-7). The cytotoxic effects of HYDAMTIQ were greater (after 240 hrs of exposure) in MS unstable HCT-8 cells than in MS stable HT29 cells. HYDAMTIQ induced higher antiproliferative effects in the ATM low expression SW620 cell line than in the ATM high expression H630 cell line. Also, the combination of HYDAMTIQ and 5-FU exerted a synergistic effect on inhibition of proliferation of SW620 cells and an antagonistic effect on that of H630 cells.

Conclusions. Our results confirm that the novel PARP inhibitor HYDAMTIQ inhibits the growth of human tumor cells with defective DNA damage response pathways and exerts synergistic cytotoxicity with 5-FU. These data provide relevant examples of synthetic lethality and evidence for further development of a novel PARPI. Supported by a grant from the Ente Cassa di Risparmio di Firenze, Florence, Italy

No conflict of interest.

522 PPAR-gamma stimulation in the prophylaxis of 5-FlUoro-Uracil induced mucositis

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Introduction. Chemotherapy induced mucositis is a major dose-limiting side effect of schedules containing 5-Fluorouracile (5FU). Rosiglitazone (RGZ) is a peroxisome proliferator activated receptor (PPAR) gamma agonist with known anti-inflammatory and antifibrotic properties and has been investigated as an antineoplastic agent for his cytostatic action in cancer cells. The aim of this study was to evaluate the prophylactic effectiveness of RGZ in a mouse model of chemotherapy-induced oral mucositis.

Material/methods. Oral mucositis was induced in C57BL/6J mice by daily intraperitoneal injection of 5FU 50 mg/kg for 5 days with or without pretreatment with RGZ (5mg/Kg/day) started 24h before 5FU administration. A third group of untreated mice was used as control. Mice were sacrificed at different time points (day 5, 8, 11, 15) : oral mucosa samples were harvested for histopathological evaluation and molecular analysis.

Results. At histopathological evaluation, control mice showed a regular organization of the epithelial layers while administration of 5FU resulted in loss of epithelial architecture, collagen fibres deposition and intense inflammatory infiltration: RGZ markedly reduced the intensity of leucocyte infiltration and significantly preserved tissue structure. Transcription levels of MMP2 and Col-1 and expression of NFkB, TGFB and CTGF, that were significantly increased after 5-FU injection at day 15, were comparable to control arm levels in mice pre-treated with RGZ.

Conclusion. This study shows that RGZ exerts a protective action on normal mucosae damaged by chemotherapy. RGZ is a promising candidate for preventing 5FU-induced mucositis in cancer patients.

No conflict of interest.

523 Overexpression of ABC family members is associated with intrinsic and acquired gemcitabine resistance in gallbladder cancer cell lines

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Introduction. Gallbladder cancer (GBC) is a highly aggressive disease with a dismal prognosis that is commonly diagnosed at advanced stages. Gemcitabine/cisplatin combination has become the standard treatment for patients with non-resectable, recurrent, or metastatic GBC, but the response rates are low and tumor cells develop resistance to chemotherapy. This is a multifactorial event; however, membrane transporters from ATP-binding cassette (ABC) and Solute Carrier (SLC) families play a main role in this process. The aim of this study was to evaluate changes in drug transporter expression associated with the development of gemcitabine (GEM) resistance in GBC cell lines.

Material and method. G415, NOZ, TGBC-1TKB and TGBC-2TKB human gallbladder cancer cell lines were used in this study and acquired resistant sublines were developed by stepwise selection that was tested by MTS assays. Gene expression of 16 ABC and SLC transporters were examined in parental and resistant cells by qPCR, and validated by immunocytochemistry (ICC) and Western blot. This gene panel was also evaluated in clinical samples (16 chronic cholecystitis and 16 tumors) using qPCR.

Result and discussion. Intrinsic drug resistance to GEM was tested in all cell lines and TGBC-1TKB was catalogued as highly resistant while NOZ was the most sensitive line. The resistant phenotype of all sublines was stable over time and the resistance index (RI) ranged from 10 to 100. All sublines acquired cross-resistance to cisplatin and 5-fluorouracil, with RI of 1.5-3 and 1-8 fold, respectively. Quantitative PCR showed higher expression of drug efflux pump genes between resistant sublines and their parental lines, with an increase of ABCC2 (MRP2) expression in TGBC1 and TGBC2, ABCC3 (MRP3) in G415 and NOZ and ABCG2 (BCRP1) in G415 and TGBC1 ($p<0.05$). These findings were corroborated by ICC and Western blot. In tissue samples, four genes were differentially expressed in tumors versus non-neoplastic tissues: ABCB3 and ABCC3 were up-regulated, and SLC28A1 and SLC29A1 were down-regulated ($p<0.05$).

Conclusion. Our findings suggest that members of the ABC family contribute to intrinsic and acquired chemoresistance to gemcitabine in GBC cell lines and are overexpressed in GBC tissues. Further experiments need to be performed to test whether selected inhibition of ABC members in addition to chemotherapy could decrease the rate of resistant gallbladder cancer.

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No conflict of interest.

524 Identification of selective inhibitors of the spindle assembly checkpoint kinase TTK (Mps1) for treatment of triple negative breast cancer

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The protein kinase TTK (commonly referred to as Mps1) is a component of the Spindle Assembly Checkpoint (SAC), a surveillance mechanism that ensures the fidelity of chromosome segregation. Defects in SAC functioning can lead to chromosome segregation errors, resulting in an abnormal number of chromosomes, or 'aneuploidy'. Aneuploidy is a common feature of solid human tumors and a predictor of poor prognosis in breast, lung, brain and colorectal cancer. TTK mRNA levels are elevated in several cancers, in particular in triple negative breast cancer (TNBC), the most aggressive type of breast cancer. A novel class of compounds that potently inhibits TTK enzyme activity and cancer cell line proliferation was identified and optimized on selectivity by EntropySelect™ [1]. The clinical candidate, NTRC 0066-0, inhibits TTK enzyme activity with subnanomolar potency (IC50) and was more than 200 times selective over 276 kinases examined, including mitotic and cell cycle dependent kinases (CDKs). The compound is characterized by slow dissociation kinetics, resulting in a long target residence time. It potently inhibits the proliferation of a wide variety of human cancer cell lines [2] with potency in the same range as marketed cytotoxic agents. The compound inhibits the phosphorylation of a TTK substrate protein at the kinetochore and induces chromosome missegregation in cell lines and mouse tumors in vivo, demonstrating target engagement. The compound was used to investigate TTK inhibition as a therapeutic approach for TNBC in a xenograft model of the human TNBC cell line MDA-MB-231 and in a mouse model of spontaneous mammary tumor formation based on genetic inactivation of the BRCA1 and TP53 genes [3]. The compound inhibited tumor growth in the MDA-MB-231 xenograft model as a single agent. In the genetic breast cancer model upon co-treatment with docetaxel we observed tumor regression and increase in mouse survival. TKK inhibition is proposed as a novel therapy for the treatment of TNBC.

References

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525 The chromatin remodeler, cohesin subunit SA-1 is a novel sorafenib target against hepatocellular carcinoma

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Introduction. Sorafenib has been an important advance in treatment of hepatocellular carcinoma (HCC). However, the survival benefit has been modest underscoring the need for the elucidation of the molecular targets in order to develop more active agents. Sorafenib has been thought to be a kinase inhibitor with major targets including Raf and VEGF receptor. However the biological activities are pleotropic suggesting alternations more globally in gene expression. There is emerging appreciation of the importance of chromatin remodelers in HCC as a modality of altering transcriptional activation (e.g. Huang et al., Nature Gen 2012). The cohesins represent a novel family genes regulator and our preliminary data indicates the product of the cohesion gene Stag-1 (SA-1) is upregulated in HCC. We now test the hypothesis that SA-1 could be a target for sorafenib.

Material and Method. Two human HCC cell lines, HuH7 and HepG2 were treated with Sorafenib 10 µm. We used standard analysis techniques including immunoblotting and real time PCR (using β-actin as loading control for both). We used SiRNA to knockdown SA-1 (achieving a ~70% reduction).

Result and discussion. Sorafenib (10uM) was treated for 24hr in a marked decrease in SA-1 level versus vehicle treated cells (HepG2 10.5 ±0.42 vs 6.59±1.5, respective; $p=0.016$ and Huh7: 2.29±35 vs 1.70±0.15) to see whether this may be linked to sorafenib efficacy, we evaluated the MEK MAPK PCNA axis and as expected (since sorafenib is a Raf inhibitor) 50-60% suppression (table 1). We then knocked down SA-1 and interrogated this pathway and found that SA-1 downregulation mirrored the effects of sorafenib. Finally by knocking down SA-1, we noted a marked ~55% decrease in the histone mark in acetyl H3K9 consonant with a decreased gene expression.

Treatment	Cell Type	Comparator	pMEK	pMAPK	PCNA
Sorafenib	HepG2	Vehicle Treated	68% reduction ($p=0.01$)	53% reduction ($p=0.013$)	61% reduction ($p<0.01$)
SA-1 siRNA	HepG2	Scramble Control	61% reduction ($p=0.02$)	36% reduction ($p=0.05$)	60% reduction ($p=4x10-4$)
Sorafenib	HepG2 with SA-1 knockdown	Vehicle Treated	9.7% induction	18% reduction	5% reduction

Conclusion. We demonstrate herein, for the first time, that the cohesin SA modulates gene expression in liver cancer and is downregulated by sorafenib. Importantly the sorafenib suppression of SA-1 appears to be paramount for sorafenib activity. This work provides key insights into the myriad of anti-neoplastic effects of sorafenib and may provide novel 'druggable' target.

Conflict of interest: Ownership: Nanocytomics LLC, Pegasus Biosolutions, American BioOptics

526 Genomic drivers of multiple myeloma and consequences for targeted drug response

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Introduction. Multiple myeloma (MM) is a heterogeneous disease that eventually becomes resistant to therapy. Determining the genomic lesions driving each stage of the tumor and identifying actionable items for novel anti-cancer drugs will improve and increase therapeutic options for the malignancy. The aim of the present work is to obtain a comprehensive catalog of driver genomic lesions of MM and linking these to the action of targeted therapeutic approaches.

Methods. A total of 15 newly diagnosed (NDMM) and 25 refractory/relapsed (RRMM) MM patients were prospectively included. Exome sequencing was performed on DNA from bone marrow derived CD138+ selected cells and from skin biopsies. Somatic

variants were identified while genomic drivers and the clonal composition of each tumor individual were determined in silico. The ex vivo selective drug response of CD138+ cells from each patient each was assessed by testing their sensitivity to a comprehensive panel of 308 approved and investigational oncology drugs.

Results. RRMM samples exhibited a larger clonal complexity as compared to NDMM mainly driven by the onset of mutations, but not CNAs during disease progression. The mutations affected genes involved in known MM mechanisms as well as other cancer hallmarks not as well described in this malignancy. Interestingly, a larger number of mutations correlated with an overall better ex vivo drug response profile, stressing that they may offer tumor vulnerabilities to be exploited by targeted therapeutic approaches. Some of these genomic lesions shaped the ex vivo sensitivity to several agents targeting key signaling pathways of MM, including MEK, CDK and mTOR inhibitors.

Conclusions. MM progression in the present cohort was related to the acquisition of a more complex landscape of mutations. Some of these genomic lesions confer sensitivity to specific targeted drugs, which may be of potential clinical interest, particularly for those patients where conventional treatment is no longer effective. Although the ex vivo drug response profile was heterogeneous across patients, several putative biomarkers of response to targeted drugs were observed. The present study is a proof of concept of the potential of the approach, but further data is necessary to confirm these findings and evaluate its benefits in a clinical setting.

No conflict of interest.

527 Selective inhibitors targeting indoleamine 2,3-dioxygenease and tryptophan 2,3-dioxygenase

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Background. Indoleamine 2,3-dioxygenase (IDO1) and tryptophan 2,3-dioxygenase (TDO) are two structurally different enzymes that both catalyze the conversion of tryptophan to N-formylkynurenine (NFK). IDO1 has been clinically validated as a small molecule drug target for cancer immunotherapy. Preclinical studies indicate that TDO may be a target for glioblastoma and neurodegenerative disease. We previously determined the selectivity of a number of drug candidates for IDO1 and showed that most inhibit both IDO1 and TDO [1]. Here we present inhibitors that are either selective for IDO1 or TDO.

Materials and Methods. We have developed a high-throughput screening assay for IDO1 and TDO based on a novel chemical probe, NFK Green™. The probe reacts specically with NFK to form a green fluorescent molecule. Using NFK Green™ assay, we have screened the 300,000 compound library of the European Lead Factory and a 87,000 diversity library of the Pivot Park in Oss. Screening resulted in a substrantial number of compounds that reproducibly and potently inhibit human TDO. The hits were further profiled in biochemical assays for human IDO1 and TDO, biochemical assays for mouse IDO1 and TDO, and cellular assays based on IDO1 or TDO-expressing cell lines. In addition, we performed modelling studies based on existing crystal structures of human IDO1 and TDO.

Results. Based on the IC50s in the IDO1 and TDO assays, many compounds found in our screen appeared to be selective for TDO. However, TDO has a higher KM,Tryptophan than IDO1, and therefore inhibitors may not necessarily bind better to TDO but may simply experience less competition with tryptophan. Furthermore, some inhibitors appreared aselective in the biochemical assays, but were selective in a cellular context. We provide evidence that this may be related to the presence of oxidative stress-modulating enzymes in cells. Other compounds show substantial differences in affinity between human and mouse enzymes. Interestingly, two inhibitors of known chemical structure give consistently selective effects in biochemical and cellular assays for human and mouse IDO1/TDO. 3D-modelling of atomic interactions reveals that each inhibitor contacts unique amino acids in the substrate binding pocket.

Conclusion. By employing an assay cascade, we have discovered selective inhibitors for IDO1 and TDO. Both compounds serve as tool compounds for further study of the biology of IDO1 and TDO.

Conflict of interest: Ownership: I am co-founder, managing director and shareholder of Netherlands Translational Research Center B.V.

528 Dextran-Catechin conjugate: Targeting copper metabolism in neuroblastoma

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Introduction. Neuroblastoma is an aggressive childhood cancer that is poorly responsive to therapy and rarely cured. Improved and less toxic therapies are urgently required. Natural antioxidants, such as Catechin, have anticancer properties with low toxicity to normal cells. However, their low stability in serum limits their use in clinical practice. To increase the stability of Catechin, we developed a novel modified form of Catechin covalently linked to Dextran (Dext-Cat). In this work, we studied the anti-tumor efficacy and the mechanism of action of Dext-Cat against neuroblastoma.

Material and Methods. Four independent neuroblastoma cell lines were selected for this study. Anticancer activity was tested by drug treated cell viability assays. Real time PCR was used for gene and western blotting for protein expression studies. Intracellular metal ion content was measured by spectrophotometric analysis. Fluorescence-lifetime imaging microscopy was used to study the NADH/NAD+ ratio to determine the induction of oxidative stress. The cellular level of the antioxidant GSH was examined by colorimetric assay. The tumour growth in a xenograft neuroblastoma model was measured using calipers.

Results and Discussion. The neuroblastoma cell lines IMR-32, BE(2)C and doxorubicin-resistant BE(2)C-ADR were sensitive to Dext-Cat (IC50 17.83 µg/ml, 35.38 µg/ml and 31.47 µg/ml, respectively) at concentrations that were not toxic to non-malignant cells. However, the Cisplatin-resistant neuroblastoma cells (IMR-32-CisRes) were 2.5-fold more resistant against Dext-Cat compared to the parental IMR-32 cells. IMR-32-CisRes exhibited 50% lower expression of copper transporter-1, which regulates Cisplatin uptake, and lower intracellular copper compared to the parental cells. On the contrary, Dext-Cat sensitive neuroblastoma cell lines showed elevated intracellular copper. We showed for the first time that Dext-Cat reacts with Copper generating reactive oxygen species and inducing cancer cell death. The decrease of NADH/NAD+ ratio and GSH level after Dext-Cat treatment confirmed the oxidative stress. Furthermore, we showed that Dext-Cat significantly reduced the tumour growth in a xenograft neuroblastoma model, without affecting healthy tissues.

Conclusion. Our data show that Dextran-Catechin mediates its effects via copper metabolism and has the potential to be used as an effective therapy for aggressive neuroblastoma.

No conflict of interest.

529 Jadomycin B is selectively toxic to triple negative human breast cancer cells in a zebrafish xenotransplantation model

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Introduction. Jadomycins are bacterial-derived cytotoxic agents that retain their cytotoxicity in multidrug resistant (MDR) MCF7 breast cancer cells that overexpress drug efflux transporters, such as P-glycoprotein (P-gp). However, jadomycins have never been tested for efficacy in MDR triple-negative (TN) breast cancer, for cancer cell selectivity, or in vivo. Our first objective was to determine the selectivity of jadomycin cytotoxicity in drug-sensitive and taxol-resistant, P-gp overexpressing TN breast cancer cells versus human mammary epithelial cells (HMECs). The second objective was to determine jadomycin host versus TN cancer cell toxicity in xenotransplanted zebrafish embryos; a preclinical model used as an in vivo platform for drug discovery that allows for the direct visualization of any therapeutic response induced by jadomycins.

Materials and Methods. TN MDA-MB-231 (231-CON) cells were cultured in gradually increasing concentrations (0.05 mg/mL to 5 mg/mL) of taxol for 7 months to generate taxol-resistant cells (231-TXL). P-gp expression was determined using quantitative PCR. MTT assays measured 231-CON, -TXL, and HMEC viability with or without jadomycin B, S, or F, or mitoxantrone or doxorubicin treatment. In vivo toxicity of jadomycins was determined in zebrafish embryos and inhibition of cancer cell proliferation was determined in zebrafish embryos xenotransplanted with 231-CON cells.

Results and Discussion. P-gp gene expression increased 122,000-fold in 231-TXL versus -CON cells. Jadomycins B, S, and F were equipotent in 231-TXL (IC50 values of 2.9 – 3.1 µM) versus 231-CON (2.7 – 3.0 µM) cells while mitoxantrone and doxorubicin were 19- and 42-fold less potent, respectively. Jadomycin B, S, and F IC50 values were 3.7, 3.6, and 2.0-fold greater in HMECs than in 231-CON cells, respectively. In vivo toxicity assays depicted maximum tolerated doses for jadomycins B, S, and F of 40, 20, and 20 µM, respectively. Jadomycin B (20 mM) significantly reduced xenotransplanted 231-CON cell proliferation over 48 hours by 72% in comparison to vehicle control.

Conclusions. Jadomycins B, S, and F retain their cytotoxic potency in MDR 231-TXL cells and may be selective for breast cancer cells in vitro and in vivo, warranting further research testing jadomycin activity against MDR TN breast cancer.

No conflict of interest.

530 Resistance to a MEK inhibitor, AZD6244: Association with increased Immunoglobulin Transcription Factor-2 through WNT-independent canonical pathway in melanoma cells

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Purpose: BRAF-mutated melanoma patients eventually develop clinical resistance to BRAF or MEK inhibitors. We tried to elucidate the resistance mechanisms.

Experimental Design. We analyzed genome-wide gene expression profiling data from NCI-60 cell line panel screening for a MEK inhibitor, AZD6244. Of 62 identified differentially expressed genes between 6 primary resistant and 6 sensitive cell lines, we selected Immunoglobulin Transcription Factor-2 (ITF-2 also known as transcription factor-4, TCF-4), which was known as a downstream target of the Wnt/Tcf pathway. We validated increased ITF-2 (mRNA and protein levels) in 6 resistant cell lines. We established an AZD6244 resistant cell line (M14/AZD-3) from an AZD6244 sensitive M14 melanoma cell line. To test ITF-2 as a resistance marker, we applied siRNA constructs against ITF-2 in both primary and acquired AZD6244 resistant cell lines. We also evaluated the signaling pathways for MAPK and GSK3β/β-catenin cascades.

Results. The expression of ITF-2 was elevated in acquired resistant cells (M14/AZD-3) as well as primary AZD6244 resistant cells (LOX-IMVI). The siRNA inhibition of ITF2 promoted the apoptosis of resistant melanoma cells by AZD6244. We observed increased level of inactive GSK3β (Ser9) and nuclear translocation of β-catenin in primary and acquired resistant cells. Interestingly, expression of phospho-p90RSK increased in the resistant cells, but treatments with Wnt/calcium modulator (cyclosporine A), GSK α inhibitor (SB216763), or GSK β inhibitor (GSK VI) did not show significant apoptotic effects in the resistant cells.

Conclusions. Our results suggest that up-regulation of ITF-2 gene expression is associated with cellular resistance to a MEK inhibitor, AZD6244 in melanoma cells. Activation of Wnt-independent beta-catenin pathway seems to be associated with increase of ITF-2 expression.

No conflict of interest.

531 JUN mediates melanoma phenotype-switching and confers cell survival during early adaptive resistance to BRAF/MEK inhibitor therapy

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Introduction. The majority of patients with BRAFV600-mutant metastatic melanoma display remarkable but incomplete responses to BRAF/MEK inhibitors. We envisaged that identifying the key mediators of cell survival in the context of early resistance, would reveal targets for combination therapy to provide more complete and durable responses.

Materials and Methods. Pharmaco-genomic analyses across 22 BRAFV600-mutant lines using 'limma' differential expression and NCI DAVID analysis identified expressed genes and GOterms significantly associated with differences in inherent drug response. Gene expression, signalling, morphology and functional analyses following drug treatment were used to determine the molecular, cellular and phenotypic characteristics of cells surviving early drug treatment. Gene expression and knockdown studies were used to link changes in MAPK signalling and the transcriptome, with phenotype-switching and cell survival. Matched patient samples pre-treatment and early on BRAF inhibitor therapy were used to validate our findings.

Results and Discussion. 4 of 22 BRAFV600-mutant melanoma lines displayed inherent resistance to BRAF/MEK inhibitors vemurafenib, dabrafenib, selumetinib and trametinib. GeneGO network analysis revealed the AP1, MITF and LEF1 transcriptional complexes as the main 'hubs' associated with differences in drug response. The resistant lines expressing high JUN, also expressed high ZEB1/low ZEB2, low MITF, low LEF1, high WNT5A and a greater capacity to migrate, indicative of a mesenchymal-like phenotype. The responsive lines expressed low JUN and epithelial-like characteristics. Early drug adaptation in the responsive lines in vitro and in xenografts was consistently characterised by increaing JUN expression together with molecular features indicative of a phenotype-switch akin to an epithelial-mesenchymal-transition (EMT). In matched patient samples, JUN was significantly increased early on therapy compared to pre-treatment. Enforced c-JUN expression was sufficient to induce an EMT-like phenotypic switch in the absence of drug treatment, while siJUN prevented the vemurafenib-induced phenotypic-switch and strongly enhanced cell death. Combination treatment with vemurafenib and JNK inhibitors also reduced cell migration and increased cell death.

Conclusion. Increased c-JUN induces melanoma phenotypic switching and mediates cell survival associated with early adaptive resistance to BRAF/MEK inhibition in melanoma.

No conflict of interest.

532 Inhibition of melanoma metastasis to the lung by fungal metabolites with peroxiredoxin-like peroxidase activity

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Metastatic malignancy is the most detrimental issue in melanoma at the vertical growth phase. Although key genes or signaling networks involved in the melanoma metastasis have been elucidated, there was no evidence of any antioxidant enzyme involvement. We have shown that the level of peroxiredoxin-2 (Prx2) inversely correlates with the metastatic ability of melanoma cells. Briefly, the SK-MEL melanoma cells with a silenced Prx2 expression were more proliferative and migratory than the A375 and G361 cells with high Prx2 expression. At a molecular level, Prx2 negatively regulated the Src/ERK activation, which in turn fortified the adherens junctions by increasing the E-cadherin expression and the Y654 phosphorylation-dependent retention of b-catenin in the plasma membrane. The mouse melanoma B16F10 cells depleted of Prx2 showed the enhanced lung metastasis in vivo. Based on these results, we attempted to employ a group of natural fungal secondary metabolites exhibiting the Prx-like activity to control melanoma metastasis to the lung. These metabolites inhibited proliferation and migration as well as lung metastasis of melanoma cells in vivo. Therefore, this study for the first time demonstrates therapeutic potential of Prx mimetic as an inhibitor of melanoma metastasis.

533 Apoptotic effect of caffeic acid phenethyl ester on chromosomally instable bub1Δ and bub3Δ saccharomyces cerevisiae strains

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Introduction. Caffeic acid phenethyl ester (CAPE) is an active component of propolis obtained from bee hives has anti-inflammatory, antioxidant, and anticarcinogenic properties that are known for many years. It has been shown in several studies that CAPE inhibits many cancer types. A recent study shows that, CAPE affects the aneuploidy, in other words chromosome instability, which is observed in 70-80% of the cancer cases and one of the hallmarks of cancer cells. Even though, it is known that CAPE can affect the chromosome instability, information available on the how CAPE acts on genetic and molecular mechanisms of chromosome instability is insufficient. Our goal is to examine the action mechanism of CAPE at genetic and molecular levels, identify the mechanisms how CAPE affect the chromosomal instability.

Material and Methods. For our research we used S. cerevisiae as a model organism. Chromosome instability is intensively studied in S. cerevisiae and the genes giving rise to this condition when they are knocked-out have been identified. In our study, effect of CAPE on these strains has been investigated. For investigating effect of CAPE, growth rate of S. cerevisiae strains relative to the control strains at 0 and 30 µg/ml CAPE concentrations have been tested by spot test. Knock-out strains exhibited growth inhibitions were further confirmed by analyzing their colony formation on solid media containing 0, 20 and 30 µg/ml CAPE. As a final step, rate of apoptosis were investigated with/without CAPE treatment by using TUNEL assay. Results and Discussions: As a result of our study, related to CAPE acting mechanism, we detected Bub1 and Bub3 are both mitotic checkpoint control proteins and strains with knocked-out Bub1 and Bub3 displayed increased chromosome instability. bub1Δ and bub3Δ strains treated with CAPE also showed increased apoptotic marker detected by TUNEL assay. **Conclusion.** CAPE affects viability of chromosomally instable cells through cell cycle checkpoint control mechanism. Based on these results, CAPE can be a potential chemotherapeutic agent for inhibition of tumor cell cycle progression. Obtained results will be used to further investigate the effect of CAPE on checkpoint control deficient cancer cell lines. This project is supported by TUBITAK (Project No: 112S254).

Poster Session: Molecular and Genetic Epidemiology I

535 Prevalence of human papillomavirus in laryngeal and hypopharyngeal squamous cell carcinomas in Northern Spain

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Introduction. Recent studies support a role for human papillomavirus (HPV) in oropharyngeal squamous cell carcinomas (SCCs); however, the significance of HPV in non-oropharyngeal head and neck cancers is uncertain. The aim of this study was to determine the prevalence of HPV in a large cohort of laryngeal and hypopharyngeal SCCs in northern Spain.

Material and Method. Clinical records and paraffin-embedded tumor specimens of 124 consecutive patients surgically treated for laryngeal (62 cases) and hypopharyngeal (62 cases) SCCs between 2002 and 2007 were retrieved. All cases were histologically evaluated, and presence of HPV was assessed by p16-immunohistochemistry followed by GP5+/6+-PCR-based DNA detection. Samples positive in both assays were subjected to HPV genotyping and HPV E6 transcript analysis.

Results and Discussion. Seventeen cases (14%) were positive for p16 immunostaining, of which 2 (1 larynx, 1 hypopharynx, 1.6% of total series) were found positive for HPV DNA by subsequent GP5+6+-PCR. Both SCCs contained HPV type 16 and showed HPV16 E6 mRNA expression.

Conclusion. HPV is only occasionally involved in laryngeal and hypopharyngeal SCC patients in northern Spain.

No conflict of interest.

536 Immunohistochemical markers of distant metastasis in laryngeal and hypopharyngeal squamous cell carcinomas

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Introduction. Metastasis remains a major cause of mortality in head and neck squamous cell carcinoma (HNSCC). Current clinicopathological features have shown limited predictability for the risk of distant metastasis in individual patients, and therefore more accurate and reliable markers are needed. The aim of this study was to investigate the ability of various molecular markers present in primary tumors to predict the risk of developing distant metastasis.

Material and Method. Restrictive clinical criteria were applied for patient selection in order to carry out a case-control study with comparable clinical features on a group-wide basis and a similar risk of metastasis. All patients were surgically treated (with postoperative radiotherapy when appropriate) and classified as stage IV disease. Immunohistochemical analysis was performed for a panel of proteins known to participate in cellular processes relevant to metastatic dissemination (E-cadherin, annexin A2, cortactin, FAK, EGFR, p53, and p-AKT).

Results and Discussion. Results showed that the loss of E-cadherin expression was significantly correlated with the risk of distant metastasis (P = 0.002; log-rank test), while the loss of annexin A2 expression was nearly statistically significant (P = 0.06). None of the other protein markers assessed were associated with the development of distant metastasis.

Conclusion. According to our data the loss of epithelial adhesion seems to play a central role in the development of metastasis in HNSCC, and more importantly, immunohistochemical assessment of key proteins involved in cell adhesion regulation, such as E-cadherin could represent a useful tool to evaluate easily and routinely the metastatic potential of these carcinomas. This determination could be easily and routinely performed on standard paraffin-embedded pathology specimens, making feasible the inclusion of this molecular marker in the diagnostic work-up and treatment planning of these tumors.

No conflict of interest.

538 Correlation among APC and MUTYH mutational spectrum, expression and phenotype in italian patients with familial polyposis and/or colorectal cancer

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Introduction. Gene dosage imbalance may influence the transcriptome. To gain insight into the role of altered gene expression in the onset of disease in the present study we performed an analysis of absolute and allele-specific expression (ASE) of the two major genes involved in hereditary gastrointestinal polyposis syndromes, APC and MUTYH.

Material and method. We analyzed DNA and RNA extracted from peripheral blood mononuclear cells (PBMC) of 50 familial polyposis and/or colorectal cancer patients and 152 healthy blood donors. Patients were studied for germline alterations in both genes using dHPLC, MLPA and automated sequencing. Controls were genotyped for the frequents SNPs rs3219489 (c.1014G>C) of the MUTYH gene and rs2229992 (c.1458 C>T) of the APC gene. APC and MUTYH mRNA expression levels were investigated by quantitative Real-Time PCR (qRT-PCR) analysis using TaqMan assay. ASE was evaluated by dHPLC-based SNuPE. Results and discussion. Patients were distributed in three different subgroups: 13 carriers of APC mutations but with a wild type MUTYH sequence (APC+/MUTYH-), 5 carriers of MUTYH mutations but with a wild type APC sequence (APC-/MUTYH+) and finally 21 patients without mutations in both genes (APC-/MUTYH-). Forty-two controls were selected and matched to cases on gender and age. Results from qRT-PCR indicated that patients with APC-/MUTYH- genotype showed a statistically reduced expression of the APC gene with a mean value of $2^{-\Delta Ct}$= 0.18+0.07 vs control group having a mean value of $2^{-\Delta Ct}$=0.32+0.12 ($P<0.05$). Absence of mutations in both genes correlated with a statistically significant reduction of APC gene. As regards MUTYH expression analysis, the APC-/MUTYH+ subgroup showed a statistically reduced expression with a mean value of $2^{-\Delta Ct}$=0.17+0.05 vscontrol group having a mean value of $2^{-\Delta Ct}$=0.56+0.22 ($P<0.05$). For MUTYH the presence of mutation may be correlated to the reduced expression. We analyzed 21 heterozygous patients (13 APC mutation-negative and 8 carriers of APC mutations) for rs2229992 (c.1458 C>T) by ASE assay. Altered germline ASE was detected in 4 out of 8 APC mutations carriers: 2 cases showed a moderate and 1 a marked variation. Finally one case showed a complete loss of one allele. Among APC mutation-negative cases, 4 out of 13 showed a moderate ASE. Analysis of MUTYH gene by rs3219489 (c.1014G>C) and rs34612342 (c.536A>G) ASE assays did not show any altered expression in the cases analyzed. Statistical analysis by Spearman's Rho Test showed a positive and significant correlation between APC and MUTYH genes both in cases and in controls (respectively Rho=0.381, p=0.020 and Rho=0.708, p<0.001).

Conclusion. Our results underline the importance of RNA-level studies in the molecular diagnosis of hereditary gastrointestinal polyposis syndromes.

No conflict of interest.

Poster Session: Molecular and Genetic Epidemiology II

543 Discovery of new potential genetic biomarkers of epithelial ovarian cancer prognosis

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Introduction. Among gynecological tumors, epithelial ovarian cancer (EOC) evinces the highest mortality worldwide. Therapy failure is often associated with late diagnosis; however, drug resistance of cancer cells is among major reasons, too. Malignancy-related alterations of gene and protein expression, as well as individual genetic variability, lead to different drug distribution and biotransformation in cancer cells, and thus to different therapy outcome. That is why we focused on novel molecular biomarkers of EOC prognosis and chemotherapy efficiency which could contribute to better guidance in ovarian cancer treatment.

Material and method. Tissue samples from 60 patients with EOC and 14 women without any signs of carcinoma (controls) were included in the study. Gene expression analysis was performed using real-time PCR with relative quantification. The data were analyzed by REST 2009 and SPSS v16.0 software.

A panel of 86 genes connected to drug transport (ABC and SLC transporters), drug biotransformation and EOC resistance and progression was explored. Related transcriptional factors were also examined.

Results and discussion. Differences in gene expression levels between tumors and controls were investigated. mRNA level of 12 genes was not detectable in ovarian tissue, thus it was not further analyzed. Compared to control samples, 13 genes were overexpressed and 30 genes were down-regulated in tumors, while expression of 31 genes did not significantly differ.

Associations between gene expression level and clinicopathological data of patients were also evaluated. The most important relationship discovered was between survival of EOC patients and ABCD3, ABCG2, ATOX1 and NR0B2 expression. There were also strong correlations between tumor stage and ABCA3 level, between grade and NR1I1, SOD2 and TP53 level and between TRAP1 level and histological type. The majority of these genes were previously linked to drug resistance in various types of cancer, including EOC. In agreement with this, results of our research suggest novel biomarkers of EOC therapy outcome.

Conclusion. In conclusion, several interesting correlations between patients´ characteristics and gene expression in ovarian tumors, as well as alteration of gene expression in EOC samples compared to controls were found. Subsequent studies on patients´ samples and on EOC cell lines (taxane-sensitive and resistant lines) will verify the role of the putative markers identified in this study in EOC therapy outcome.

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No conflict of interest.

545 Vimentin is a new molecular partner of LASP-1 in human hepatocellular carcinoma cells

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Introduction. Hepatocellular carcinoma is the third most common cause of cancer-related mortality worldwide. We have previously reported that LASP-1 is a downstream protein of the urokinase type plasminogen activator (uPA) and its mediator in the migration of HCC cells. LASP-1 is overexpressed in some human malignancies and it is involved in tumor development and metastases, but its role in HCC is poorly understood. In the present study we investigated the biological role of LASP-1 in HCC by a molecular and biological characterization of LASP-1 expression in human HCC specimens and in cultured HCC cells. We have ascertained the expression level of LASP-1 mRNA in HCC with different hepatic background disease and we have

biologically characterized the ectopic LASP-1 overexpression in HCC cells.

Materials and Methods. The expression levels of LASP-1 mRNA were evaluated by qRT-PCR in tissues from biopsies of 55 HCC patients with different hepatic background disease. The molecular interactors of LASP-1 were identified by the proteomic analysis of the co-immunoprecipitated fractions using MALDI-TOF mass spectrometer. The co-localization of the LASP-1 and its putative partner vimentin, was examined by confocal immunofluorescence analysis.

Results and Discussion. We identified 3 groups of patients with high, equal or low LASP-1 mRNA levels in HCC tissues compared to their correspondent adjacent peritumoral (PT) tissues. In particular we found that i) the HCCs displayed a higher LASP-1 mRNA level in HCC compared to PT tissues; ii) the expression levels of LASP-1 mRNA in female HCCs were significantly higher compared to male HCCs; iii) the cirrhotic HCCs displayed a higher LASP-1 mRNA. Further, the biological characterization of the ectopic LASP-1 overexpression in HCC cells, using MALDI-TOF mass spectrometer on the LASP-1 co-immunoprecipitated fractions, displayed vimentin as a novel putative partner of LASP-1.

Conclusion. Our results suggest that LASP-1 mRNA overexpression may be mainly implicated in female HCCs and cirrhotic HCCs; and that LASP1 may play its role with vimentin in HCC cells. The identification of groups of HCC patients with shared molecular and clinical characteristics is important to set up the follow-up of the patients and to study better therapies. The finding that LASP-1 can collaborate with VIM and uPA in aggressive HCC cells may be of help in future studies of innovative therapies targeting these molecules alone or in combination.

No conflict of interest.

546 Association between the vitamin D receptor BsmI polymorphism and breast cancer risk in Pakistan

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Introduction. Vitamin D is hypothesized to lower the risk of breast cancer by inhibiting cell proliferation via the vitamin D receptor (VDR). Two common single nucleotide polymorphisms (SNPs) in the VDR gene, rs1544410 (BsmI) and rs2228570 (FokI) have been shown to be inconsistently associated with breast cancer risk in Caucasians, while data in Asians are scarce. Here we investigated whether these SNPs contribute to breast cancer risk in Pakistani breast cancer patients and controls participating in the hospital-based breast cancer case-control study PAK-BCCC.

Material and method. Genotyping of the BsmI and FokI VDR SNPs was performed in 463 genetically enriched female breast cancer cases with known BRCA1/2 status and 1,012 controls from Pakistan by PCR-based restriction fragment length polymorphism (RFLP) analysis. The association between SNP genotypes and breast cancer risk was investigated in a logistic regression model adjusted for potential breast cancer risk factors. Odds ratios (ORs) and 95% confidence intervals (CIs) were reported stratified by BRCA1/2 status and family history.

Results and Discussion. We observed an association of the BsmI b allele with an increased breast cancer risk (per allele OR 1.28, 95% CI 1.09-1.49, P=0.003). Subgroup analysis by BRCA1/2 status revealed that the association was restricted to BRCA1/2 non-carriers (per allele OR 1.33, 95% CI 1.11-1.59,P=0.002). In the non-carrier group, the risk was further increased in women, who reported a positive family history of breast and/or ovarian cancer in first-degree relative(s) (per allele OR 1.61, 95% CI 1.20-2.22, P=0.002). No association with breast cancer risk was detected for the FokI SNP.

Conclusion. The b allele of the VDR BsmI SNP is associated with an increased breast cancer risk in Pakistani women negative for BRCA1/2 germline mutations.

No conflict of interest.

Poster Session: Prevention and Early Detection I

550 Chemopreventive and chemosensitizing properties of N-(4-hydroxyphenyl) retinamide on Cancer Stem Cells (CSCs)

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Introduction. Cancer Stem Cells (CSCs) are transformed cells able to preserve the tumor heterogeneity by retaining self-renewal and differentiation capacities. CSCs, due to their very slow replication rates, are able to escape standard chemotherapeutic treatments, thus being clearly implicated in tumor relapse and metastatic disease. The ability of several phytochemicals to inhibit tumor onset, growth and angiogenesis, has been extensively documented and several mechanisms suggested. Many of these compounds, in fact, exert anti-oxidant, anti-proliferative, anti-angiogenic and pro-apoptotic effects on a variety of cancers, including leukemia, prostate, breast, colon, brain, melanoma, and pancreatic tumors. Further, they also affect cellular metabolism, targeting cancer cells though their metabolic derangements as well.

Material and methods. We assessed the ability of N-(4-hydroxyphenyl) retinamide (4-HPR/Fenretinide) to selectively target CSCs from breast (BC) (MCF-7, MDA-MB-231), colon (CRC) (HT-29, HTC-116) and medulloblastoma (MB) (DAOY, ONS-76) tumor cell lines as far as its capacity to sensitize CSCs to chemotherapy. To address these point, spheroid assay, flow cytometry analysis for surface antigens and migration/invasion assay were performed.

Results and Discussion. We found that 4-HPR decreased spheroids number and length in all the tumor cell lines assessed, and this was associated with the downregulation of CD44+CD24low cell population (breast) and CD133+ ABGG2+ cells (MB). Moreover, 4-HPR treatment is associated with down regulation ofOct-4 gene expression both in BC and MB-derived spheroids. Finally, 4-HPR was able to impair breast and MB-CSC ability to invade basement membrane matrix (Matrigel). Interestingly, the combination of 4-HPR with chemodrugs like Doxorubicin (BC) or 5-Fluorouracil [(5-FU) CRC] significantly enhance chemotherapy effect on CSCs in term of spheroid formation, stemness-related marker expression and invasive abilities.

Conclusion. Taken together, 4-HPR can be considered a valid compound for CSCs targeting and a promising agent to sensitize the chemoresistant-CSC to chemotherapy.

No conflict of interest.

551 miRandola database: The future of non-invasive diagnosis through circulating miRNA biomarkers

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Introduction. Non-coding RNAs (ncRNAs) such as for example microRNAs (miRNAs) are frequently dysregulated in cancer and have shown great potential as tissue-based markers for cancer classification and prognostication. ncRNAs are present in membrane-bound vesicles, such as exosomes, in extracellular human body fluids. Circulating miRNAs are also present in human plasma and serum cofractionate with the Argonaute2 (Ago2) protein and the High-density lipoprotein (HDL). Since miRNAs and the other ncRNAs circulate in the bloodstream in a highly stable, extracellular forms, they may be used as blood-based biomarkers for cancer and other diseases. A knowledge base of non-invasive biomarkers is a fundamental tool for biomedical research.

Material and method. Data is manually collected from ExoCarta, a database of exosomal proteins, RNA and lipids and PubMed. Articles containing information on circulating miRNAs are collected by querying PubMed database using the keywords 'microRNA', 'miRNA', 'extracellular' and 'circulating'. Data is then manually extracted from the retrieved papers. General information about the miRNAs is obtained from miRBase. The aim of miRandola is to collect data concerning the miRNAs contained not only in exosomes but in all kind of circulating miRNAs functionally enriched with information such as their family, diseases, processes, functions, associated tissues, and their potential roles as biomarkers.

Result and discussion. Here, we present an updated version of the miRandola database, a comprehensive manually curated collection and classification of extracellular circulating miRNAs. miRandola contains 2366 entries, with 599 unique mature miRNAs and 23 types of samples, extracted from 139 papers. miRNAs are classified into four categories, based on their extracellular form: miRNA-Ago2 (173 entries), miRNA-exosome (862 entries), miRNA-HDL (20 entries) and miRNA-circulating (1311 entries). Moreover, the database contains several tools, allowing users to infer the potential biological functions of circulating miRNAs, their connections with phenotypes and the drug effects on cellular and extracellular miRNAs.

Conclusion. miRandola is the first online resource which gathers all the available data on circulating miRNAs in a unique environment. It represents a usufeul reference tool for anyone investigating the role of extracellular miRNAs as biomarkers as well as their physiological function and their involvement in pathologies. miRandola is constantly updated by the staff as soon as new data is available and the online submission system is a crucial feature which helps ensuring that the system is always up-to-date.

The future direction of the database is to be a resource for all the potential non-invasive biomarkers such as lncRNAs, cell-free DNA and circulating tumor cells (CTCs). miRandola is available online at: http://atlas.dmi.unict.it/mirandola/

No conflict of interest.

Poster Session: Prevention and Early Detection II

553 Circulating free DNA: A 'liquid biopsy' for the early detection of cancer?

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Introduction. Circulating free DNA (cfDNA) is under intense investigation as a 'liquid biopsy' for the early detection of cancer. Clinically relevant mouse models of cancer serve as ideal platforms for analysis of cfDNA in early stage disease, since disease onset and tumour burden can be controlled, and matched tissue and blood can be isolated at defined time points. This proof-of-concept study aimed to characterise cfDNA isolated from small quantities of mouse blood in conditional V600EBraf- and G12DKras-induced models of early lung adenocarcinoma, activated via intranasal infection with adenoviral Cre recombinase.

Material and method.cfDNA was isolated from plasma using Qiagen kits and analysed by PCR (recombined Lox-V600EBraf and Lox-G12DKras alleles) and real-time quantitative PCR (total cfDNA levels). Micro-computed tomography (μCT) imaging was used to provide a longitudinal in vivo assessment of lung tumour burden. After 10 weeks, lung tissue was harvested, sectioned and stained with haematoxylin and eosin to confirm the histological nature of the lesions.

Result and Discussion. Braf+/LSL-V600E and Kras+/LSL-G12D mice developed bronchial epithelial hyperplasia, alveolar hyperplasia and papillary adenomas; however, no lung adenocarcinomas were evident. At 10 weeks post-infection, the recombined Lox-V600EBraf and Lox-G12DKras alleles were detected in cfDNA isolated from 100μl plasma of Braf+/LSL-V600E (n=3) and Kras+/LSL-G12D mice (n=8), respectively, but not wild-type mice. Total cfDNA levels were also higher in Braf+/LSL-V600E and Kras+/LSL-G12D mice than in wild-type mice (n=7).

Conclusion. This pilot study demonstrates the use of cfDNA analysis in the Braf+/LSL-V600E and Kras+/LSL-G12D mouse models, with clear evidence of the recombined alleles in cfDNA at early, premalignant stages of disease and warrants further investigation in other genetically engineered mouse models. Longitudinal blood sampling and μCT imaging will allow V600EBraf-/G12DKras-driven lung tumours to be monitored and correlated with changes in cfDNA concentration, integrity and sequence composition over time.

No conflict of interest.

Poster Session: Prevention and Early Detection II

555 Pirc rat, mutated in Apc, as a tool to study colon cancer and to identify drugs interfering with the early or late events of carcinogenesis

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Introduction. Pirc rats (F344/NTac-Apc am1137)(1), carry a mutation in the Apc gene, mutated in familial adenomatous polyposis (FAP) and in most sporadic colorectal cancers (CRC). At variance with the existing Apc-based models developing tumours mostly in the small intestine, Pirc rats develop several colon tumours, thus potentially standing as a faithful model of colon cancer. The early phases of carcinogenesis in this strain are not known, therefore we studied the apparently normal colon mucosa (NM) and the presence of microscopic precancerous lesions in Pirc rats at various ages (1- 8 months). The response to apoptotic stimuli, and the effect of drugs and natural compounds on colon carcinogenesis were also studied. **Materials and Methods.** Pirc rats, from our own colony, were sacrificed at various ages to study precancerous lesions (mucin depleted foci (MDF) (2). Apoptosis and proliferation were studied (immunohistochemistry) under basal conditions and after 1,2-dimethylhydrazine (DMH) (75 mg/kg), to induce apoptosis. Results and Discussion. MDF are already present at weaning and their number increases with age. Like colon tumours, MDF show Wnt signaling activation (nuclear β-catenin accumulation) and are dramatically decreased by Sulindac (320 ppm), a drug with chemopreventive activity. Sulindac, as well as a combination of 3,3'-diindolylmethane (250 ppm) and curcumin (2000 ppm), was also able to decrease macroscopic tumour multiplicity, suggesting a potential chemopreventive effect also of these natural compounds. Colon proliferation in the NM was higher in Pirc rats compared with wt rats. Myc and Birc5 (survivin) genes were significantly up-regulated in the NM of Pirc rats. Notably, Pirc rats showed lower apoptosis after DMH, indicating resistance to apoptosis even in the apparently normal mucosa, a phenomenon that will be further investigated and that may also help to understand resistance to chemotherapic drugs. **Conclusions.** The overall results put forward PIRC rat as a robust model of colon carcinogenesis, either to study the process itself or to test in vivo chemopreventive agents in both short- and long-term studies.

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Poster Session: Radiobiology/Radiation Oncology I

556 PARP inhibition radiosensitizes human soft tissue sarcoma cells

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Introduction. Locally advanced soft-tissue sarcomas (STS) have a poor prognosis, thus there is a clear need for novel strategies. Inhibitors of poly-ADP ribose polymerase (PARPi) have shown to enhance the cytotoxic effect of irradiation (IR) in several tumors, and evidences suggest that PARPi could be used to selectively kill cancers defective in DNA repair. Some STS are translocation-related and have defect in DNA repair systems, so there is a rationale for using PARPi in STS. We investigated the effect of PARP inhibition on survival after exposure to IR in four STS cell lines.

Materials and Methods. Clonogenic assays were performed on fibrosarcoma, liposarcoma, leiomyosarcoma and rhabdomyosarcoma cell lines. Cells were irradiated with a 2, 4 or 6 Gy, with or without olaparib (1 μM) iniparib (10 μM) or veliparib (5μM) pre-treatment. Non-irradiated cells were used as controls. Cells were incubated for 15 days, then stained with crystal violet, and colonies of more than 50 cells counted. Surviving fraction was calculated by the formula: (mean colony counts/seeded cells) × plating efficiency (PE = mean colony counts/cells seeded for non-irradiated controls). The PE of the combined treatments were normalized to PARPi-treated cells. The linear-quadratic survival expression was fitted to the data by nonlinear regression. The radiosensitization enhancement ratio for the PARPi at 50% survival (ER50) was as follows: ER50 = Dose at 50% survival without PARPi/Dose at 50% survival with PARPi.

Results and discussion. Significant radiosensitization was observed in all human STS cell lines using PARPi, with an ER50 ranging from 1.2 to 3.41.

Rhabdomyosarcoma showed the greatest increase in radiosensitivity, with an ER50 of 3.41 with veliparib. Fibrosarcoma was also highly responsive to PARPi radiosensitizing effect, with an ER50 of 2.29 with olaparib and 2.21 with veliparib. Leiomyosarcoma and liposarcoma showed similar radiation responses after PARP inhibition, with the higher radiosensitization in presence of veliparib (ER50 1.62 and 1.46, respectively).

We demonstrated that PARPi are potent radiosensitizers on human STS in vitro models. The different PARPi radiosensitizing effects observed in various cell lines may be explained by the presence of genomic aberrations in DNA repair machinery in specific STS subtypes.

Conclusion. These preliminary data encourage to further study PARPi and suggest that PARPi therapy, mostly if associated with IR, can be a promising treatment for STS.

No conflict of interest.

557 Effect of alpha lipoic acid on radiation-induced thyroid injury in rats

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Introduction. Exposure of the thyroid to radiation is often unavoidable, although the thyroid gland is a non-target organ during radiotherapy of head and neck cancers. Radiotherapy-induced thyroid disorders include hypothyroidism, thyroiditis, Graves' disease, adenoma, and carcinoma. In our previous study, α-lipoic acid (ALA) attenuates radiation-induced acute injury in rat thyroid. The present study aims to demonstrate more delayed protective effect of α-lipoic acid (ALA) on radiation-induced thyroid injury in long term period. Material and method: Rats were assigned to four groups: controls (CTL), irradiated (RT), given ALA before irradiation (ALA+RT), and received ALA only (ALA, 100 mg/kg, i.p.). ALA was administered at 24 h and 30 minutes prior to irradiation. The neck area including the thyroid gland was evenly irradiated with 2 Gy per minute (total dose of 18 Gy) using a photon 6-MV linear accelerator. Rats were sacrificed on week 4 and 8 after irradiation. We evaluated activity index including follicular size, colloid density and cell height by H&E and PAS stain. We are also assessed the tissue fibrosis and expression of TGF-β1 and collagen. To investigate effect of ALA on radiation-induced oxidative stress, immunohistochemical staining for 8-OHdG and MDA and PCR for NOX subunit mRNA level were performed. And also, GSH level was measured.

Result and discussion. All of these were induced in RT group compared to other groups and ALA pretreatment ameliorates these expression, mRNA level, and GSH level induced by radiation.

Conclusion. Therefore, this data provide that ALA pretreatment was effective not only acute injury but also delayed damage in irradiated rat thyroid model and ALA could be therapeutic drug for treatment of patients with thyroid injury during radiotherapy in head and neck.

No conflict of interest.

558 The DNA damage-induced phosphoproteome is modulated by inhibition of the MET receptor

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Introduction. Ionizing radiation (IR) is frequently used in the treatment of a variety of malignant tumors of different origins and stages. In recent years, numerous studies have demonstrated that interfering with signaling via growth factor receptor tyrosine kinases (RTKs) can increase the sensitivity of certain tumors to IR. The RTK for hepatocyte growth factor MET is aberrantly activated in numerous types of human malignancies. MET inhibition (METi) has been shown to synergize with DNA damaging agents in generation of DNA damage and interfere with damage repair. In this study, we aimed to explore how the cellular response to ionizing radiation is modulated by METi.

Material and Methods. We have recently conducted an immunoaffinity-based LC-MS/MS phosphoproteomics survey study to explore the cellular phosphoproteome following exposure of MET-addicted cancer cells to METi alone and in combination with IR. Phosphorylation sites of interest have been examined using selected reaction monitoring (SRM) and further validated in vitro and in vivo by Western blotting and immunohistochemistry, respectively.

Results and Discussion. Analysis of the survey data has identified more than 300 phosphopeptides, which have changed by at least 4-fold in one experimental condition or more. Among these, we have identified using clustering analysis a group of phosphopeptides, in which the phosphorylation is increased by the combination of METi and IR compared to IR alone. Several of these phosphorylation changes have been confirmed and further investigated by targeted proteomics in selected cell lines.

Conclusions. We have identified a sub-network of the DNA damage response (DDR) that is activated in MET-addicted cancer cells upon DNA damage and METi, and could be responsible for synergism between these two modalities. We believe that these results will aid in understanding as how MET signaling crosstalks with the DDR with subsequent translational therapeutic clinical applications.

No conflict of interest.

559 Protective effects of alpha lipoic acid on radiation-induced salivary gland injury in rats

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Introduction. Radiation therapy is a widely used treatment for patients with solid tumor. However, radiation exposure to the head and neck often induces salivary gland dysfunction. This study aims to investigate effects of alpha lipoic acid (ALA) on the radiation-induced salivary gland injury. **Material and Method.** Rats were assigned to four groups: controls (CTL), irradiated (RT), given ALA before irradiation (ALA+RT), and received ALA only (ALA, 100 mg/kg, i.p.). ALA was administered at 24 h and 30 minutes prior to irradiation.The neck area including the salivary gland was evenly irradiated with 2 Gy per minute (total dose of 18 Gy) using a photon 6-MV linear accelerator.Rats were sacrificed on day 4 and 7after irradiation. We observed that ALA restores radiation-induced histopathological changes (acini cell vacuolization, dutual cells necrosis, fibrosis) and reduces radiation-induced apoptosis in the salivary gland.

Result: Radiation-induced expression of TGF-1 and inflammation-related factors (pNF-κB, p-Iκ B-α, iNOS) and MAPKs activation were attenuated by ALA pretreatment. And also, ALA reduced radiation-induced expression of 8-OHdG, a DNA oxidative stress marker. Moreover, ALA inhibited mRNA expression of NADPH oxidase subunit induced by radiation.

Conclusion. This study suggests that ALA could be a therapeutic drug for treatment of salivary gland damage to radiation during radiotherapy of the head and neck.

No conflict of interest.

560 MET targeting sensitizes glioblastoma stem-like cells to ionizing radiation

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Introduction. Glioblastoma, the most aggressive and common type of brain tumor, is relatively rare but virtually incurable, in spite of an aggressive treatment protocol including surgery, radiotherapy, and chemotherapy. Primary resistance to DNA-damaging agents, mostly ionizing radiation, has been associated with inherent properties of the glioblastoma stem-like cell (GSC) subpopulation, but it relies on molecular mechanisms still poorly understood.

Materials and Methods. Cultures enriched in GSCs (neurospheres) were isolated from primary glioblastomas and their mutational and gene expression profile was analyzed. Neurospheres were treated with therapeutic doses of ionizing radiation (IR) and cell viability and clonogenicity were analyzed in vitro. Neurosphere subpopulations positively selected by IR were identified and characterized by flow-cytometry and cell sorting. The cell response to IR was evaluated in the presence of specific small-molecule MET inhibitors. Glioblastomas were generated by neurosphere transplantation subcutis or intracranially and treated with radiotherapy protocols in association with MET inhibitors. Tumor growth was monitored by intravital imaging.

Results and discussion. Neurospheres display extensive radioresistance if compared with their differentiated counterparts, immortalized glioblastoma cell lines, or normal astrocytes. Radioresistance is independent of the mutational status and gene expression profile. In a subset of neurospheres, characterized by low EGFR expression, ionizing radiation positively selects a subpopulation of cells that express high levels of the HGF receptor MET, and retain stem-like properties. The MET tyrosine kinase actively sustains GSC radioresistance, by promoting the DNA repair pathway. Conversely, pharmacological inhibition of MET impairs the mechanisms of GSC radioresistance and helps ionizing radiation to deplete the GSC subpopulation. Treatment of tumors generated by neurosphere transplantation shows that MET inhibitors cooperate with radiotherapy in reducing tumor volume, prolonging mouse survival and reducing the GSC frequency.

Conclusions. These findings provide preclinical evidence that MET inhibitors can radiosensitize the cancer stem cell component of glioblastoma.

No conflict of interest.

562 The role of neutrophil myeloperoxidase in radiation-induced lung damage

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Introduction. A mainstay of lung cancer treatment is radiotherapy, although its efficacy is limited by toxicity to surrounding normal tissue. A number of therapeutic approaches to overcome radiation-induced lung damage have been investigated, although none have an impact in the clinic. After irradiation, immune cells are recruited, initiating an inflammatory response that can be persistent and result in irreversible damage (fibrosis). Neutrophils, which play a major role in inflammation, abundantly express myeloperoxidase (MPO), which generates reactive oxygen species to kill pathogens. The aims of this project are to establish a model of radiation-induced fibrosis and investigate the role of MPO in radiation-induced fibrosis. Material and method: Normal lung bronchioepithelial (NBE1) and lung adenocarcinoma (A459) cells were incubated with 10 nM of MPO for 30 min and analysed by immunofluorescence and alkaline comet assay. 6-8 week old female C57BL/6 MPO wild-type (WT, n=44) or MPO knockout (KO, n=18) were irradiated (whole-thorax) using a Gulmay RS320 irradiator (1.82 Gy/min). Following radiation, lung tissue was fixed in 4% PFA and embedded in paraffin or snap frozen for subsequent protein extraction. 5 µm sections were stained by immunohistochemistry or Masson's trichrome and analysed using Aperio ImageScope software. Blood was collected at the time of sacrifice and plasma cytokines levels were analysed by ELISA.

Results and Discussion. After six months, a single dose of 10 Gy induced mild lung fibrosis, while 20 Gy and 22.5 Gy (in fractionated doses) induced the formation of dense fibrotic areas. In MPO KO mice, 10 Gy single dose induction of g-H2AX was more effectively repaired 24h after irradiation compared with MPO WT animals. Moreover, radiation-induced recruitment of neutrophils and cytokine (KC and IL-6) induction were lower in MPO KO mice compared with WT animals 1h after irradiation. In vitro, MPO was internalised and remained active in both A549 and NBE1 cells, but only induced DNA damage in A549 cells.

Conclusion. We have established a model of radiation-induced fibrosis that we will use to investigate potential therapies to overcome radiation toxicity. Currently, our data suggest that MPO activity may play a role in the acute inflammatory responses following radiation, whereas persistent unrepaired DNA double strand breaks may contribute to later effects such as fibrosis.

No conflict of interest.

Poster Session: Radiobiology/Radiation Oncology II

563 Tumor-macrophage fusion hybrids contribute to tumor radiation resistance via epithelial mesenchymal transition

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Introduction. The molecular pathways contributing to resistance to radiation remains poorly understood. Recent evidence has shown that irradiation may even promote malignant phenotypes of cancer cells. Clinical trials to improve tumor radiation sensitivity using several chemotherapy and biologic agents meet with limited success. Other novel targets should be developed to overcome resistance of tumor to radiation therapy. Cell fusion is believed to be a relatively rare and strictly regulated phenomenon taking place in fertilization, formation of placenta and muscle or bone homeostasis. Recent discoveries of broader role of cell fusion in tissue regeneration, particularly during inflammation, have revitalized the interest in cell fusion as one of the driving forces of cancer progression. Chronic inflammation or radiation has been shown to dramatically increase the frequency of cell fusion between hematopoietic cells and various somatic cells. Definitive and direct

observation of cell fusion in human cancer has been reported in renal cell carcinoma and metastatic melanoma to brain after allogeneic bone marrow transplantation from donors of the opposite gender.

Material and method. Murine pancreatic ductal adenocarcinoma cells, Panc-02, and lewis lung carcinoma (LLC) cells were labeled with PKH26. Murine bone marrow cells or macrophages were labeled with PKH67. Cell fusion phenomenon, colony assay and expression of epithelial, mesenchymal, stem cell protein markers were evaluated in parental, indirect and direct co-culture system. Cre-recombinase transfected LLC and Panc-02 cells were subcutaneously implanted into dual bioluminescent reporter transgenic mice (loxP-DsRed-STOP-loxP-EGFP). Cell fusion were measured by GFP cells using immunoluorescence staining and flowcytometry within tumors with or without macrophage depleting agents, carrageenan, before local radiation.

Results and discussion. Cancer cells cultured directly with macrophages showed higher colony formation after radiation compared to that of indirectly co-cultured or parental cancer cells. Isolated fusion hybrids revealed more radio-resistance and expressed higher level of vimentin, CD133 with significantly decreased E-cadherin proteins compared to those of indirectly co-cultured or parental cancer cells.

Progressively increased GFP cells were noted in tumors of cre-loxP murine model. After carraggeenan treatment, the frequency of fusion decreased significantly. Flowcytometry revealed decreased mesenchymal and stem cell markers of tumor cells after carrageenan treatment.

Conclusion. In this study, we demonstrate that macrophage is a principle fusion partner of cancer. After fusion between cancer cells and macrophages, cancer cells undergo epithelial mesenchymal transformation, express cancer stem cell phenotypes and become resistance to radiation.

No conflict of interest.

564 Radiosensitization with combined use of olaparib and PI-103 and underlying mechanism in triple-negative breast cancer

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Introduction. Triple-negative breast cancer (TNBC) shows aggressive clinical behavior, but the treatment options are limited due to lack of a specific target. TNBC shares many clinical and pathological similarities with BRCA-deficient breast cancer, for which poly(ADP-ribose) polymerase (PARP) inhibitor is effective, but PARP inhibitor alone failed to show clinical effects in patients with sporadic TNBC. Radiation induces DNA double-strand breaks, and the phosphoinositide 3-kinase (PI3K) signaling pathway has been known to regulate steady-state levels of homologous recombination. A recent preclinical study showed that PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient TNBC to PARP inhibition. Therefore, we assessed the radiosensitizing effect, and the underlying mechanism of combination treatment with PARP inhibitor olaparib and PI3K inhibitor PI-103 in BRCA-proficient TNBC cells.

Material and Method. MDA-MB-435S cells were divided into four treatment groups, irradiation (IR) alone, olaparib plus IR, PI-103 plus IR, and olaparib plus PI-103 plus IR. Cells were exposed to the drugs for 2 hours prior to irradiation, and the cell survival curve was obtained using a clonogenic assay. Western blotting and immunofluorescent detection of γH2AX foci were performed. Xenograft and bioluminescence imaging were carried out to assess in vivo radiosensitivity.

Results. Combined use of olaparib and PI-103 enhanced radiation-induced death of MDA-MB-435S (sensitizer enhancement ratio[SER]0.05,1.7) and MDA-MB-231-BR (SER0.05,2.1) cells and significantly reduced tumor volume in a xenograft models (P<0.001). Treatment with PI-103 showed persistent γH2AX foci, indicating delayed repair of DNA strand breaks. PI-103 alone increased levels of poly(ADP-ribose) and phosphorylated extracellular signal-regulated kinase, and downregulated BRCA1.

Conclusions. Combined use of olaparib and PI-103 enhanced radiation-induced cell death in BRCA-proficient MDA-MB-435S and MDA-MB-231-BR cells and xenografts. TNBC patients have high incidences of locoregional relapse and distant metastasis, and radiation therapy targets both locoregional control and treatment of distant recurrences such as brain metastasis or other oligometastasis. Targeting of the PI3K signaling pathway combined with PARP inhibition maybe a feasible approach to enhance effects of radiation in BRCA-proficient TNBC. [Work supported by grant (#2012-0004867 & #2013R1A1A2074531) from National Research Foundation, Korean Ministry of Future Creative Science to In Ah Kim.]

No conflict of interest.

565 Early positive detection of tumor radiotherapy effect by mitochondrial complex I activity using a novel PET probe [18]F-BCPP-EF

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Introduction. Anaerobic glycolysis is the main pathway of energy production in the tumor (Warburg effect) while oxidative phosphorylation in mitochondria is suppressed. Radiotherapy is reported to switch this 'Warburg effect' to aerobic metabolism to induce apoptosis in the tumor. We developed a novel PET probe, 18F-2-tert -butyl-4-chloro-5-{6-[2-(2-fluoroethoxy)-ethoxy]-pyridin-3-ylmethoxy}-2H-pyridazin-3-one (18F-BCPP-EF), for positive imaging of mitochondrial complex I (MC-I) activity. In this study, early detection of the effect of tumor radiotherapy was evaluated using 18F-BCPP-EF as a new PET probe.

Materials and Methods. C3H/HeN mice inoculated with murine squamous cell carcinoma were treated with a single dose of x-ray irradiation; 0, 6, 15 or 30 Gy on day 0. Tumor uptake of 18F-BCPP-EF and 18F-FDG was serially examined using PET in 2, 3, 4, 5, 7 days and 2, 7, 10, 14 days after irradiation, respectively (n=4 for each timing and dose). Apoptosis incidence in the tumor tissue was determined by TUNEL staining.

Results and Discussion. Tumor growth suppression was dose-dependent after irradiation; 6 Gy was mildly effective to suppress growth by half of non-treated (0 Gy) control, 15 Gy and 30 Gy were effective to suppress tumor growth as the original size (15 Gy) and undetectable size (30 Gy) 14 days after treatment. While 18F-FDG uptake was initially increased and eventually decreased 10 days after 30 Gy radiation when tumor size was already reduced. In contrast, 18F-BCPP-EF uptake was significantly increased as early as 2 days (15 Gy) or 3 days (30 Gy) after irradiation when tumor size showed no difference regardless of radiation doses. Apoptosis incidence in the tumor tissue paralleled to the changes in 18F -BCPP-EF uptake by PET.

Conclusion. Tumor uptake of 18F-BCPP-EF was increased early after effective doses of irradiation when 18F-FDG uptake remained elevated due to radiation-induced inflammation. The results suggest that 18F-BCPP-EF is a promising 'positive' MC-I imaging PET probe for early detection of adequacy of tumor radiotherapy.

No conflict of interest.

567 Effect of alpha lipoic acid on radiation-induced small bowel injury in mice model

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Introduction. Radiation therapy is a highly effective treatment for patients with solid tumor. However it causes to a normal tissue damage including inflammation and deteriorates quality of life as well as decrease of the treatment efficacy. Tolerance dose for a normal tissue is limiting factor in radiation therapy and also an important factor for determining the occurrence and severity of radiation-induced complication. This study aims to investigate effects of alpha lipoic acid (ALA) on enhancing the tolerance dose of normal tissue and to increase the effectiveness of radiation therapy by reducing the radiation-induced complication.

Materials and Methods. Forty-three Balb/c mice (7-8 week, male) were randomly assigned to four groups: healthy control (CTL, n=9), alpha lipoic acid alone (ALA, n=9), irradiated (RT, n=12), and received ALA before irradiation (ALA+RT, n=13). Whole abdomen was evenly irradiated with 2 Gy per minute (total dose of 15 Gy) using a 6-MV linear accelerator with 1.5 cm thick tissue-equivalent bolus on day 0. Mice were treated with either ALA (100 mg/kg, i.p.) or saline (equal volume, i.p.) for three days on day -2 to 0. Mice were sacrificed on day 3, 7, 14 after irradiation. Body weight, food intake, histopathology and biochemical analysis were evaluated. This experiments were approved (GNU-130621-M0039) by the Gyeongsang National University Institution Animal Care & Use Committee.

Results and Discussion. A significant difference for body weight and food intake was observed between the RT and the ALA+RT group. Loss of body weight was much more definite in the RT than other groups. Recovery of body weight was more delayed and did not recovered as much as initial weight in the RT alone than ALA+RT group. There was also a significant pathologic difference between RT and the ALA+RT group. Moreover, crypt cells were more conserved and recovered in the ALA+RT group. Inflammation was decreased and recovery time was shortened in the ALA+RT group compared to RT group. Expression of inflammation-related factors (pNF-kB, pIkB-a, MMP-9) and MAPKs were significantly decreased in the ALA+RT group compared to RT alone.

Conclusions. The present study showed that ALA pretreatment decreased the severity and duration of radiation-induced enteritis by reducing inflammation and enhancing the recovery. Thus, ALA could be one of therapeutic drugs for treatment radiation-induced enteritis.

Key Words: alpha lipoic acid, radiation, small bowel, radio-protection, inflammation

No conflict of interest.

569 Attenuation of radiation-induced lung damage by a PPAR-gamma agonist

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Introduction. Peroxisome proliferator activated receptor (PPAR)-gamma agonists have anti-inflammatory, anti-fibrotic and antineoplastic effects. These properties make PPAR-gamma agonists interesting drugs to investigate for prevention and therapy of radiation-induced toxicities. The aim of this study was to evaluate effect of rosiglitazone, a PPAR-gamma agonist, on a murine model of pulmonary damage induced by irradiation. In addition, we aimed to verify if the radioprotective effect of RGZ was selectively exerted in normal tissues or it occurred also in tumour tissues.

Material and methods. Mice were irradiated selectively on the lungs with a single dose of 19 Gy, with or without 5 mg/Kg/day rosiglitazone started 24 h before irradiation. During the observation period, CT was performed and Hounsfield Units (HU) from lung regions were determined. After 16 weeks lungs were collected for histological and molecular analysis. A549 tumour xenografted mice were irradiated (15 Gy) with or without rosiglitazone; tumour volumes were measured for 35 days.

Results and discussion. Rosiglitazone markedly reduced radiologic and histologic signs of fibrosis, inflammatory infiltrate, alveolar structures alteration and HU lung density increase induced by irradiation. Moreover rosiglitazone significantly decreased radiation-induced Col1, NF-kB, TGFβ protein expression and reduced A549 xenografted tumour growth in mice.

Conclusions. We demonstrated that rosiglitazone exerts a protective effect on normal tissues and reduces inflammation and density in radiation-induced pulmonary damage. Moreover, we confirmed rosiglitazone antineoplastic properties and demonstrated its radiosensitizing effect. Thus, PPAR gamma agonists should be further investigated as radioprotective agents in lung cancer.

No conflict of interest.

Poster Session: Signalling Pathways I

570 Peptide-guided targeting of GPR55 for new therapeutic strategies of cancer

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Introduction. Increasing evidence indicates that G-protein-coupled receptors (GPCRs) are 'druggable' targets in cancer treatment and prevention1. GPR55 was recently identified as the lysophosphatidylinositol receptor, and several aspects of its physiology are still under investigation2. GPR55 has a role in the regulation of cancer progression, being its expression correlated with the invasive potential of metastatic cells and bone metastasis formation2. In order to develop innovative cancer therapy, this study is aimed to interfere the GPR55 function in cancer cells with peptidic binders.

Material and method. Whole-cell-based screening of M13-phage-displayed random library3 was performed using as bait the GPR55 receptor heterologously expressed in HEK293 cells. This method has been largely used in reverse pharmacology to identify ligands of orphan receptors. The 7-mer insert peptides in M13-phage library were flanked by a pair of cysteine residues resulting in cyclized peptides stabilized by disulfide bond. Binding specificity to GPR55-positive cells was verified using synthetic FITC-labelled peptides by FACS and confocal microscopy. The effect of peptide binding to GPR55 was analysed in lysphosphatidyinositol-triggered GPR55 signalling, including intracellular calcium, AKT phosphorylation, modulation of actin cytoskeleton, and receptor internalization.

Results and discussion. By screening M13-phage-displayed random library, we have selected a peptide that binds specifically to HeLa cells expressing GPR55 and not to cells interfered for GPR55. Binding specificity of the FITC-labelled peptide was further investigated in competition assays with unlabelled peptide, and confirmed by the use of scramble analogues. The identified peptide did not affect significantly the GPR55 signalling; however, it showed a significant modulation of both basal and agonist-induced GPR55 internalization. This analysis has been extended to lymphoblastoid leukemic cells.

Conclusions. Based on preliminary results, we propose the use of GPR55-peptidic binders for in-vivo diagnosis of GPR55-positive tumor cells, specific delivery of chemotherapeutic agents to cancer cells, and direct modulation of receptor activities for therapeutic applications.

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No conflict of interest.

571 Integrated quantitative experimental and computational approach for characterizing non-genetic drug resistance mechanisms

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Introduction. Targeted cancer therapeutics such as tyrosine kinase inhibitors (TKIs) have seen constraint in clinical efficacy due to both intrinsic and acquired resistance. Indicators for sensitivity to TKIs may include genetic mutations or protein-level overexpression of targeted or bypass receptor tyrosine kinases (RTKs). While the latter is often attributed to gene amplification, genetic characterization of tumor biopsies has failed to explain substantial proportions of resistance; e.g., 30% of drug resistant tumors to EGFR TKIs in non-small cell lung carcinoma (NSCLC)[1]. Therefore we hypothesize that post-translational mechanisms governing RTK levels, including receptor trafficking and proteolytic cleavage by pericellular proteases, may represent underappreciated contributors to drug resistance.

Materials and Methods. An experimental model of acquired drug resistance was generated in the NSCLC cell line HCC827 as previously described [2]. Principal component analysis (PCA) was performed to compare RTK lysate and supernatant measurements between sensitive and resistant cell lines with and without gefitinib (EGFR TKI) treatment. Samples were measured quantitatively by multiplexed bead-based ELISA. Steady state and dynamic RTK internalization was measured with cleavable cell permeable and impermeable biotin labels respectively. A differential equation (DE) model was formulated and fit to experimental data using a direct search algorithm in MATLAB, permitting estimation of changes in cellular parameters associated with resistance.

Results and Discussion. PCA analysis indicates that sensitive and resistant cells have a differential response in RTK levels and shedding to gefitinib. Lysate RTK levels increased in resistant cells while supernatant levels increased in sensitive cells, with MET prominent among the separating variables in the PCA analysis; MET RTK levels increased and percentage shed MET decreased in resistant cells after treatment. DE analysis supports the finding that post-translational mechanisms most strongly influenced the change in MET lysate levels, potentially sensitizing these cells to MET combination therapy.

Conclusion. We have demonstrated a combined computational and experimental framework novel in its quantitative assessment of the contributions of various mechanisms involved in resistance to EGFR inhibition in NSCLC cell lines. Ongoing work includes extending the model to downstream bypass signaling effects.

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Conflict of interest: Advisory board: DAL: Merrimack Pharmaceuticals
Other substantive relationships: DAL: consultant for Genentech

572 Effects of KEAP1 genetic and epigenetic silencing in SCLC cell lines

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Introduction. Nuclear factor erythroid-2 related factor 2 (Nrf2) is a redox-sensitive transcription factor that positively regulates the expression of genes encoding antioxidants, xenobiotic detoxification enzymes, and drug efflux pumps, and confers cytoprotection against oxidative stress and xenobiotics in normal cells. Kelch-like ECH-associated protein 1 (Keap1) negatively regulates Nrf2 activity by targeting it to proteasomal degradation. We have just described a Keap1/Nrf2 axis full genetic and epigenetic characterization of SCLC cell lines that revealed unreported molecular alterations of the Keap1/Nrf2 axis.

Materials and Methods. The downstream effects of the genetic and epigenetic alterations of the KEAP1 gene were investigated in 12 cell lines derived from human small cell lung carcinoma (SCLC) in terms of modulation of the KEAP1 transcript and protein levels, nuclear accumulation of Nrf2 and enhancing transcriptional induction of xenobiotic metabolism enzymes. Additional functional analysis of short interfering RNA (siRNA) inhibition of the KEAP1 and treatment with 5-aza-2'-deoxycytidine (DAC) were performed.

Results and discussion. Our analysis revealed that the Keap1 mRNA and protein level decreased significantly in genetic and epigenetic alterated SCLC cell lines compared with those without any alterations. Conversely Nrf2 levels and protein nuclear localization were increased and these modifications were associated with a parallel increase in the expression of AKR1C1, TXN1 and NQO1 at the cellular level. Silencing RNA experiments in vitro in H1184 and H69V were performed to confirm the cause-effect relation between the gain of Nrf2 and the increase in AKR1C1, TXN1 and NQO1 expression. Treatments with 5-aza-2'-deoxycytidine restored the expression of KEAP1 in SCLC cells and replace the functional Keap1/Nrf2 equilibrium.

Conclusions. Our data provide new insights into the potential downstream effects of genetic and epigenetic Keap1/Nrf2 molecular deregulation in SCLCs, suggesting that the impairment of Keap1 activity actually induces the expression of cytoprotective enzymes also in SCLC cells. Validations on tissues from SCLC patients combined with in vitro pharmacological studies are required to establish new combination of therapeutic strategies in targeted cancer treatments of this aggressive lung tumour type.

No conflict of interest.

573 Nrf2-keap1 axis: Uncovers molecular profile in Lung Carcinoids

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Introduction. The Keap1/Nrf2 pathway is a master regulator of antioxidants and cellular stress responses implicated in resistance of tumour cells against chemotherapeutic drugs. Recent data suggest that genetic and epigenetic mechanisms may play a pivotal role in the regulation of KEAP1 expression in Non

Small Cell Lung Cancer. At present, data concerning the mechanism of alteration of Nrf2-Keap1 pathway in carcinoids of the lung remain almost incomplete.

Materials and Methods. Here we report a comprehensive molecular characterization of Keap1/Nrf2 axis in cell lines and 48 tissues from patients with carcinoid tumors of the lung by integrating data from alterations at DNA, transcript and protein levels.

Results. A hypermethylation of the CpGs island located in the P1 promoter region of KEAP1 was detected in 16 out of 32 typical carcinoids (50%) and 8 out of 16 atypical carcinoids (50%). No somatic mutations were detected either in the kelch-repeats regions of the KEAP1 gene or in the Nhe2 domain of NFE2L2gene, whereas LOH at the KEAP1 locus (19p13.2) was found in more than 50% of the cases, suggesting that biallelic inactivation of KEAP1 in lung cancer is a common event in lung carcinoids. Decreased of the KEAP1 expression in methylated cancer cells induced greater nuclear accumulation of Nrf2, causing enhance of transcriptional xenobiotic metabolism enzymes.

Conclusions. This is the first study to our knowledge that provides new insights into the mechanism of deregulation of the Nrf2/Keap1 detoxification pathway in lung carcinoids. Loss of KEAP1 function leading to constitutive activation of Nrf2-mediated gene expression in cancer suggests that deregulation of the Keap1/Nrf2 system could play a pivotal role in the pathogenesis of carcinoids.

No conflict of interest.

Poster Session: Signalling Pathways I

576 The Extracellular signal-regulated Kinase 5 is required for human melanoma cell growth

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Introduction. Melanoma is the most aggressive and lethal among skin cancers, known for its high metastatic potential, enhanced heterogeneity, and resistance to chemotherapy. Mitogen-Activated Protein Kinases are often targets for the treatment of cancer, including melanoma. Nevertheless, available treatments for melanoma, especially in its intermediate or advanced stages, are unsatisfactory. The possibility of an involvement of the MAPK Extracellular signal-Regulated Kinase 5 (ERK5) in the growth of melanoma is an unexplored issue.

Material and method. Cell lines and patient-derived primary melanoma cells (SSM2c, M32 and M26c, expressing wild type B-RAF, A375, M32 and M5 cells expressing V600E mutated B-RAF) have been used for in vitro experiments and xenografts in mice. ERK5 inhibition was obtained using an ERK5 specific inhibitor, XMD8-92, or lentiviral vectors encoding for ERK5 specific shRNA.

Result and discussion. In silico data analysis indicated that components of the ERK5 pathway (MEKK2, MEKK3, MEK5, ERK5, MEF2D) may be altered (including increase in gene copy number, mutations - irrespectively of their effect on kinase activity or other biological functions -, and mRNA upregulation) in up to 40% melanoma patients. We found that ERK5 protein is consistently expressed in melanocytes, A375 melanoma cell line and in primary melanoma cells derived from 7 patients. Moreover, ERK5 was constitutively phosphorylated in Thr218/Tyr220 in 24 hours serum-starved A375, M5 and M32 cells pointing to a possible link between B-RAF activation and ERK5 phosphorylation. Pharmacologic ERK5 inhibition dose-dependently decreased melanoma cell number in culture in either cells expressing wt (IC50 after 72 hours 3-3.5 μM) or V600E B-RAF (IC50 after 72 hours 2.1-2.5 μM). Consistently, genetic inhibition of ERK5 markedly decrease the number of cells in culture in patient derived melanoma cells expressing wt or V600E mutated B-RAF. Treatment with XMD8-92 induced G2/M arrest of wt B-RAF expressing cells. On the other hand, we found an increase of the percentage of cells in S phase, at the expense of those in G0/G1 in cells expressing V600E B-RAF. Genetic inhibition of ERK5 in SSM2c and M5 markedly or totally, respectively, impaired melanoma cell growth in xenografts in mice.

Conclusion. ERK5/BMK1 may be a suitable target for the treatment of melanoma. The recent development of ERK5 inhibitors allows translating experimental findings to a preclinical setting.

No conflict of interest.

577 RET targeting with small kinase inhibitors in ER positive breast cancer xenografts sensitive to aromatase inhibitors

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Introduction. The majority of breast cancers are estrogen receptor positive (ER+) and depend on estrogen for growth and survival. Blockade of estrogen biosynthesis by aromatase inhibitors (AIs) is the first-line endocrine therapy for post-menopausal women with ER+ breast cancers. Despite providing substantial improvements in patient outcome, resistance to such therapy remains a major clinical challenge. We have previously demonstrated that upregulated GDNF/RET signaling pathway in a subset of ER+ breast cancers is responsible for AI resistance. Here we assess the efficacy of different small kinase inhibitors known to target RET and investigate the potential of combining in vivo and in vitro a RET inhibitor with the AI letrozole.

Material and Method. MCF7 cells were subjected to different concentrations of small kinase inhibitors known to target RET: sunitinib, XL-184, NVP-BBT594 or NVP-AST487. For in vivo studies, ovarectomized mice were inoculated with MCF7-AROMA cells and given daily subcutaneous injections with the aromatase substrate androstenedione. Once tumors were detectable, mice were treated with either letrozole, RET inhibitor or the combination of the two drugs.

Results and Discussion. NVP-BBT594 and NVP-AST487 were shown to inhibit GDNF-induced RET downstream signaling at similar concentrations, within the nanomolar range. NVP-AST487 was chosen for further experiments due to the better tolerability and less cytotoxic effects in mice. The lower dose of NVP-AST487 demonstrated to inhibit RET downstream signaling was also able to inhibit GDNF-enhanced growth of 3D MCF7-AROMA tumor spheroids in vitro. Surprisingly, letrozole, NVP-AST487 and the combination of the two drugs were similarly effective in impairing tumor volume in MCF7-AROMA cells xenografts. Importantly, immunohistochemical analysis revealed that xenografts treated with the NVP-AST487 inhibitor were characterized by high necrotic areas. These data are consistent with previous studies (Gattelli et al., 2013) demonstrating in a different in vivo model that NVP-AST487 was as effective as the pure ER-antagonist, fulvestrant, in reducing primary mammary tumor growth. Crucially, our 3D in vitro assays demonstrate that letrozole treatment did not inhibit GDNF-stimulated tumor spheroid growth while Gatteli et al. demonstrated that NVP-AST487 was superior to fulvestrant in impairing metastatic dissemination.

Conclusion. Targeting basal RET signaling in AI-sensitive xenografts is as effective as using the current therapeutic regimen with endocrine therapy. However, these data suggest that when tumors display enhanced RET signaling as a result of GDNF stimulation, RET inhibition in combination with endocrine therapy is required to reduce ER+ breast primary tumor growth and delay cancer cells dissemination.

No conflict of interest.

579 Interplay between metabolism and the Hippo signaling pathway in T cell leukemia

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The general mechanism underlying the tumor suppressor activity of the Hippo signaling pathway remains unclear. The Hippo signaling pathway comprises a central kinase cassette (CKCA) and consists of MST1/2 kinase, LATS1/2 kinase, and two scaffold proteins, known as SAV1 and MOB1A/1B. CKCA activity inhibits complex formation between the TEAD transcription factors and the YAP/TAZ effectors. In this study, we explore the molecular mechanisms connecting the Hippo signaling pathway with metabolism in T-ALL cells. Using bioinformatic approach and molecular biology techniques we have identified two key regulators of glycolysis, C-MYC and GLUT1, as targets of the Hippo signaling pathway in human T-ALL cells.

Our results revealed that activation of MST1 inhibited the expression of GLUT1 and C-MYC. Furthermore, RNAi experiments confirmed the regulation of GLUT1 and C-MYC expression via the MST1-YAP1-TEAD1 axis. Surprisingly, YAP1 was found to positively regulate C-MYC mRNA levels in complex with TEAD1 but negatively regulate C-MYC levels in cooperation with MST1. Hence, YAP1 serves as a rheostat for C-MYC, which is regulated by MST1. Moreover, the specific inhibition of MST1 expression resulted in an increase in lactate production in T-ALL cells, whereas the specific inhibition of TEAD1 expression resulted in a reduction of lactate production. Additionally, we found out simvastatin as inhibitor of proliferation and C-MYC expression in T-ALL cells and this inhibition depends on the Hippo pathway activity. Simvastatin represents an FDA approved drug largely used to lower the cellular cholesterol levels in patients with hypercholesterolemia by inhibiting the enzyme HMG-CoA reductase.

Altogether, our results promote connections between the Hippo signaling pathway and metabolism of T-ALL cells as a new target for leukemia treatment.

No conflict of interest.

580 Combinations of ARQ087 with chemotherapeutic agents are safe and show a striking antitumor activity in different xenograft models

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Introduction. ARQ087 is a non ATP-competitive tyrosine kinase inhibitor with activity against the FGFR receptor family, and currently in Phase I clinical studies for the treatment of advanced solid tumors. It potently inhibits FGFR1, 2 and 3, as well as mutant FGFR2 (N549H) and FGFR3 (K650E/M) with IC50 values in the low nanomolar range in biochemical assays. ARQ087 has shown potent antiproliferative activity in a panel of cancer cells with deregulated FGFR1 and FGFR2 pathways and exerted a dose-dependent antitumor activity in in vivo systems with FGFR1/FGFR2-driven xenografts model (H1581 and SNU16 respectively) and models harboring FGFR2-activating mutation (AN3CA and MFE296). Given orally in a 15 daily administration, ARQ087 has shown a very safe profile while inducing tumor regressions in FGFR-driven models. The feasibility of combining ARQ087 with chemotherapy was investigated in H1581 FGFR1-amplified lung xenograft and in SNU16 FGFR2-driven gastric xenografts.

Material and method. Tumor bearing nude mice were randomized to receive vehicle, ARQ087, paclitaxel, carboplatin as single agents, ARQ087 and paclitaxel, ARQ087 and carboplatin, paclitaxel and carboplatin as doublets, and the triple combination of ARQ087 with carboplatin and paclitaxel. Similar experimental setting was applied in mice bearing SUN16 xenografts, but instead of paclitaxel, capecitabine was given. The

choice of the anticancer agents to be combined with ARQ087 was supported by the fact that these drugs are currently used for the treatment of various cancers clinically.

Results and discussion. In both experimental settings the drugs given as single agents showed moderate antitumor activity. Combinations of two agents was more active than the single ones alone and combination of three agents showed the best antitumor activity. In particular, combination of ARQ087 with paclitaxel and carboplatin in H1581 bearing mice resulted in tumor regression and only 1/8 tumor re-grew after 30 days from the last drug administration versus 6/8, 4/8 e 5/8 in mice treated with paclitaxel alone, combination of ARQ087 with paclitaxel, and combination of paclitaxel with carboplatin respectively. Of note, no toxic deaths nor premature stopping nor delaying of drug administration occurred.

Conclusion. These data clearly suggest that ARQ087 can be safely combined with chemotherapy and that the combinations are much more active than the monotherapies.

No conflict of interest.

581 miR-30a inhibits endothelin A receptor expression and acquisition of chemoresistance of ovarian carcinoma

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Introduction. Chemotherapy is the preferred therapeutic approach for the therapy of epithelial ovarian carcinoma (EOC), but a successful long-term treatment is prevented by the development of drug resistance. Endothelin-1 (ET-1)/ETA receptor (ETAR) axis is upregulated in chemoresistant EOC cell lines and associated with acquisition of chemoresistance and epithelial-mesenchymal transition (EMT) phenotype and poor prognosis. Therefore, interference with ET-1/ETAR signaling pathway has emerged as a promising strategy for cancer therapy. Because recent works have underlined the involvement of microRNAs (miRNAs) in cancer development and drug-resistance, in this study we determined whether in EOC cells an aberrant expression of miRNAs might regulate ETAR expression and downstream effects.

Materials and Methods. miRNAs profile was assessed in A2780 and 2008 EOC cell lines and their cisplatinum-resistant variants, and in human sensitive and cisplatinum-resistant EOC tumors, by qPCR. ETAR expression was revealed by WB and IHC. miRNA specific ETAR 3'UTR binding was evaluated by luciferase reporter assay. The biological effects of miRNA in EOC cell lines were analyzed by cell viability, TUNEL, invasiveness and vasculogenic mimicry assays.

Results and Discussion. Based on bioinformatics tools, we selected putative miRNAs able to recognize the 3'UTR of ETAR. Among these, only miR-30a resulted downregulated in chemoresistant EOC cells compared with parental cells and directly suppressed the ETAR protein expressionvia sequence-specific interaction with 3'-UTR of ETAR. Moreover, miR-30a overexpression was able to restore drug sensitivity by enhancing the susceptibility of these cells to cisplatinum-induced apoptosis. In addition, miR-30a re-introduction in chemoresistant EOC cells, impaired their capacity to promote the invasive process, reverted EMT phenotype and reduced the vasculogenic mimicry. Similarly, we demonstrated that the treatment with macitentan, a potent ETAR antagonist with significant affinity for ETBR, counteracted the effects elicited by ETAR pathway in chemoresistant EOC cells. In resistant EOC xenografts macitentan reduced tumor growth and metastatic progression and sensitized to chemotherapy. Finally, in specimens from EOC patients low expression of miR-30a was associated with poor clinical outcome and inversely correlated with ETAR expression.

Conclusion. This study for the first time identifies miR-30a dependent regulation of ETAR in human chemoresistant ovarian cancer, further supporting that the regulation of ETAR represents a valid therapeutic opportunity for circumventing chemoresistance in EOC patients.

No conflict of interest.

583 Overcoming TKI resistance in chronic myeloid leukemia and EGFR mutant glioblastoma by targeting a Nox2-Egr1-Fyn pathway

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Introduction. Targeting of oncogenic kinases for cancer therapy has yielded important translational milestones, and has significantly impacted patient care, particularly for malignancies bearing BCR-ABL1 (the oncogene responsible for chronic myeloid leukemia, (CML)) and EGFR (which is amplified or mutated in a number of tumors). Challenges, however persist, particularly with regard to resistance or lack of response to the small molecule tyrosine kinase inhibitors (TKI). Lessons learned from the targeting of BCR-ABL1 in CML have been translated to other malignancies characterized by constitutively active kinases. Amongst the tumors bearing constitutively active EGFR, a subset of glioblastoma (GBM) that carries mutant EGFR (vIII), is amongst the worst, prognostically and displays low TKI responses, with a mean survival of fourteen months. Therefore strategies to enhance TKI action are highly relevant to many tumor types with CML and EGFR mutant GBM as prototypic examples.

Materials and Methods. Human TKI sensitive and resistant CML cell lines, as well as human glioblastoma cells expressing either wild type EGFR, or mutant EGFR (vIII or R108K) were used. Oxidative stress, oxygen consumption, transcript and protein expression of Fyn, Egr1 and the Nox2 component, p47phox, were evaluated in all lines and compared across sensitive and resistant isogenic cell lines. Effects of knockdown of Fyn, Egr1, and p47phox on cell viability and proliferation in the presence and absence of TKI were evaluated.

Results and Discussion. Using BCR-ABL1 and EGFRvIII expressing models, and stem cells from CML and GBM patients, we have identified a novel pathway that can be targeted to overcome TKI resistance or sensitize cells to TKI. This Nox2-Egr1-Fyn pathway is transcriptionally upregulated in both CML and EGFR amplified GBM. Interestingly, this pathway is dependent upon oxidative stress stemming from the active kinase in both CML and GBM models. Our preliminary data indicates that in both BCR-ABL1 expressing and EGFRvIII expressing cells, the major source of oxidative stress is the NADPH oxidase. Compellingly, a subunit of the Nox2 isoform, p47phox, is upregulated in both systems, and knockdown reduces expression of Egr-1 and Fyn.

Conclusion. Targeting the p47 component of Nox2 or the transcription factor Egr-1 directly may be a conserved route for sensitization to TKI in refractory CML and GBM.

No conflict of interest.

584 PGE2 via EP4 receptor trans-activates the Colony-Stimulating Factor-1 (CSF-1) receptor and via the kinase ERK1/2 synergizes with CSF-1 in the induction of macrophage migration

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Introduction. Prostaglandin E2 (PGE2) is a key mediator of immunity, inflammation and cancer. PGE2 exerts its autocrine/paracrine effects on target cell binds to four specific G-protein-coupled (GPCRs) termed EP receptors (EP1-4). The complexity of PGE2 signaling is further increased by the existence of a cross-talk between EP receptors and tyrosine kinase receptors (TKR) such as Fibroblast Growth Factor Receptor 1 (FGFR1) or Epidermal Growth Factor Receptor (EGFR). The Colony-Stimulating Factor-1 Receptor (CSF-1R) is a TKR that supports the survival, proliferation, and motility of monocytes/macrophages, which are essential components of innate immunity and cancer development. The aim of this study was to investigate on a possible cross-talk between EP and CSF-1R.

Materials and Methods. The experiments were performed with two murine macrophage cell lines, BAC-1.2F5 and RAW264.7 that express physiologically high amounts of CSF-1R protein. CSF-1R genetic inhibition was performed using specific siRNA. Pharmacologic inhibitors used were: PP2 and SU6656 (1 μM) for Src family kinases (SFK); CI-1040 (1 μM) and UO126 (10 μM) for EK1/2; GW2580 (1 μM) for CSF-1R, L-161982 (1 μM) for EP4 antagonist. To evaluate which EP receptors were involved in the cross-talk we used specific agonists and antagonists of EP (1 μM). Migration assays were performed with modified Boyden chambers.

Results. In either BAC-1.2F5 or RAW264.7 1 μM PGE2 induced rapid CSF-1R phosphorylation, wich was dependent on SFK activation. Also, in fibroblast expressing ectopic CSF-1R we found that 1 μM PGE2 induced CSF-1R phosphorylation and its downstream signaling indicating that the effect of PGE2 on CSF-1R is not restricted to macrophages. Genetic and pharmacolgical inhibition of CSF-1R reduced PGE2-elicited ERK1/2 phosphorylation and macrophage migration, indicating that CSF-1R plays a role in PGE2-mediated immuno-regulation. The inhibition of EP4 receptor prevented PGE2-induced macrophages migration and CSF-1R phosphorylation, indicating that EP4 is responsible for the cross-talk between PGE2 and CSF-1R.

Furthermore, at low concentrations (i.e. below IC50) PGE2 synergized with low concentrations of CSF-1 in inducing macrophage migration, as well as ERK1/2 phosphorylation. ERK1/2 inhibition completely blocked the migration induced by the combination CSF-1/PGE2. These results were confirmed in primary murine macrophages.

Conclusions. Our results indicate that PGE2, through its EP4 receptor, trans-activates CSF-1R via SRC and synergizes with CSF-1R-dependent signaling at the level of ERK1/2 in promoting macrophage migration. This synergistic interaction is likely to play an important role in the initiation and progression of inflammatory diseases as well as cancer.

No conflict of interest.

585 TRIB2 promotes resistance to chemotherapeutics by deregulating the AKT signalling network

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Introduction. Intrinsic and acquired cancer cell resistance to conventional and targeted chemotherapy is the primary reason for treatment failure in many patients. The identification of molecular mechanisms involved in drug resistance that could be targeted is of enormous clinical importance. The constitutive activation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway are often found in tumors and as a result, specific PI3K or AKT inhibition has become a key pharmaceutical objective. As such, crucial transcription factors such as forkhead box O (FOXO) proteins and p53 have been shown to mediate the action of multiple anti-cancer drugs, including PI3K pathway inhibitors.

Materials and Methods. We extensively evaluated cancer cell line sensitivity to a broad range of chemotherapeutic agents. Using standard molecular biology methodologies including immunoblot analysis, qRT-PCR, MTS viability assays, FACS, immunofluorescence, co-IP and ChIP analysis we examined the stress signalling

cascades in cells exposed to various chemotherapeutics including a range of novel PI3K inhibitor compounds that are currently in clinical trials. We also conducted the detailed analysis of ex vivo melanoma, colon and pancreatic patient samples and correlated patient prognosis to tribbles homolog 2 (TRIB2) expression.

Results and Discussion. Here we reveal that TRIB2 expression ablates FOXO activation, disrupts the p53/MDM2 regulatory axis thus confers resistance to anti-cancer drugs that include PI3K inhibitors. We show that TRIB2 expression is significantly increased in melanoma, colon and pancreatic cancers and correlates with extremely poor clinical prognosis. We report that TRIB2-mediated suppression of FOXO and p53 activity is indirect and rather, is via the activation of AKT. Mechanistically, the TRIB2 protein is stabilized by reduced proteasome-dependent degradation and promotes AKT activation via its COP1 domain. Altogether, our study reveals a novel regulatory mechanism underlying drug resistance in a range of cancers, and suggests that TRIB2 functions as an important component within the AKT signaling network, particularly in cancer cells.

No conflict of interest.

587 What about a dual inhibition of epidermal growth factor receptor and notch pathway in non-small cell lung cancer cells?

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Introduction. The contribution of Epidermal Growth Factor Receptor (EGFR) signaling in lung cancer development is well established. Monoclonal antibodies and inhibitors of tyrosine kinase targeting EGFR are widely used in non-small cell lung cancer (NSCLC) therapy. Despite the promising action of new drugs, it is known in advance that all patients are going to develop drug resistance, underlying the great importance to identify novel and effective combinations of agents based on different molecular mechanisms. Notch may behave both as an oncogene or a tumor suppressor gene in lung cancer cells. Notch receptor undergoes cleavage by enzymes, including γ-secretase, generating the active Notch intracellular domain (NICD), which interacts with specific transcription factors in the nucleus. The aim of the present study was to investigate the effect of both EGFR and Notch blockage in NSCLC cells, using the irreversible ErbB family blocker afatinib and the γ-secretase inhibitor DAPT.

Material and Method. In vitro experiments were conducted in H661 and H23 human NSCLC cell lines, expressing different EGFR and NICD protein levels. Collective cell migration and single cell migration were determined by using the wound-healing assay and the boyden chamber assay, respectively. Secretion of MMP-9 and MMP-2 levels was detected using gelatin zymography. Cell adhesion and spreading dynamic were determined. Immunofluorescence assay was used to illustrate the actin cytoskeleton localization.

Results and Discussion. The combination of DAPT and afatinib inhibited both collective and single cell migration in H661 cells, expressing both low EGFR and NICD protein levels while it had no effect on H23 cells that express both high EGFR and NICD protein levels. These results were in accordance with changes in MMP-9 and MMP-2 secretion. In addition, H661 cells did not present any intense spreading dynamic after agents' combination while this effect was not observed in H23 cells. This result was supported by studying the actin cytoskeleton distribution. However, as far as cell adhesion's experiments are concerned, the agents' combination had no significant impact on both cell lines. The effect of the combination seems to depend on the cell type.

Conclusion. The current study presents indications that a dual inhibition of EGFR and Notch pathway might be promising in NSCLC cells that express low EGFR and NICD protein levels. However, these findings merit further investigation.

No conflict of interest.

588 Combination treatment with All-Trans Retinoic Acid prevents cisplatin-induced enrichment of CD133+ tumor initiating cells and reveals heterogeneity of cancer stem cell compartment in lung cancer

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Introduction. The existence of specific cellular subpopulations within primary tumors with increased tumorigenic potential and chemotherapy resistance (Cancer Initiating Cells, CICs) holds great therapeutic implications. Resistant cells can remain quiescent for long periods and be responsible for local relapses and metastasis. We and others have previously described in non-small cell lung cancer the presence of cisplatin resistant CD133+ cells with tumor-initiating potential and co-expression of CXCR4 as possible indicator of CICs with disseminating potential. In this study, in order to overcome CDDP resistance of CD133+NSCLC cancer stem cells, we tested the capacity of differentiating agent All-Trans Retinoic Acid (ATRA), in vitro and in vivo, to mobilize CICs to a more CDDP susceptible phenotype

Methods To assess the presence of a quiescent subset, lung cancer cell lines were analyzed in vitro with PKH67 cell fate tracing system. Lung cancer cell lines and ex vivo cultures termed cancer tissue originated spheroids (CTOS), established from patients derived xenografts (PDXs), were treated with ATRA and/or cisplatin (CDDP) and tested by FACS analysis to assess CICs modulation. In vivo, Patient Derived Xenografts (PDXs) were treated with ATRA and/or CDDP, tumor growth measured by caliper and treated tumors tested by FACS analysis to assess CICs modulation.

Results In vitro cell fate tracing systems allowed us to identify heterogeneity within the CICs compartment with a highly quiescent pool and a slowly dividing subpopulation, respectively enriched for CD133+/CXCR4- and CD133+/CXCR4+ cells. Pre-treatment with differentiating agent All-Trans Retinoic Acid (ATRA) specifically counteracts cisplatin-induced enrichment for chemoresistant slowly dividing compartment, indicating effect on CD133+/CXCR4+ cells. The same effects are appreciable also in vivo in PDXs, where several cycles of ATRA and cisplatin treatment are able to stably reduce this fraction of CICs and tumor dissemination.

Conclusions Mobilization of CICs compartment after treatment with differentiative agent ATRA has promising effects in counteracting cisplatin resistance of CD133+ cells, partially affecting the slowly dividing CICs, enriched in disseminating CD133+/CXCR4+ and resulting in a reduction of both local tumor growth and dissemination. In addition, our approach discloses a further level of complexity of chemotherapy resistant CD133+ CICs, revealing phenotypical and functional heterogeneity of the cancer stem cells compartment in lung cancer.

No conflict of interest.

589 Harnessing reactive oxygen species and cell death pathways for the treatment of pediatric T-ALL

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Introduction. Reactive oxygen species (ROS) homeostasis results from the balance between ROS-generating pathways and the concerted action of scavenging systems. In the context of the control of cell turnover, the effects of ROS have been compared to a rheostat. According to this theory, a moderate increase in ROS stimulates normal resting cells to proliferate, while a further increase will favour tumor transformation, and excessive ROS production will lead to DNA damage and activation of apoptotic cell death. One important implication of this model is that tumor cells would be particularly vulnerable to cell death in response to agents that increase ROS levels. We propose to develop new strategies based on the manipulation of ROS homeostasis to treat patients with acute lymphoblastic leukemia of the T-cell subtype (T-ALL), 20% of whom do not respond to the standard prednisone-based therapies.

Material and methods. Experiments were carried out on the T-ALL cell lines Jurkat, TALL-1 and on explants of pediatric T-ALL cells grown in NOD/SCID mice. Mitochondrial ROS production was increased using NS1619, a small molecule acting on the BK mitochondrial potassium channel. ROS scavenging was impaired by inhibiting NADPH production by the pentose phosphate cycle using dehydroepiandrosterone (DHEA), an inhibitor of glucose-6-phosphodeheydrogenase, the rate-limiting enzyme of this pathway.

Results and Conclusions. NS1619 induced death of TALL-1 and Jurkat cells in a dose-dependent manner. Cell death was diminished by pretreatment with the ROS scavenger N-acetylcysteine, indicating that ROS were necessary for the observed effects on cell death.

Treatment with DHEA increased both ROS levels and cell death. The combination of NS1619 and DHEA had an additive effect and induced significant cell death of the 2 cell lines and of primary cells isolated from patients with prednisone-resistant T-ALL but did not kill normal PBMC.

Our results suggests that the combination of drugs that increase mitochondrial ROS production and decrease the activity of ROS-scavenging pathways may prove to be effective for treating patients with prednisone-resistant T-ALL.

No conflict of interest.

590 mPGES-1 and EGFR in prostate cancer progression

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Introduction. Inflammation is associated to cancer development and progression. Prostaglandin E2 (PGE2) has been reported to modulate the tumor aggressiveness and the epidermal growth factor receptor (EGFR)-driven oncogenicity (1, 2, 3). In prostate cancer, over-expression of EGFR promotes metastatic progression. Here, we investigated whether the expression of microsomal PGE synthase-1 (mPGES-1) enhanced aggressiveness of prostate cancer and might be critical for EGFR-mediated tumor progression.

Methods and results. In human prostate tissues, immunohistochemical data revealed that mPGES-1 correlates with EGFR expression. In vitro, in DU145 and PC-3 cell line, two models of EGFR-dependent metastatic castrate-resistant prostate cancer (CRPC), expressing mPGES-1 (mPGES-1SC), we demonstrate that mPGES-1 knock down (mPGES-1KD) or pharmacological inhibition attenuates the overall oncogenic drive. Indeed, mPGES-1SC cells express stem cell-like features and mesenchymal transition markers. Further, mPGES-1SC cells show increased capability to invade and high EGFR expression compared to mPGES-1KD cells. mPGES-1 expression correlated with in vivo tumor growth and metastasis. Although EGFR inhibition reduces both mPGES-1SC and mPGES-1KD cell tumor growth, we show that mPGES1/PGE2 signaling contributes to sensitize tumor cells to EGFR inhibitors.

Conclusion. We propose that mPGES-1 might be a novel predictive signature gene of tumor progression in prostate cancer.

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No conflict of interest.

Poster Session: Signalling Pathways II

591 Afatinib inhibits EGFR wild-type NSCLC cells through CIP2A to reactivate protein phosphtase 2A

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Afatinib has anti-tumor effects on non-small cell lung carcinoma (NSCLC) bearing epidermal growth factor receptor (EGFR) mutation. In this study, we report the similar anti-tumor effects on NSCLC cells without EGFR mutation through CIP2A pathway. Four NSCLC cell lines (H358 H441 H460 and A549) were used to explore the anti-tumor efficacy of afatinib. Using flow cytometry, we found afatinib induced significant cell death and apoptosis in H358 and H441 more than in H460 and A549. Afatinib-induced apoptosis was associated with dose- and time- dependent reactivation of PP2A and downregulation of cancerous inhibitor of protein phosphatase 2A (CIP2A) and p-Akt. Inhibition of PP2A or ectopic expression of CIP2A or Akt in H358 cells abolished the effects of afatinib. Furthermore, we demonstrated the Elk1, the potential transcription factor of CIP2A, could meditate the afatinib-induced anti-tumor efficacy. Using ChIP assay, Elk-1 binds to the CIP2A promoter downregulating transcription of CIP2A. In vivo efficacy of afatinib against H358 and H460 xenografts tumors were also determined in nude mice. Importantly, afatinib-induced tumor inhibition was associated with reactivation of PP2A and downregulation of CIP2A and p-Akt in vivo. Our findings disclose the therapeutic mechanism of this novel targeted agent, and suggest the therapeutic potential and feasibility of developing PP2A enhancers as a novel anti-cancer strategy.

No conflict of interest.

592 Global mapping of tyrosine kinase-regulated signaling dynamics by combined use of SILAC- quantitative proteomics and RNAi screening: The TK-SILACepedia

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Introduction. Cancer is a heterogeneous disease harbouring aberrant genomic alterations. Although extensive transcriptome and epigenomic analyses have provided us with data at the genetic level and their translation to specific phenotypes, the proteomic portrait and mapping of signaling pathways in cancer is potentially more insightful. Tyrosine kinases (TKs) are important regulators in cellular activities and perturbations of the TK signaling can contribute to oncogenesis. However, less than half of the TKs have been thoroughly studied and a global functional analysis of their proteomic portrait is lacking.

Material and method. Stable isotope labeling with amino acids in cell culture (SILAC)-based quantitative proteomics combined with RNAi screening was performed in order to identify and quantify the TK-regulated proteome upon individual silencing of all TKs expressed in MCF7.

- Western blotting was also used to further validate the expressions of significantly modulated proteins.
- Subsequently, a series of bioinformatics analyses were used to characterize the proteomic signature and functional portrait for each identified TK cluster.
- GO (gene ontology) and protein-protein interaction network analyses to elucidate the effects of clusters on known interaction networks was also implemented.
- KM Plotter analysis was utilized to assess the clinical relevance of each classified TK cluster in different molecular subtypes of breast cancer.
- The profile of each classified TK cluster for drug sensitivity and resistance in breast cancer cell lines was also assessed.

Results and discussion. As a result, a broad proteomic repertoire modulated by TKs was revealed.

- This yielded 10 new distinctive TK clusters according to similarity in TK-regulated proteomes, each characterized by a unique signaling signature.
- Analysis of different breast cancer subtypes demonstrated distinct correlations of each cluster with clinical outcome.
- From the significantly up- and down-regulated proteins, we identified a number of markers of drug sensitivity and resistance.
- Various biochemical networks were uncovered that will help us further understand and elucidate the cross-talk between cellular signaling pathways implicated in breast cancer.
- Our data supports the role of TKs in regulating major aspects of cellular activity, but also reveals redundancy in signalling, explaining why kinase inhibitors alone often fail to achieve their clinical aims.

The TK-SILACepedia provides a comprehensive resource for studying the function of TKs in cancer.

Conclusion. To our knowledge, this is the first globally proteomic signature of TKs in breast cancer and it emphasizes a rationale for the development of a combination of multi-targeted TKs as antitumour drugs. Overall, the TK-SILACepedia provides a comprehensive resource for studying the function of TKs in cancer.

No conflict of interest.

595 TRAIL-based therapeutics in osteosarcoma: Involvement of bone tumor microenvironment in TRAIL resistance

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Introduction. Osteosarcoma (OS) is the most common pediatric bone tumor. OS patients have not seen major therapeutic advances these last thirty years and the survival rate of 70 % at five years for a localized tumor still falls to around 20 % in the case of a metastatic tumor or resistance to chemotherapy. The pro-apoptotic cytokine TNF-Related Apoptosis Inducing Ligand (TRAIL) can selectively kill tumor cells representing a promising therapeutic approach for patients at high risk. However, therapeutic use of TRAIL in OS patients seems limited since several OS cell lines showed high resistance towards TRAIL sensitivity.

Material and method. In vitro and in vivo studies identified several molecular mechanisms involved in TRAIL resistance in OS that could be targeted for subsequent therapeutic strategies. Different levels of TRAIL regulation signaling pathways have been explored: death (DR4 and DR5) and decoy (DcR1, DcR2, Osteoprotegerin) receptor expression, involvement of inhibitory proteins of apoptosis (c-FLIP, IAP1/2), activation of TRAIL-induced surviving, migration or invasion pathways (NF-kB, MAPK, PI3K/Akt...).

Result and discussion. We hypothesized that the bone micro-environment may provide a favorable niche for TRAIL resistance due in particular to hypoxia, inflammation or acidic extracellular pH. Therapeutic perspectives are linked to the possibility to overcome TRAIL resistance by combining drugs targeting the bone micro-environment with TRAIL or death receptor agonist antibodies. Therefore, this area might be targeted by new re-sensitizing agents. For example, zoledronic acid already used as an antiresorptive agent in OS, shows a sensitizing effect to TRAIL by inhibition of IAPs in in vitro synergy studies. However the transition of these observations to nude mouse models reveals that zoledronic acid is not sufficient to overcome TRAIL resistance mechanisms, largely because of the induction of the TRAIL non-canonical pathway in OS cells which overrides the pro-apoptotic canonical pathway. Activation of this second signaling pathway leads to increased tumor cell proliferation, survival, migration and invasion. It is more importantly observed in vivo and may be linked to the particular bone tumor micro-environment observed in OS.

Conclusion. We propose that a combinatory therapy based on a selective TRAIL activating apoptosis pathway (APG880) associated with zoledronic acid may overcome TRAIL resistance of OS models.

No conflict of interest.

596 The Organic Cation Transporter OCTN1, genetically linked to inflammatory bowel disease, promotes Il-1beta processing and prevents colon cancer in Apc/Min mice

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Introduction. The L503F variant of the cation transporter OCTN1, linked with genetic susceptibility to IBD, is hyper-represented in ulcerative colitis patients progressing to colorectal cancer (CRC), and also in young CRC patients without overt bowel inflammation. Thus, this molecule may have a role in cancer, but lack of knowledge on the underlying molecular mechanisms limits its potential as a novel anticancer drug target.

Materials and Methods. In order to get a mechanistic insight in the potential role of OCTN1 in colorectal neoplasm, OCTN1 KO mice and their wild type controls were subdued to the AOM+DSS paradigm for colitis-associated cancer, or backcrossed into the Apcmin/+ background as a non-IBD model of intestinal carcinogenesis. Moreover, the two OCTN1 variants 503L and 503F were over-expressed in 293T cells and endogenous OCTN1 was knocked down in colon carcinoma Caco-2 cells (homozygous for the 503F variant) and in the human monocytic THP-1 cell line (homozygous for the 503L allele). Processing/secretion of Interleukin 1β, a phenomenon largely involved in IBD and CRC genetic risk, was examined in these cell populations as well as in freshly isolated WT and OCTN1 KO monocytes.

Results and discussion. OCTN1 KO were not differently susceptible to CAC compared to WT littermates, but their survival was drastically shorter in the Apc/min background, consistent with a tumor suppressive action of the transporter. OCTN1 overexpression in 293T promoted unconventional processing and secretion of Il-1 beta, with Crohn's associated 503F variant having the strongest effect; conversely, Il-1 beta release was impaired in OCTN1-depleted THP-1 cells and in OCTN1 KO macrophages. Interestingly, OCTN1 co-localized with the lysosomal marker Cathepsin B, and inhibition of autophagy reduced the processing of pro-Il1β indicating that non-conventional (inflammasome-independent) processing of Il-1 may lie in the cascade linking OCTN1 to cancer. Of note, these effects were largely unaffected by the known OCTN1 substrate Ergothioneine.

Conclusions.. These results suggest an unexpected role for OCTN1 in colon cancer suppression. This action may be related to Il1β processing and release, especially in the context of overall poorly inflammatory tumors. Validation of this circuitry and identification of the OCTN1 ligands/substrates involved may help the prevention of sporadic and IBD-associated CRC.

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597 Feedback autocrine loop of FGF-FGFR activates ERK rebound to MEK inhibition in HNSCC

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Background. Therapeutic resistance to the antitumor agents is a critical problem that has limited the clinical benefit of targeted therapies. However, the molecular mechanisms underlying resistance have not yet cleared. Recent reports that described the mechanism of resistance focused on the feedback activation of specific receptor tyrosine kinases (RTKs). We aimed to investigate the cause of the limited therapeutic effect of MEK inhibitor in head and neck squamous cell carcinoma (HNSCC) because MEK/ERK reactivation has been shown inevitably occurs.

Methods. We investigated the ERK rebound using a selumetinib (AZD6244), the selective MEK1/2 inhibitor. We tested in several HNSCC cell lines and in human tumor xenograft mouse model. Activation of fibroblast growth factor receptor 3 (FGFR3) by selumetinib was selected using a receptor tyrosine kinase (RTK) signaling array. The relation between FGFR3 and ERK rebound tested using a FGFR3 inhibitor (PD173074) or FGFR3 siRNA. The expression of FGF ligand after treatment of selumetinib was assessed by real time PCR and ELISA. The effect on tumor growth was identified in orthotropic nude mouse models of HNSCC.

Results. We showed that selumetinib induced the ERK reactivation transiently in HNSCC cells. The rebound in ERK and AKT pathway in HNSCC cells is accompanied by increased phospho FGFR3 signaling after treatment of selumetinib. Feedback activation of FGFR3 may be due to an autocrine secretion of FGF2 ligand. Knockdown of FGF2 inhibits FGFR3 activation and ERK rebound, which decreases HNSCC cell proliferation. The FGFR3 inhibitor PD173074 or FGFR3 siRNA prevents ERK rebound and sensitize HNSCC cells to selumetinib, which induces apoptotic cell death and suppresses cell proliferation as well as tumor growth.

Conclusions. In several HNSCC, tumor cells overexpress FGF2, FGFR1, and FGFR3 signals, especially after MEK inhibition. This study suggests that a rationale for combining MEK inhibitor with inhibitors of feedback activation of FGF2-FGFR3 signaling in HNSCC cells. Our results provide the rational therapeutic strategies for advanced HNSCC with resistance to the targeted therapy.

No conflict of interest.

598 cMET and its ligand stromal HGF secretion induce innate resistance to BRAF inhibitor in BRAF mutant anaplastic thyroid cancer

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Mutationally activated kinases define a clinically validated class of targets for cancer drug therapy. However, innate or acquired resistance to targeted therapy presents a challenge to the treatment of intractable cancer patients. Cancer cells typically express multiple receptor tyrosine kinases (RTKs) that mediate signals that converge on common downstream signals. Recently, it was reported that stroma-mediated ligand secretion and RTKs activation result in immediate resistance to targeted agents. We aimed to characterize further the stroma-mediated resistance of BRAF-mutant anaplastic thyroid cancer to BRAF inhibitors because most patients with this type of cancer show some degree of innate resistance.

We analyzed the mechanism of drug resistance of 8505C, BCPAP anaplastic thyroid cancer cell harboring BRAF mutation to BRAF inhibitor (PLX4032, Vemurafenib), especially focusing on RTK (c-Met) and its ligand (hepatocyte growth factor, HGF). 8505C cells showed more resistant to PLX4032 than BCPAP cells, relatively. Activation of c-Met by PLX4032 was selected in 8505C cells using a receptor tyrosine kinase signaling array. Combination of PLX4032 with c-Met inhibitor or c-Met siRNA prevents AKT signaling as well as cell proliferation and induces apoptotic cell death, which sensitizes 8505C cells to PLX4032. These combination leads to reduce a thyroid tumor volume, significantly, than PLX4032 alone in a nude mice orthotopic 8505C model. Also, stromal cell secretion of HGF resulted in activation of the HGF receptor cMET, reactivation of PI3K–AKT signaling pathways, and immediate resistance to BRAF inhibition. Dual inhibition of BRAF and cMET resulted in reversal of drug resistance, suggesting BRAF plus HGF or cMET inhibitory combination therapy as a potential therapeutic strategy for BRAF-mutant anaplastic thyroid cancer.

No conflict of interest.

599 Phosphoproteomics unravels drug-able signaling networks in gastric cancer

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Introduction. Gastric cancer is the 2nd leading cause of cancer related deaths killing close to 800,000 lives annually and the 4th commonest cancer with about 1 million new cases reported each year. The two key pillars to improve the outcomes of gastric cancer patients are early detection and therapy. Chemotherapy, alone or in combination, has reached a plateau. Consequently, there is an emerging trend to combine chemotherapy with targeted therapeutics. However, drug targets like c-Met and Her2 receptor tyrosine kinases have penetrance of only about 20-30% of all gastric cancer cases.

Materials and Methods. To identify new drug targets with higher penetrance, we conducted phosphoproteomic profiling of a large panel of gastric cell lines via phosphoprotein enrichment coupled to iTRAQ-based ESI-LC/MS/MS.

Results and Discussion. From more 1000 proteins detected, about 250 proteins, including tyrosine kinases, serine/threonine kinases and their phosphatases were found to be differentially phosphorylated in gastric cancer cells compared to normal gastric cells. Gene ontology revealed that the dataset was enriched for genes involved in translational regulation, metabolism, integrin signaling, junction adhesion and E-cadherin mediated cell adhesion. Our data will also demonstrate the power of phosphoproteomics in unraveling critical networks of signaling cascades like Wnt, JAK/STAT and VEGF pathway in gastric cancer as well as reveal a couple of top ranking genes in our dataset as potential drug targets with up to 85% penetrance in more than 200 clinical specimens.

Conclusion. Phosphoproteomics provide a comprehensive snapshot of the network of aberrant signalling pathways in gastric cancer that has implications in gastric cancer therapy.

No conflict of interest.

601 The HSP90 inhibitor, onalespib (AT13387), overcomes acquired resistance to erlotinib and crizotinib in resistance models of NSCLC

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Background. Targeted therapies are revolutionising the treatment of certain tumour types. The tyrosine kinase inhibitors (TKIs), erlotinib and crizotinib, have been successful in the treatment of EGFR-mutant and ALK-positive non-small cell lung cancer (NSCLC), but resistance arises rapidly. Understanding acquired resistance provides insight into the adaptability of signalling pathways and has clinical relevance. Onalespib is a potent, second generation HSP90 inhibitor currently in Phase 2 clinical trials. EGFR and the ALK fusion proteins are sensitive clients for HSP90, making inhibition of HSP90 a potential approach to treating EGFR and ALK-driven tumours. Here we investigated the effects of onalespib on acquired resistance to erlotinib and crizotinib in NSCLC cell lines.

Methods. In vitro models with acquired resistance to erlotinib or crizotinib were generated from the HCC827 (EGFR+) or H2228 (ALK+) NSCLC cell lines by continuous exposure with the relevant TKI. Resistant cell lines (R) were characterised using proliferation assays, western blotting, phospho-receptor tyrosine kinase (RTK) arrays and whole-exome sequencing.

Results. The resistant cell lines showed increased resistance to the relevant TKI compared to their parental lines (see table) and their signalling pathways remained active on TKI treatment. Western blots showed loss of ALK in the H2228R cells which was confirmed by exome sequencing and an induction of MET in the HCC827R cells, confirmed by phospho-RTK arrays.

Cell line	Activation status	Erlotinib inhibition of proliferation IC50 (nM)	Onalespib inhibition of proliferation IC50 (nM)
HCC827	EGFR Exon19 Del	57	33
HCC827R	N/D	>10 000	24
Cell line	Activation status	Crizotinib inhibition of proliferation IC50 (nM)	Onalespib inhibition of proliferation IC50 (nM)
H2228	ALK+	400	48
H2228R	N/D	2700	41

However, the resistant lines remained sensitive to onalespib treatment (see table). The client proteins EGFR and AKT were depleted on treatment with onalespib and, in contrast to treatment with the TKIs, signalling through both the AKT and MAPK pathways was inhibited, indicated by decreases in phosphoAKT, phosphoS6 and phosphoERK.

Conclusions. We have demonstrated that onalespib can overcome acquired resistance in both erlotinib-resistant and crizotinib-resistant NSCLC cell lines in vitro, suggesting that HSP90 inhibition may be a promising approach for combating multiple mechanisms of resistance to kinase inhibitors in the clinic.

No conflict of interest.

602 Discovery of a new isoform of BRAF 3'UTR in melanoma

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Introduction. In the past years the activity of BRAF protein has been extensively studied, but the regulation of BRAF expression has been largely neglected. Here, we report that BRAF mRNA exists in at least 2 isoforms that differ in the length and sequence of their 3'UTRs. We also discuss the implications that this discovery might have in terms of BRAF biology.

Material and method. BRAF c- and X1-3'UTR were identified by 3'RACE. The expression levels of the two 3'UTRs were evaluated in a large panel of melanoma cell lines using isoform-specific qRT-PCR primers and in 78 melanoma samples taking advantage of RNA-seq data (SKCM dataset, TCGA).

Results and discussion. By performing 3'RACE in A375 metastatic melanoma cells, we discovered that BRAF mRNA exists in 2 3'UTRs isoforms: the 121nt long canonical (c) 3'UTR that is reported in the most common databases and a 1352nt long 3'UTR that belongs to a predicted BRAF X1 transcript variant. The X1 3'UTR sequence is completely different from the c 3'UTR sequence and is transcribed from an additional 19th exon located several kbp downstream of the last canonical exon. The difference in sequence extends to the very last part of the open reading frame, so that the C-term of c-BRAF and X1-BRAF proteins differ as well.

The quantification of the c and the X1 levels performed by qRT-PCR on several human melanoma cell lines, as well as the analysis of the RNA-seq data of 78 melanoma samples available at TCGA revealed that the X1 isoform is expressed at much higher levels than the c isoform, irrespectively of the BRAF and NRAS mutational status. Furthermore, we observed that the X1 isoform is highly conserved across species, contrary to the c isoform that is restricted to H. sapiens.

Conclusion. We have identified a new BRAF 3'UTR that is completely unrelated from the canonical one and accounts for the majority of the BRAF molecules present within cancer cells. These findings call for an update of the reference human BRAF sequence. Furthermore, they prompt to study the distinct post-transcriptional networks in which the X1 and the c 3'UTR are involved and have the potential to unravel new aspects of the regulation of BRAF expression.

No conflict of interest.

603 Novel small molecule inhibitor effectively inhibits cell viability on wide spectrum of cancer cells

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Introduction. Cancer arises from uncontrolled proliferation of cells. Although both chemo- and radio-therapy induce cancer cell death and reduce tumor size, the recurrence of disease is common. Studies over the years have revealed that cancer mass is heterogeneous, made up of many different cell types that include the tumor cells, immune cells, fibroblast and a small group of tumor initiating cells or cancer stem cells (CSCs). The latter is found to be resistant to various pharmacology therapies through intrinsic resistance mechanisms and can result in aggressive proliferating tumor mass with metastasis to distant sites. This contributed significantly to the overall mortality of the disease. Current target identification focus mainly on the proliferating tumor cells with little knowledge on CSCs in part due to the low frequency in solid tumor. To increase the success of cancer treatment, it is important that the new therapeutic is also able to eradicate the CSCs.

Material and Method. 18 cancer cell lines of different tissue origins were used as initial cell viability screen with in-house small molecule library. Hits were subjected to clonogenic assay. Potential compounds were assessed for its toxicity on primary epithelial cell and fibroblast. Next, these compounds' anticancer effects were demonstrated on CSC isolated from NSCLC patients. Lastly, kinase profiling, in vitro Cellular Thermal Shift Assay (CETSA) and in silico docking model were used to identify cpd10's target(s).

Results and Discussion. Through the series of cell viability screen and clonogenic assay, we identified a novel anticancer compound (cpd10). Cpd10 demonstrate effective anti-cell viability capability across a panel of different cancer cell lines (IC50<1 uM) including 8 lung-, 4 pancreatic- and 24 ovarian- cancer cells. Cpd10 also effectively reduced cell viability of the lung CSC tumour sphere isolated from 3 patients. More importantly, cpd10 is non-toxic to primary lung epithelial cells and fibroblast. Interestingly, cpd 10 also possess anti-angiogenic capability. To date, we have identified five potential targets of cpd10 that are in the pipeline of validation. Currently, preparations are made to assess the efficacy of cpd10 in the mouse xenograft model.

Conclusion. We have identified a novel anticancer compound capable of targeting wide spectrum of proliferating tumor cells and potentially CSCs.

No conflict of interest.

605 The reciprocal regulation between TRAP1 and BRAF is responsible for control of cell cycle progression and modulation of mitochondrial apoptotic pathway

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Introduction. Human BRAF-driven colorectal carcinomas (CRCs) are aggressive malignancies with poor clinical outcome and lack of sensitivity to therapies. TRAP1 is a HSP90 molecular chaperone deregulated in human tumors and responsible for specific features of cancer cells, i.e. protection from apoptosis, drug resistance, metabolic regulation and protein quality control.

Materials and Methods. Since BRAF is under the control of HSP90 chaperones, the relationship between TRAP1 and BRAF signaling was evaluated in vitro and in human CRCs.

Results and discussion. A reciprocal regulation between TRAP1 and BRAF was observed. TRAP1 regulates BRAF synthesis/ubiquitination, without affecting its stability, being BRAF synthesis facilitated in a TRAP1-rich background and its ubiquitination upon disruption of TRAP1 network. Remarkably, BRAF downstream pathway is modulated by TRAP1, since TRAP1 silencing induces i) ERK phosphorylation attenuation, ii) cell cycle inhibition with cell accumulation in G0-G1 and G2-M transitions, and iii) wide reprogramming of gene expression with cell growth and cell cycle regulation identified as the most significant biofunctions controlled by TRAP1 network. Noteworthy, this TRAP1 regulation on BRAF is conserved in human CRCs, with the two proteins significantly co-expressed. Furthermore, BRAF and TRAP1 interact and the activation of BRAF signaling results in increased TRAP1 levels in mitochondria and enhanced TRAP1 serine-phosphorylation, both conditions associated with resistance to apoptosis. In such a context, TRAP1 targeting by the dual HSP90/TRAP1 inhibitor HSP990 or the mitochondria-directed inhibitor, gamitrinib resulted in high cytostatic activity, inhibition of colony formation and high apoptotic cell death mostly in BRAF-mutated CRC cells.

Conclusions. The reciprocal regulation between TRAP1 and BRAF favors features responsible for the aggressive phenotype of BRAF-driven CRCs and TRAP1 may represent a novel therapeutic target in these malignancies.

No conflict of interest.

606 Identification of regulatory networks associated with survival of patients suffering from stage I epithelial ovarian cancer

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Introduction. Despite good therapeutic results, more than 20% of patients with stage I epithelial ovarian cancer (EOC) relapse. Currently, clinical and pathological parameters do not successfully predict patient outcome at diagnosis; it is therefore crucial to find molecular features to optimize patient stratification and our understanding of tumor mechanisms. Here, integrating miRNA and gene expressions using an innovative approach (Calura et al., NAR 2014), we identified a tumor-specific deregulated circuit predictive of patient outcome.

Patients and Methods. 203 patients with stage I EOC with median follow up of 7 years were gathered from three tumor tissue collections. 157 samples were used as training set for the identification of the prognostic miRNA-gene expression circuit, through micrographite procedure (Calura et al., NAR 2014). And, the remaining 46 patients were used as external and independent validation set. qRT-PCR measurements of the circuit expressions in all patients were used to confirm the circuit expressions and its association with survival.

Results. Analyzing the expression levels in the tumors at the time of surgery, we identified a cell circuit composed of 11 miRNAs and 8 genes, wiring cell cycle, Activins and Hedgehog signaling. The expression levels of the network components can be translated in an index reflecting the activation state of the circuit in each patient. We find that this index is a proxy of the patient survival and, thus, can be used to efficiently stratify the patients on their risk of poor prognosis. But mainly, we find that, in the external and independent validation set of patients, the efficiency of the here-presented risk assessment procedure (progression-free survival: 87% sensitivity and 87% sensitivity) is higher than those obtained in clinical practice using the clinical variables alone.

Conclusion. The proposed circuit enhances our understanding of the disease processes, our capability to assess the patient risk at time of diagnosis and, therefore, our possibility to define personalized treatments.

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No conflict of interest.

607 DJ-1, a PTEN inhibitor, is needed for cancer invasion in many tumor types through the activation of Rho GTPases

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DJ-1 is a 20-KDa protein that was first identified as an oncogene product responsible for a subset of familial Parkinson's disease; hence its name PARK 7. Previous studies showed that DJ-1 negatively regulated the tumor suppressor gene PTEN expression; thus promoting the phosphorylation of the PI3K/Akt signaling pathway, and activating cell proliferation and transformation. Therefore, DJ1 can be used as an indication of cancer metastasis and can be a potential therapeutic target. However, the detailed mechanism and the role of DJ-1 in cellular motility are still not fully understood. PBA or phenylbutyrate is a low molecular weight fatty acid that was shown to increase expression of many genes including anti-apoptotic genes, and it does so by binding to the Sp1 binding sites in the genes promoter. Knowing that DJ-1 has the Sp1 binding site, studies showed that phenylbutyrate can increase DJ-1 gene expression and cause growth arrest of malignant tumors as well. However, the effect of PBA on cellular motility is not fully understood. Therefore this study showed that DJ-1 increased cellular motility and migration in lung, brain, breast, and skin cancer cells by activating the PI3K pathway. We showed that this activation leads to the phosphorylation of Akt and thus activation of RhoGTPases (RhoA, cdc42 and rac) which are known to increase cellular motility. Moreover, this study also showed that PBA increase DJ-1 gene expression and leads to the decrease in cancer cells motility and migration.

No conflict of interest.

609 Investigating the role of annexin A2 in epidermal growth factor (EGF) induced signalling

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Introduction. Over the past decade increasing evidence has shown that the reactive oxygen species (ROS), H_2O_2 is an important second messenger in cell signal transduction, due to its high diffusion and ability to target reactive cysteine residues in proteins. H_2O_2 is induced by various signalling proteins, including growth factors, cytokines, hormones and neurotransmitters through the activation of NADPH oxidases. Currently, H_2O_2-dependent signalling has been implicated in fundamental processes such as cell proliferation, differentiation, migration and apoptosis.

Cancer cells typically exhibit increased ROS levels compared to normal counterparts that gives them a proliferative advantage and promotes malignant progression. To balance the advantage of low ROS levels (proliferative signalling pathways) versus its damaging effect (as an oxidant at high concentrations), cancer cells induce the cellular antioxidant response.

Our laboratory identified a novel redox regulatory protein, annexin A2 (ANXA2) and showed that ANXA2 antioxidant function plays a crucial role in supporting tumour growth and chemoresistance. As a logical continuation to this research we investigated the role played by ANXA2 in the regulation of oncogenic signalling pathways induced by the epidermal growth factor, EGF.

Materials and Methods. We established We established cancer cell lines (A549 lung cancer, MDA breast cancer and HT1080 fibrosarcoma) knockdown for ANXA2 and respective control cells and investigated by western blotting the activation of signalling pathways; using the probe 2',7' dichlorodihydrofluorescein diacetate (DCF-DA) we analysed levels of intracellular ROS; and using CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega®) we studied cell proliferation, all upon treatment with 15nM EGF. In order to determine if ANXA2 interacts with PTEN in vivo we performed co-immunoprecipitation (co-IP) studies. Cell lysates were immunoprecipitated with antibodies against ANXA2, PTEN or IgG (control).

Results and Discussion. Our results showed that ANXA2 knockdown cells have enhanced levels of ROS and over activation of the PI3K signalling pathway (increased phosphorylation of Akt) compared to control cells in response to EGF treatment, which in turn resulted in enhanced proliferation of ANXA2 knockdown cells.

The PI3K pathway is negatively regulated by the protein phosphatase and tensin homolog (PTEN). PTEN has two cysteine residues within its catalytic domain that can be readily oxydized by ROS, inactivating its phosphatase function. The enhanced levels of ROS observed in the ANXA2 knockdown cells might lead to PTEN oxidation and consequent activation of the PI3K pathway observed in these cells. Through co-IP studies we observed that ANXA2 and PTEN formed a complex, thus ANXA2 could be responsible for PTEN reduction and PI3K pathway inactivation. Additional research work needs to be done to prove this hypothesis.

No conflict of interest.

610 ErbB3 receptor plays a fundamental role in the early and long time response to BRAF/MEK inhibitors in melanoma

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Introduction. The treatment of advanced melanomas bearing activating mutations in the BRAF oncogene has been improved by BRAF inhibitors in combination with MEK-inhibitors, but resistance inevitably occurs. Hence, understanding the mechanisms at the basis of development of resistance is fundamental to the discovery of new therapeutic approaches. We have recently shown that the ErbB3 receptor is involved in the activation of an early feedback survival loop upon cell exposure to BRAF and/or MEK inhibitors. Upregulation of pErbB3, due to enhanced production of its ligand neuregulin-1 (HRG), causes increased AKT phosphorylation and cell survival. Furthermore, we demonstrated that activation of the ErbB3/AKT axis is abrogated by co-treatment with anti-ErbB3 mAbs previously generated in our laboratory.

Materials and Methods. We tested a panel of 11 melanoma cell lines bearing different BRAF mutations. Western blot analysis was performed using anti-ErbB3, anti-AKT and anti-ERK 1/2 antibodies. The growth inhibitory effects of multiple combinations of BRAF and MEK inhibitors and/or anti-ErbB3 mAbs were evaluated by colony formation assays. The expression of several genes after vemurafenib and/or trametinib treatments was assessed by Real Time PCR and Western blotting. Mouse xenograft studies were carried out with M14 cells injected s.c. Drug treatments began when tumors reached a mean volume of 100mm3 and tumor growth was measured by caliper.

Results. We show that ErbB3 is strongly phosphorylated in response to BRAF or MEK inhibition in the 10 out of 11 of cell lines tested. Moreover we demonstrate that neuregulin mRNA is increased in all cell lines tested upon exposure to both BRAF and MEK inhibitors. Conversely ErbB3 transcription is not increased. Most importantly anti-ErbB3 mAbs combinations with BRAF/MEK inhibitors strongly reduce cell growth and induce apoptosis better than single antibody treatments. Moreover ErbB3 mAbs impair the establishment of resistance and restore drug sensitivity to vemurafenib in resistant melanoma cells. Finally we show that ErbB3 activation is evident also in vivo in response to treatments with BRAF and MEK inhibitors and this activation is abrogated by anti-ErbB3 mAbs combination. Moreover the quadruple combination treatments strongly reduce tumor relapse.

Conclusions. Feedback activation of ErbB3/AKT phosphorylation is a common response of melanoma cells to BRAF and/or MEK inhibitors that is abrogated by anti-ErbB3 mAbs. We demonstrate that this activation is linked to the autocrine production of neuregulin in a wide panel of BRAF mutated melanoma cells. Moreover we show for the first time that the ErbB3 receptor is a key-player also in long-term establishment of drug resistance. These data pave the way for the development of triple therapies cotargeting BRAF, MEK and ErbB3 for the treatment of BRAF mutated metastatic melanoma.

No conflict of interest.

611 Protein tyrosine phosphatase receptor type gamma increases Jak2 and beta catenin degradation in chronic myeloid leukemia

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Introduction. Protein Tyrosine Phosphatase Receptor Type γ (PTPRG) is a tumor suppressor in Chronic Myeloid Leukemia (CML), a disease characterized by the t(9:22) translocation known as the Philadelphia chromosome and the resulting rearrangement of the BCR and ABL genes. Forced overexpression of PTPRG in CML cell line is correlated with an inhibition of the oncogenic BCR/ABL1 kinase activity.

The search for new PTPRG molecular interactors among key proteins belonging to BCR/ABL1 driven pathways is crucial to understand the molecular role of this PTP as BCR/ABL1 antagonist.

We focused on PTPRG regulation of two CML proteins, β Catenin and Jak2, and on interaction with them.

Material and method. Cellular models: K562 cell line, transfected with full length PTPRG or with empty vector, and LAMA-84 cell line, with a PTPRG endogenous expression.

A pull-down assay using inactive mutant (D1028A) PTPRG and K562 cell line lysates was performed to investigate the binding between PTPRG and its putative interactors.

Protein levels of β-Catenin, Axin1 and Jak2 were analyzed through Western Blotting and quantitative PCR was used to evaluate the mRNA expression of β-Catenin transcription targets, Cyclin D1 and cMyc,

LAMA-84 cells were transfected with PTPRG siRNA to downregulate endogenous PTPRG.

Result and discussion. Pull down assay with mutant PTPRG D1028A demonstrated a molecular interaction between PTPRG and JAK2 and β-Catenin indicating these proteins as possible PTPRG substrates.

The analysis of Jak2 expression in K562 cell line showed a substantial decrease of protein level in K562 transfected with PTPRG despite a higher transcription of the gene. We hypothesized an involvement of PTPRG in the degradation of Jak2.

We observed a significative β-Catenin downregulation, both at mRNA and protein level, in presence of PTPRG in K562 and LAMA-84 cell lines and a similar expression trend for β-Catenin transcription targets, Cyclin D1 and cMyc. The specific involvement of PTPRG in this regulation was confirmed by the restored expression of β-Catenin after PTPRG siRNA downregulation in LAMA-84 cell line. Furthermore, we demonstrated a stronger binding between β-Catenin and its degradation complex in our cellular model transfected with PTPRG compared to negative control.

Conclusion. PTPRG increases degradation of two key proteins belonging to disease-promoting pathways activated by BCR/ABL1, Jak2 and β Catenin, reinforcing its role as a key tumor suppressor gene in CML.

No conflict of interest.

612 ERK1/2 phosphorylation and Bcl-xL modulate cell death and drug resistance in edelfosine-treated glioblastoma cells

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Introduction. Edelfosine is an antitumor ether lipid that induces apoptosis in different cancer cells. Glioblastoma is a highly aggressive tumor, characterized by apoptosis resistance and constitutive survival signaling expression, yet it is a necrosis-prone neoplasm. Here, we studied the effect of edelfosine on the U118 glioblastoma cell line and how the triggering of different programs for cellular demise affected overall survival and drug resistance development.

Materials and Methods. U118 cells were treated with edelfosine alone or following preincubation with the selective mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase 1/2 (MEK1/2) inhibitor U0126. Edelfosine-treated resistant cells were also challenged with the BH3 mimetic small-molecule inhibitor ABT-737. Cell death was determined by flow cytometry, fluorescence microscopy and biochemical approaches.

Results and Discussion. We found that 10 μM edelfosine triggered a fast and massive necroptotic cell death in U118 cell line, whereas apoptosis induction was scarce. Preincubation of U118 cells with U0126 changed the necrotic type of cell death to apoptosis and dramatically reduced the number of surviving cells. Combined treatment of U0126 and edelfosine inhibited ERK1/2 phosphorylation, and led to receptor interacting protein kinase-1 (RIPK1) and NFκB degradation, together with a strong activation of caspases-3, -8 and -9. When incubated with edelfosine alone, ~20% of the cells remained intact, regarding mitochondrial membrane potential ($\Delta\Psi$m), and did not die through apoptosis or necrosis. These surviving cells expressed Bcl-xL and incubation with ABT-737 after edelfosine treatment induced $\Delta\Psi$m loss and increased cell death. U0126 preincubation resulted in Bcl-xL degradation with practically all the cells undergoing total $\Delta\Psi$m loss (~95%), and strong caspase-9 activation and apoptosis.

Conclusion. Inhibition of ERK phosphorylation changed the type of cell death following edelfosine treatment from necroptosis to apoptosis, favoring cell demise. ERK and Bcl-xL inhibition resulted in increased apoptotic rates and decreased survival of resistant cells. A better understanding of the molecular regulatory features of cell death pathways, as well as of the switching mechanisms leading to different types of cell death, could help to delineate new approaches to trigger and modulate these processes, which could be of critical importance in the treatment of glioblastoma.

No conflict of interest.

Poster Session: Translational Research I

614 The "panta rhei" of breast cancer: Gene expression timeline analysis during progression of microinvasive breast cancer microenvironment

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Introduction. Tumors develop by progression through a series of stages. It is now widely accepted that cancer is attributed to the accumulation of genetic alterations in cells. Every cells of the tumor microenvironment is constantly changing in the flow of the cancer progression. A number of genes have been identified as having functions in various stages of progression in promoting cancer progression in experimental models. However, the association between gene expression alterations and resulting phenotypic alterations with respect to the aggressiveness and migration potential of cancer cells is not fully understood. Therefore, elucidation of genotype–phenotype correlation will be required to further understand the complex process of progression and invasion. All tumors require at least some stroma to meet their needs of nutrition, waste removal, and structure. It has become clear in recent years that stroma is essential for tumor maintenance and growth and has potential as a therapeutic target. Here, we aimed to give a chronological order of gene expression changes given in the dynamical framework of microinvasive breast cancer microenvironment.

Materials and Methods. RNA-seq (Ion Proton technology) was performed on three microinvasive breast cancers, applying new modifications to the usual protocol. For each of them we microdissected 7 different portions of the tumor (around 200 cells), 4 related to the breast epithelium and 3 to the stroma. The regions were selected on the basis of their grade of progression. Breast epithelium was chronologically subdivided in normal breast epithelium (NBE), carcinoma in situ (CIS), emerging invasive fingers (EIF) and invasive breast cancer (IBC). For each of the breast epithelium subdivisions we collected the adjacent stroma (S) except for the in situ portion: S-NBE, S-EIF and S-IBC.

Results. Whole transcriptome analysis performed on each microdissected regions reveals a series of gene expression changes occurring during cancer progression in the breast epithelium along with the adjacent stroma.

Discussion and Conclusions. More thorough analyses are needed to give a clear view of the flow of molecular events starting from the normal breast epithelium to the microinvasive stage, as well as to give a better understanding of the stroma-epithelium molecular means of communication. The analysis of all the molecular changes occuring in the breast epithelium and in the stroma of microinvasive cancer could lead to the development of new therapeutic targets.

No conflict of interest.

615 Dual inhibition of PI3K and mTOR strongly induces apoptosis of uveal melanoma cell lines and leads to tumor stasis in vivo in patient-derived xenografts

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Introduction. Uveal melanoma (UM) is the most frequent ocular tumor in adults. While the primary tumor is efficiently removed by enucleation, no treatment for the metastastic disease is currently available. Mutations activating the G proteins GNAQ/GNA11 are present in 80% of UM tumors and lead to hyperactivity of PKC and MAPK pathways. Other signaling cascades, such as AKT/mTOR, have been involved in UM proliferation. However, none of the therapeutic approach targeting those pathways has been shown effective so far. In this work, we tested novel combinations of several targeted agents with the goal to identify more effective therapeutic strategies for UM patients.

Material and Method. We performed a drug combination screen using a panel of ten UM cell lines representative of the molecular background of the disease. We tested seven targeted agents inhibiting PKC, MEK, AKT, PI3K and mTOR signaling cascades and assessed all possible 2 by 2 drug combinations. To identify the most synergistic combination, cell viability was first measured in all cell lines. Then, regulation of cell cycle and induction of apoptosis were monitored for the most promising ones. Finally, the highest synergistic combination was tested in vivo in UM patient-derived xenografts (PDX).

Results. The strongest synergy was observed between the mTOR inhibitor Everolimus and the PI3K inhibitor GDC0941, which resulted in an increase in apoptosis in several UM cell lines compared to monotherapies. Furthermore, dual inhibition of mTOR and PI3K strongly enhanced the antitumor effect of each single agent in UM patient-derived xenografts, leading to stabilization of tumor growth.

Conclusion. Altogether, our results highlight a novel and effective combination strategy in UM models, which can be highly beneficial for UM patients.

No conflict of interest.

616 Prognostic microRNA signature in malignant pleural mesothelioma patients

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Introduction. Malignant pleural mesothelioma (MPM) is an aggressive tumor mainly associated with asbestos exposure. MPM patients have a poor outcome (median overall survival (mOS) <1 year), therefore novel diagnostic and therapeutic approaches are needed. MicroRNA (miR) play a role in tumorigenesis and progression in MPM. This study aimed to identify miRNAs associated with poor prognosis.

Material and Method. We identified 26 un-resected MPM patients (11 long survivors (LS) OS>3 years and 15 short survivors (SS) OS<1 year). MiR expression in 26 FFPE biopsies and 3 normal pleura (NP) was studied using the Agilent Human miR Microarray including 2006 miR. Expression data were normalized by GeneSpring software (v.12.6). Class-comparison analysis between MPM/NP and SS/LS was performed using a t-test adjusted for multiple comparisons using Benjamini-Hochberg. OS curves were estimated using the Kaplan-Meier method and compared with the log-rank test. In silico validation was performed using miRseq data from TCGA portal based upon 16 patients (LS: 8; SS: 8). Candidate miR were assessed by univariate analysis using Kaplan-Meier method and median as cutoff.

Result and Discussion. Patients' characteristics: median age 67 years (53-77); 81% males, 19% females; 73% epithelioid histotype, 12% sarcomatoid, 12% biphasic

(1 unspecified MPM). No differences in age, gender and histotype were observed among LS and SS. By class-comparison analysis, 30 miRNAs were significantly up-regulated and 11 down-regulated in MPM vs normal pleura (adjusted p-value <0.05). In the univariate survival analysis 14 miRNAs were differentially expressed in MPM and significantly associated with outcome. A miRNA signature, based on the top 6 prognostic miRNAs (unfavorable, miR-1224; favorable, miR-99a, miR-125b, let-7b, let-7c and let-7i) classified patients into low or high-risk. High-risk MPM patients had a significantly shorter median OS (4.1 months, 95% CI 2.2-5.9) as compared with low-risk patients (median not reached, Log-rank p<0.001). In silico validation analysis confirmed that low expression of mir-99a, miR-125b and let-7c was associated with shorter OS. Relevant pathways, such as PI3K/AKT, WNT were associated with these top miRNAs by pathway analysis.

Conclusion. A prognostic miR signature was identified by profiling a cohort of un-resected MPM, underlying the clinical potential of miR as predictors of survival. An additional validation in a larger independent cohort of MPM is ongoing.

No conflict of interest.

617 Metabolic and morphofunctional imaging of trabectedin treatment effects in a preclinical model of HER-2 overexpressing ovarian cancer

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Introduction. Magnetic Resonance Spectroscopy (MRS) and imaging (MRI) offers powerful tools to detect metabolic and morphofunctional alterations occurring in tumour following treatment and to identify novel biomarkers of tumour response (Podo et al NMR Biomed 2011; Canese et al, CMIR 2009). Trabectedin (ET-743) is a new marine-derived antitumor agent, which has shown in vitro and in vivo activity in multiple tumor types, including ovarian cancer (Sehouli et al, Annals of Oncology 2012; Del Campo, Med Oncol 2013). Purpose of this work was to investigate novel non-invasive in vivo biomarkers of trabectedin response in xenografts of human in vivo passaged HER2-over-expressing epithelial ovarian cancer (EOC) cells.

Methods. Xenografts derived from s.c. implantation of SKOV3.ip cells (1x 106) in SCID mice were treated weekly with trabectedin (0.2 mg/kg, n=11) or saline (n=9) per three weeks, starting from day 13 post injection.

In vivo MRI and MRS measurements were performed at 4.7 T (Agilent) (Canese et al, NMR Biomed 2012). Ex vivo MRS analyses were performed at 9.4 and 16.4 T (Bruker). Histological analysis of xenograft sections was performed on biopsies.

Results and Discussion. Compared with the parental EOC cell line, the highly tumorigenic in vivo passaged SKOV3.ip cells showed an increased content of total choline-containing metabolites (tCho) and a higher HER2 level. Tumour water diffusivity (measured by MRI) increased in trabectedine- vs saline-treated SKOV3.ip xenografts. In vivo and ex vivo MRS experiments showed a decrease in tCho level and an increase in lactate (Lac) content. These alterations are peculiar of cytotoxic effects, while no changes in tCho and Lac contents were found in the same SKOV3.ip model after a conventional cytostatic treatment (Pisanu et al, Br J Cancer 2014). The observed decrease in tCho suggests that this signal could act as a potential biomarker of trabectedine response, while the increase in Lac (also found in the serum of patients) likely reflects the activation of lactic acid dehydrogenase, as a consequence of the cytotoxic insult induced by trabectedin on cancer lesion cells. Histological analyses are ongoing on xenografts-derived samples.

Conclusion. To the best of our knowledge this is the first report in which a multidisciplinary approach combining MRS and MRI techniques and molecular analyses were used to characterise different effects of trabectedin on human EOC xenografts, as a basis for further developing clinical, non invasive imaging methods suitable for follow-up of EOC patients. We acknowledge partial support by the Oncotechnology Program of the Istituto Superiore di Sanità and PharmaMar for providing the drug

No conflict of interest.

618 Paclitaxel drives response to combination therapy with bevacizumab in ovarian cancer preclinical models

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Introduction. The choice of dose and schedule of chemotherapy combined with angiogenesis inhibitors is a relevant clinical issue. Carboplatin plus paclitaxel is the standard chemotherapy and the addition of bevacizumab is recommended for patients with ovarian cancer. Weekly schedule of paclitaxel (dose-dense) combined with carboplatin has shown relevant benefits (progression free survival and overall survival) compared with conventional tri-weekly paclitaxel, and it is well tolerated. However the advantage of adding bevacizumab (BEV) to chemotherapy with dose-dense paclitaxel (PTX) compared to conventional chemotherapy remain to be shown. The aim of our study was to compare the antitumor activity of different schedules of chemotherapy (i.e. conventional or equi/high dose-dense PTX) with or without bevacizumab in models of patient-derived epithelial ovarian cancer xenografts (EOC-PDX) with known sensitivity to cisplatin (DDP).

Material and method. Two high grade serous EOC-PDX were used, MNHOC18 and MNHOC84. Mice bearing EOC-PDX were randomized to receive: i) vehicle; ii) chemotherapy (DDP/PTX conventional; DDP/PTX dose-dense); iii) chemotherapy plus bevacizumab (BEV+DDP/PTX conventional; BEV+DDP/PTX dose-dense). Dose-dense regimen was administered at two drug concentrations: equi-dose (total dose of PTX as for conventional) and high-dose (total dose of PTX 1.5 higher than conventional). Antitumor activity was evaluated as the best T/C% and partial/complete responses; computer assisted analysis of tumor growth curves allowed to calculate scores of Log Cell Kill (LCK), re-growth doubling time and absolute growth delay, supported by DNA flow cytometric analysis. The effect on tumor vasculature was evaluated as number of CD31 positive vessels. Result

Dose-dense schedules of PTX were more effective in reducing tumor growth than conventional dose on both EOC-PDX models, with the high dose-dense being the most active. Bevacizumab added to conventional PTX was more effective than chemotherapy alone and when added to dose-dense PTX the outcome was improved in a dose-dependent manner, resulting in complete tumor responses. Analysis of the growth curves suggested that the triple treatment in the dose-dense regimen caused long term impairment of the re-growth kinetics of the tumors.

Conclusion. Dose-dense PTX was more efficacious than weekly PTX on EOC-PDX models and the response was improved by the addition of bevacizumab. The administration of dose-dense PTX combined with bevacizumab is likely offering the opportunity of better response than conventional treatment, with less toxicity for ovarian cancer.

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No conflict of interest.

619 Uncovering mechanisms of resistance to cetuximab by insertional mutagenesis in heterotopically-engrafted human colorectal cancers

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Introduction. Colorectal cancer is the third most common cause of cancer-related death worldwide with over 50,310 estimated deaths in 2014. The anti-Epidermal Growth Factor Receptor (EGFR) monoclonal antibody, cetuximab, has proven to be effective in combination with chemotherapy or as a single agent in a subpopulation of colorectal cancers (CRCs) lacking oncogenic alterations in downstream effectors of the EGFR pathway (i.e. KRAS, BRAF, PIK3CA and PTEN). Despite a massive initial tumor reduction, responses are transient and tumors become refractory within 12-18 months. We take advantage of a lentiviral vector (LV)- based insertional mutagenesis platform to induce cetuximab-resistance in Patient-Derived Xenografts (PDXs) of CRC, with the final goal of uncovering molecular mechanism of acquired resistance.

Materials and method. Established PDXs from cetuximab-sensitive liver metastatic CRC lesions have been transduced with a genotoxic LV (LV-SF-LTR) or a non-genotoxic LV, as a control. After re-engraftment in NSG mice to reconstitute the tumor, mice were divided in two groups receiving either cetuximab or the vehicle. LV-SF-LTR transduction at high vector copy number hit genes responsible for the resistance to the tumor-specific targeted therapy and deregulates their expression. Therefore, exposure to the treatment did not arrest tumor growth. LAM-PCR and deep sequencing analysis performed on drug-sensitive and drug-resistant tumors are ongoing to map the integration sites of the LVs and consequently to identify LV-induced gene deregulations responsible for the pharmacological resistance.

Results and discussion. Recent findings linking the KRAS mutational status to cetuximab primary resistance prove that early screenings are essential to predict the outcome of the therapy. Given the heterogeneity of CRCs in cetuximab-sensitive as well as cetuximab-resistance settings, an extensive knowledge of the genes and pathways involved in drug-resistance are pivotal in treatment decision-making. Insertional mutagenesis has been successfully used to shed light on novel genes involved in the resistance to lapatinib in breast cancer and to erlotinib in pancreatic cancer, underlying the forcefulness of this method in pursuing the culprits of resistance to targeted therapies.

Conclusion. The identification of the biomarkers of cetuximab-resistance will enhance screening for rational drug combinations aimed at delaying or overcoming acquired resistance in CRC patients.

No conflict of interest.

620 Thymidylate-synthase poly-epitope peptide vaccination in pretreated metastatic cancer patients; a multi-arm phase Ib trial

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Introduction. Thymidylate-synthase (TS) poly-epitope (27-mer) peptide (TSPP) is a vaccine containing the amino-acidic sequences of three CTL epitopes with HLA-A2.1-binding motifs of TS, an enzyme over-expressed in cancer cells, which plays a crucial role for DNA repair and replication and inhibited by 5'-fluorouracil. Preclinical characterization granted the rationale to design a dose-finding multi-arm phase Ib trial (TSPP/VAC1) to test in metastatic cancer patients TSPP vaccination alone (arm A) or in combination with the IG1 immunomodulation regimen1 (with GM-CSF scIL2) (arm B) or in combination with the GOLFIG poly-chemoimmunotherapy2 (arm C)

Patients and Methods. This trial was designed to test the safety and immunobiological activity of TSPP vaccination in different therapeutic conditions. Forty-nine pretreated metastatic cancer patients, with a good performance status (ECOG < 2) were enrolled in the study between April 2011 and July 2013 (12 in arm A, 9 in arm B, 29 in arm C). All patients received every 2/3 weeks sc. injections of TSPP/montanide (1:1 emulsion) at escalating dosage [9, 100µg (DL-1); 9, 200 µg (DL-2) and 31, 300 µg (DL-3)]. Dosage and schedules of IG1 and GOLFIG regimen have been published in previous reports1,2.

Results and Discussion. TSPP resulted safe and its MTD was not achieved. There was no grade 4 toxicity. The most common adverse events were grade 2 dermatological reactions; cough, rhinitis, fever, poly-arthralgia, gastro-enteric symptoms and to a lesser extent, moderate hypertension and hypothyroidism. The majority of adverse events recorded in the arm C were related to GOLFIG regimen and consisted G1-2 haematological (16 cases) and gastro-enteric events (12). TSPP vaccination was associated with rise in auto-antibodies and TS-epitope-specific CTL precursors with substantial differences in the expression of regulatory-T-cells, CTL subsets, and cytokine functional phenotype among the arms. TSPP vaccination showed evidence of antitumor activity with a disease control rate of 66.7% in arm A, 33.3% in arm B, and 79.3% in arm C with a median PFS of 6.4 (95%CI=3.66-9.2), 3.69 (95%CI=1.55-5.82), and 4.93 (95%CI=3,79-6,065) months respectively, and an OS of 10.98 (95% CI=7.56-14.4), 5.9 (95% CI=4.11-7.69), and 11.96 (95% CI= 8,92-14.98) months, respectively.

Our findings provide the framework to evaluate TSPP anti-tumor activity in further trials.

Ref. 1P Correale, et al, Eur J Cancer.2001, 37:892-902; 2P. Correale, et al, J Clin Oncol. 2005, 23:8950-8

No conflict of interest.

621 Identification of circulating miRNAs in DIPG patients as predictors of response to targeted therapy and as classifiers of clinical outcome

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Introduction. Diffuse intrinsic pontine gliomas(DIPG) are the most common brainstem tumors of childhood and represent one of the most challenging paediatric tumours to treat. A non-randomized, open label phase II pilot study was conducted at Fondazione IRCCS Istituto Nazionale Tumori (Milan) to assess the efficacy in terms of objective response rate according to the RECIST criteria of combining nimotuzumab and vinorelbine with radiation in newly-diagnosed DIPG.

Methods. Vinorelbine at 25 mg/m2 was given any other week, with the same dose of nimotuzumab, thereafter until tumor progression or for a total of two years. Twenty-five children (median age 7.4) were enrolled according to the standard MRI inclusion criteria and a follow-up with a median observation time of 21 months was achieved. A response was observed in 24/25 patients (96 %). One-year PFS rates was 30 ± 10 % respectively; the median PFS were 8.5 months (Massimino M et al., J Neurooncol. 2014). Serum specimens were collected at baseline and during follow-up. We used a high-throughput microRNA screening approach to assess miRNA profile in serum samples obtained from 24 DIPG patients with the main aim to identify novel non-invasive biomarkers able to improve the prediction of disease outcome and therapeutic sensitivity.

Results. Here we present the results of serum miRNA profiling at baseline. microRNA expression profiling was performed using Agilent platform and Human miRNA SureSelect 8x60K containing 2006 miRNAs annotated on miRBase19.0. Primary data analysis yielded a matrix containing 330 detectable miRNA. Association with PFS allowed us to disclose a signature of 10 miRNAs able to stratify high and low risk patients (HR=4.33, 95%CI 1.49-12.54; p=4.27E-05). Validation of the 10-miRNA signature was performed by RTqPCR and our signature was challenged against a cohort of children not affected by neurological pathologies to ascertain the accuracy to predict the response to treatment in DIPG patients.

Conclusions. We identified a promising miRNA signature that can help able to stratify high and low risk DIPG patients. At present, this is the first study that aims at investigating circulating miRNA levels in DIPG pediatric patients.

No conflict of interest.

622 Metastasis- related characterisation of tumorspheres derived from circulating epithelial tumor cells (CETCs) in colorectal cancer patients

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Background. Colorectal cancer is one of the most commonly diagnosed and lethal cancers worldwide. Tumor recurrence and metastasis are two critical survival-influencing factors. Circulating cancer stem cells are a rare subpopulation of tumor cells circulating in the blood of cancer patients. They are suspected to be responsible for tumor growth and metastasis, resistance towards therapy and thus progression. Culture of tumorspheres has been used to detect cancer stem cells from primary tumor material or cell lines. In contrast, we established an effective method for identification of cancer stem cells from circulating tumor cells in the blood of patients with colorectal cancer with subsequent phenotypic and genotypic characterization.

Methods. 30 colorectal cancer patients with detectable CETCs were enrolled in the present study. CETCs were enumerated with the maintrac® approach and subsequently cultured under conditions favoring growth of tumorspheres. Immunofluorescence and qRT-PCR were applied to examine the metastatic ability of tumorspheres in vitro.

Results. Only a small fraction of CETCs had the ability to form tumorspheres. Patients with metastatic disease had more tumor spheres, then non metastatic patients (median 15 vs 48 spheres per 100 µl blood) indicating that the number of tumor spheres is dependent on stage of disease. Analysis of surface markers showed high levels of EpCAM and CD 133 expression. Furthermore, spheres had high enzymatic activity for ALDH 1. Array qRT-PCR analysis revealed that tumorspheres express genes of pluripotency such as SOX2, OCT4 and NANOG. There was no sphere formation in 50 healthy subjects.

Conclusion. This study demonstrates that tumor stem cells are present in peripheral blood from metastatic and non-metastatic colorectal cancer patients. A more complete understanding of the biology of cancer stem cells will translate into the development of better chemotherapeutic and biological agents for the treatment of colorectal cancer.

No conflict of interest.

623 Afatinib plus cetuximab delays resistance compared to single agent erlotinib or afatinib in mouse models of TKI-naïve EGFR-mutant lung adenocarcinoma

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Introduction. EGFR tyrosine kinase inhibitors (TKIs), like erlotinib and afatinib, have improved the outcome of patients with advanced EGFR-mutant lung adenocarcinomas. Despite the initial response, almost all patients develop acquired resistance to the agents on average one year after starting therapy. Resistance is most commonly due to the emergence of a secondary mutation in EGFR (EGFRT790M). We previously found that the combination of the EGFR TKI afatinib and the EGFR antibody cetuximab could overcome T790M-mediated resistance in preclinical models. This drug combination has shown a promising 30% response rate in a clinical trial in patients with acquired resistance to first-generation TKIs. A major outstanding question in the field is whether this regimen is beneficial when used as first-line therapy for patients and whether it delays or prevents the emergence of drug resistance.

Materials and Methods. To test if the afatinib plus cetuximab combination was more effective than single agent TKI in the initial treatment of EGFR mutant lung cancer, we measured tumor relapse following 4-weeks of treatment with the different regimens in transgenic mice bearing EGFRL858R-induced lung adenocarcinomas. In the same mice, long-term treatment was used to evaluate and compare the emergence of resistance within the three treatments arms and to study underlying mechanisms of resistance.

Results and discussion. Afatinib plus cetuximab delivered upfront to mice with TKI-naïve EGFRL858R-induced lung adenocarcinomas delayed tumor relapse compared to single agent erlotinib or afatinib. 100% of the mice treated with erlotinib or afatinib developed drug-resistant tumors in contrast to 55% of mice treated with afatinib plus cetuximab. Mechanisms of tumor escape observed in afatinib plus cetuximab resistant tumors include the EGFRT790M mutation (53.8%) and Kras mutations (38.5%).

Conclusion. Our data indicate that afatinib plus cetuximab is superior to afatinib or erlotinib when used as front-line therapy. Nevertheless the combination effectively lead to the regression of tumors harboring the EGFRT790M mutation, suggesting a potential treatment scenario in which afatinib plus cetuximab are given upfront followed by 3rd generation TKI treatment if and when resistance emerges. Currently, a Cooperative group Phase II/III trial of afatinib plus cetuximab vs. afatinib alone in patients with TKI-naïve EGFR mutant lung cancer is planned.

No conflict of interest.

624 Gene expression profile of primary squamocellular oral cavity tumors to disclose biological features in nodal invasion

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Background. The nodal/extranodal extension of disease in oral cavity squamocellular cancer (OCSCC) is not easily detectable by clinical and radiological exams. We analyzed the gene-expression (GE) profile of specimens obtained on primary tumor (T) in order to identify biological determinants of nodal invasion that eventually could predict nodal status.

Methods. Archival surgical specimens from patients with stage III-IV OCSCC from 1989 to 2008 were collected. In the present work we included 87 patients balanced among pathological negative nodes (pN0; n=34), positive nodes with (pN+ECS+; n=29) or without (pN+ECS-; n=24) extranodal extension. The histological specimens of primary disease (T) were microdissected to obtain one sample representative of the central area of the tumor (pTcent) and one from the peripheral area (pTperiph). The samples of primary tumors were profiled on DASLwg Illumina BeadChips. Pathway analysis was performed through GSEA v2.0.14.

Results. GE profile yielded a data matrix containing 17,138 detected genes. Imposing a significance threshold of false discovery rate (FDR) <10%, distinct gene expression patterns for pN0, pN+ECS- and pN+ECS+ were disclosed. Interestingly, we noticed that the gene expression differences were associated to the specific tumor area with larger expression modulation when pTperiph is analyzed compared to pTcent. GSEA analysis was performed between pN+ECS+ and pN+ECS- to investigate the biologic pathways altered in pTperiph specimens. We then applied an enrichment map tool allowing the visualization of coherent clusters of enriched gene sets. The analysis revealed a large number of biological networks deregulated between pN+ECS+ and pN+ECS- groups. The most relevant pathways enriched in ECS+ include immune response, positive regulation of cell proliferation and cell cycle, negative regulation of apoptosis, while ECS- cases show enrichment in pathways linked to cell signaling.

Conclusion. Different molecular patterns on pTperiph samples of primary tumor were identified among for pN0, pN+ECS- and pN+ECS+ that could be useful to predict nodal status in OCSCC.

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No conflict of interest.

625 Cationic amphiphilic antihistamines sensitize non-small cell lung cancer cells to chemotherapy and improve the survival of cancer patients

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Introduction. Acquired resistance to apoptosis is a hallmark of cancer and, thus, other cell death programs must be targeted in cancer therapy. Cationic and amphiphilic drugs (CADs) have the ability to induce a cancer-specific cell death by targeting lysosomes. Many drugs used in treatment of other diseases have cationic and amphiphilic properties.

Material and Methods. 74 clinically relevant CADs were screened for their ability to kill A-549 non-small cell lung cancer (NSCLC) cells, and the ability of the selected hits to induce lysosomal cell death was addressed. The effect of the concomitant use of antihistamines and chemotherapy in all Danish residents diagnosed with cancer during 1995-2011 were evaluated in a retrospective pharmacoepidemiological study.

Results. Two antihistamines—astemizole and terfenadine—were among the identified top hits in the screen, which prompted us to investigate the antihistamine drug class further. We found that astemizole, terfenadine, and loratadine sensitized NSCLC cells and multidrug resistant Du145 prostate cancer cells to chemotherapy in sub-micromolar doses. Fexofenadine, an antihistamine without CAD properties, did not have similar effects. Examination of the association between use of antihistamines and cancer survival revealed an inverse association between use of loratadine and mortality in patients with distant stage non-small cell lung cancer and between use of astemizole or loratadine and mortality in all patients with non-localized cancer combined.

Conclusion. The joint results from the experimental and pharmacoepidemiological studies indicate that adjuvant use of cationic amphiphilic antihistamines to standard cancer therapy may improve cancer survival.

No conflict of interest.

626 Effect of c-MET and MTOR inhibitors in malignant pleural mesothelioma (MPM) cells

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Introduction. Malignant pleural mesothelioma (MPM) is an aggressive tumour with poor prognosis. Heterogeneity of the tumour and resistance to current therapeutic modalities suggests an urgent need for effective targeted therapy. The PI3K/mTOR pathway is a major intracellular cascade stimulated by different growth factor receptors including the hepatocyte growth factor receptor (c-MET). Studies have shown that c-MET inhibition blocks tumour growth and metastasis. Trials of PI3K/AKT/mTOR pathway inhibitors have been reported in MPM with limited results attributed to feedback mechanisms and crosstalk between signalling pathways. Single agent therapy in MPM has proven futile hence the need for multiagent regimens. We hypothesized that combining a PI3K/AKT/mTOR inhibitor with a c-MET inhibitor in vitro might provide an enhanced cytotoxic effect.

Material and method. Expression of c-MET was assessed in 71 mesothelioma patients by immunohistochemistry (IHC). Growth inhibitory effects of Tivantinib, NVP-BEZ235 or VS-5584 alone in MPM cell lines (NCI-H2452, NCI-H2052, MSTO-211H) and a non-small cell lung cancer cell line (A549) were examined. The combination efficacy of Tivantinib with either NVP-BEZ235 or VS-5584 was further evaluated using the Chou-Talalay combination index. The IC50 values of Tivantinib alone and in combination with either NVP-BEZ235 or VS-5584 were also compared using the paired student's t-test.

Results and discussion. c-Met expression was demonstrated in 81.7% (58/71) of MPM cases and there was a significant association with survival (p= 0.017; Kaplan-Meir survival analysis). c-Met expression was identified as a prognostic indicator in the epithelial mesothelioma subtype using COX regression analysis (p=0.016; HR=0.248). Tivantinib inhibited the growth of MPM cell lines with IC50 values of 0.27 -1.55 μM. NVP-BEZ235 and VS-5584 also inhibited the growth of MPM cell lines with IC50 values 0.0048 – 0.0125 μM and 0.09 – 0.29 μM respectively. In MSTO-211H and H2052 cell lines, the combination indexes (CI) at fraction (Fa) 0.5 were 0.55 and 0.67 respectively when Tivantinib was examined in combination with NVP-BEZ235 (10 nM) indicating synergism. We also observed significant differences in the IC50 values of Tivantinib alone and Tivantinib combined with VS-5584 (10 nM) in the H2052 and H2452 cell lines ($p<0.05$).

Conclusion. Our findings suggest that Tivantinib can inhibit MPM cell growth in vitro at therapeutically relevant doses. The combination of Tivantinib and an mTOR inhibitor might be a potential targeted therapy for MPM patients.

No conflict of interest.

627 Epigenetic sensitization of respiratory tract cancer cells to paclitaxel

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Introduction. Respiratory Tract Cancers (RTCs) are responsible for more than a quarter of cancer related deaths world-wide. Poor prognosis is related with late diagnosis and low response to currently used chemotherapeutic drugs. Paclitaxel is an antimitotic agent widely used in the treatment of RTCs; however resistance is an important problem. As epigenetic therapies have already been introduced into clinical cancer management, we tested two epigenetic modulators for their ability to sensitise cancer cell lines to paclitaxel; DNA methyltransferase (DNMT) inhibitor Decidabine and histone deacetylase (HDAC) class I inhibitor Valproic acid (VPA).

Materials and Methods. The IC50s of all compounds used to treat A549, SKLU1 (lung adenocarcinoma) and BHY (oral squamous carcinoma) cell lines were evaluated using the MTT assay. We examined the cytotoxic effects of VPA (1 mM) and Decitabine (100nM) when combined with the IC50 concentration of paclitaxel for each cell line (13.6 nM A549, 16.7nM SKLU1 and 14.3 nM BHY). mRNA expression analysis was carried out by Taqman-based qPCR. Pyrosequencing analysis was used to determine the DNA methylation status of LINE1 transposable element.

Results. Decitabine treatment efficiency was determined by measuring the global methylation levels (LINE1 element). Decitabin did not sensitise any of the cell lines to paclitaxel when used in either a synchronous or preceding manner. The synchronous treatment of VPA and paclitaxel produced only a minor additive effect. In contrast, when VPA was used to treat the cell for 48 hours prior to paclitaxel addition, a significant increase of the paclitaxel toxicity was observed in the subsequent 72 hours. Interestingly, mRNA expression of AURKA was reduced by VPA treatment.

Conclusion. Our results demonstrate that HDAC inhibitors could be beneficial in sensitising RTC cells to paclitaxel, which is a very common and inexpensive chemotherapeutic agent. Such a sensitisation could lead to lowering the effective dose of paclitaxel and subsequently reducing the adverse effects of this drug to the patient. Appropriate clinical trials are required to confirm this. The great advantage of VPA is that it demonstrates minor side effects.

While the mechanism behind VPA-mediated sensitisation to paclitaxel is still unclear, the reduction of ARUKA expression may be one of the mediators. It is known that Aurora A inhibitors produce a similar effect. Additional research is required to establish the exact molecular mechanisms modulating this epigenetic sensitisation of cancer cells to paclitaxel.

No conflict of interest.

628 Molecular profile based approach in targeted therapy for Head and Neck Cancer- A xenograft study

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Introduction. Pathway-based adaptive therapy is an approach that has not been adopted in Head and neck squamous cell carcinoma (HNSCC), wherein therapy is still administered in a 'one treatment fits for all' basis. Patient-derived xenografts (PDX) facilitate preclinical investigations. The objective for this study was to assess the efficacy of pathway-based therapy using a patient derived xenograft model.

Methodology. PDX (NOD.CB17-Prkdcscid/NCrHsd) were generated from HNSCC tumors and their molecular profiling for known drug targets was carried out in parallel. Biomarkers were selected from the knowledge database based on their association with targeted therapy. The expression/mutation status of mTOR (Sirolimus), COX-2 (Celecoxib), RARβ (ATRA) and EGFR/KRAS (Nimotuzumab) was analyzed. The selected PDX (N=1) was treated (passage 3) with monotherapy (N=5 drugs) or combinational therapy (N=2 arms) along with standard of care (Cisplatin) for 4 weeks and observed for 2-3 weeks post treatment and correlated with marker profile.

Results and Discussion. Among the PDX generated, 18% (15/83; passage 3) showed tumor development and the xenograft of the patient with highest expression of markers was taken for further treatment. PDX of the sample (Stage IV, treatment naive) with up regulation of RARβ (>100fold), COX2 (>40fold) and mTOR (>1.5fold) and wild type EGFR/KRAS was treated with the corresponding targeted therapy. Tumor growth inhibition (TGI) was highest in the Cisplatin (95%), Sirolimus (83%) and Cisplatin + ATRA + Nimotuzumab (69%) arms. A severe loss of body weight (25%) was observed in the Cisplatin arm while the mortality rates were high in the Celecoxib (4/5) and the Sirolimus arms (3/5). Assessment of PDX 18 days post-treatment showed a reduction in TGI in Sirolimus (83%-62%) and Cisplatin/ATRA/Nimotuzumab (69-55%) arms while Cisplatin arm showed a further regression of the tumor. Correlation of the molecular profile to drug response showed that Group VIII (combination therapy) including Nimotuzumab, ATRA and Cisplatin showed the best correlation.

Conclusion. This preliminary study indicated that the combination arm (Cisplatin/ATRA/Nimotuzumab) was probably the best option (high TGI + low mortality/minimal body weight loss) compared to Cisplatin (high TGI and body weight loss). Currently the study is being expanded by increasing the sample cohort.

Key words: Patient-derived xenografts (PDX), Biomarkers, Tumor growth inhibition (TGI)

No conflict of interest.

629 Discovery and validation of predictive protein biomarker candidates in CRC

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Introduction. Chemotherapeutic agents are still the backbone of colorectal cancer (CRC) therapy, but molecular determinants of chemoresistance are still lacking. Chemoresistance of cancer cells is a main obstacle to a successful outcome especially in first line therapy, but also in later stages. Chemoresistant cancer cells are most likely to survive chemotherapy and arise as recurrence disease. Therefore, the discovery of predictive, protein biomarkers may lead a better stratification of patients, and personalized chemotherapy that spares patients from unnecessary side effects and ineffective treatments. Since the proteome of each cell is responsible for fundamental biological processes and also makes up the bulk of pharmaceutical targets and potential biomarkers, we choose a label-free proteomic workflow for biomarker discovery.

Material and method. In this study, we discovered protein biomarker for intrinsic chemoresistance to FOLFOX chemotherapy in CRC using a newly developed top down LC-MALDI-MS-MS workflow to analyze the proteome of chemosensitive and chemoresistant cell lines. Subsequently, we have built a validation cohort of clinical biospecimen and used Laser Capture Microdissection to enrich tumor contents of the samples. An initial validation was conducted with isoform specific NanoPro1000 assays which were established for the corresponding biomarkers.

Results and discussion. The proteomic discovery study resulted in the discovery of biomarker candidates from various cellular compartments. The comparison of chemosensitive vs. chemoresistant cells revealed biomarker candidates which were differentially regulated. Biomarker candidates up regulated in the chemosensitive cells were the Heat shock cognate 71 kDa protein (HSPA8) which was up regulated 2.94 fold and the UBX domain-containing protein 1 (UBXN1), up regulated 2.06 fold. Biomarker upregulated in the chemoresistant group were the Superoxide dismutase [Cu-Zn] (SOD) which was up regulated 1.86 fold and Eukaryotic translation initiation factor 2C 1 (AGO1); up regulated 2.72 fold. Furthermore, we successfully established antibody based NanoPro1000 assays for these biomarker candidates for an initial validation in an independent cohort of clinical biospecimen.

Conclusion. In the validation phase of biomarker candidates the quality of biospecimen (e.g. ischemia time and tumor content), as well as the quality of the used antibodies are essential parameter to be able to elucidate the true biological value and meaning of biomarker candidates. Nevertheless, the discovery of predictive biomarkers for chemoresistance and the identification of molecular mechanisms underlying chemoresistance could tremendously promote individualized chemotherapy and the development of new anticancer drugs to overcome chemoresistance.

No conflict of interest.

630 A plasma microRNA-based test predicts lung cancer outcome and disease status at follow-up in low-dose computed tomography (LDCT) screening trials

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Introduction. Despite low-dose computed tomography (LDCT) screening, patients developing interval or incidental lung cancer and those with higher stage had a poor outcome. To improve the cost-benefit ratio efficacy of LDCT screening, several trials are investigating the value of non-invasive biomarkers. We analyzed the capacity of a three level (low, intermediate, high) miRNA signature classifier (MSC) to predict outcome and to monitor follow-up of lung cancer patients within the LDCT screening trials.

Materials and Methods. For 10 years (2000-2010), 3411 heavy smokers older than 50 years were enrolled in a two independent LDCT screening trials. For 84 out of the 111 individuals who developed lung cancer in the first five years of screening, plasma samples were available to perform the MSC test. The five-year overall survival according to clinico-pathological characteristics and MSC was calculated, with a median follow-up of 3.6 years. In addition, changes of MSC risk profile at follow-up was assessed for 31 patients with longitudinal plasma samples (n=86) collected before and after surgical resection.

Results and Discussion. In the subset suitable for MSC analysis, five-year survival was 78% (95%CI: 59% - 89%) for low to intermediate risk, and 35% (95%CI: 19%-51%) for high risk (p for log rank test=0.0008). The prognostic power of MSC persisted when the analysis was restricted to 75 LDCT-detected cases after exclusion of interval cancers (5-years survival: 87% vs. 38% respectively, p<0.0001), as well as to 48 patients with incidental cancers (5-years survival: 81% vs. 10% respectively, p=0.0008). Within stage I, the 5-years survival was 100% in low to intermediate risk MSC and 77% in high risk MSC (p=0.09). Reduction of MSC risk profile after surgery was observed in 73% of the analyzable subjects who remained disease free, at median time of 11.3 months (IQR=15.7). In all relapsing patients the MSC test returned to intermediate or high risk at the time of second primary or metastatic progression.

Conclusions. Plasma MSC predicted lung cancer outcome and tumor recurrence at follow-up, and might improve the individual risk assessment and performance of LDCT screening in the near future.

Conflict of interest: Corporate-sponsored research: Gensigna

631 Role of AGTR1 in the response to bevacizumb treatment in solid tumors

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Introduction. Only some patients get benefit of the antiangiogenic therapy with bevacizumab, which is a humanized monoclonal antibody that selectively binds vascular endothelial growth factor (VEGF) that has shown efficacy in combination with chemotherapy in several cancer types. There is an imperative need for reliable biomarkers to help predict which patients will respond to treatment. Significantly, secondary hypertension is as a sign of response to anti-angiogenic treatment. The renin-angiotensin system (RAS) controls renal homeostasis and vascular tone, and one key molecule for this regulation is AGTR1, which is one of the receptors for angiotensin II. There is experimental evidence that AGTR1 is involved in processes of carcinogenesis, invasion, metastasis and angiogenesis. Therefore, the aim of this study was to explore the role of AGTR1 in the response of cancer cells to bevacizumab

Material and Methods. MCF-7 cells stably overexpressing AGTR1 were treated with angiotensin II, the AGTR1 antagonist losartan, and bevacizumab, alone or in combination, and cell viability and proliferation was tested by trypan blue exclusion. MCF7 cells stably overexpressing AGTR1 were orthotopically injected in the mammary fat pad of NOD.SCID mice and animals were treated with bevacizumab or control vehicle. Tumor samples were analyzed by IHC using anti-CD31 antibody and angiogenesis expression profiles were analyzed using a RT Q-PCR array.

Results and Discussion. A significant antiproliferative effect of bevacizumab was only observed in cells overexpressing AGTR1 when stimulated with angiotensin II. Our in vivo assays confirmed this effect as only in mice bearing tumors overexpressing AGTR1 the treatment with bevacizumab was capable of exert a marked reduction of tumor volume. Microvascular density was higher in AGTR1-overexpressing tumors and showed a distinct angiogenesis related genes expression

profile. Of special relevance is the increased expression in AGTR1-overexpressing tumors of CXCL10, which is an antiangiogenic chemokine.

Conclusion. AGTR1 expression levels clearly impact the response of cancer cells to bevacizumab, and may constitute a valuable predictive biomarker of response to bevacizumab in solid tumors.

No conflict of interest.

632 Molecular differences between cabazitaxel and docetaxel resistance in castration-resistant prostate cancer cells

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Introduction. Docetaxel (D) is the standard first line chemotherapy used in metastatic castration-resistant prostate cancer (mCRPC). Cabazitaxel (CZ) is a new taxane analog of D that has demonstrated activity in both D-sensitive and D-resistant tumours. CZ is used as a second line treatment of patients with mCRPC after D failure. In this study we investigate the main molecular mechanisms responsible for CZ-resistance in comparison with D-resistance in two different castration resistant prostate cancer cells (CRPC) cells lines.

Material and Method. DU-145 and PC-3 cell lines were converted to CZ-resistant cells (DU-145CZR and PC-3CZR, respectively) by means of exposing parental cells to stepwise increasing doses of CZ. D-resitant cell lines (DU-145DR and PC-3DR) were generated similarly in a previous work in our group. Affymetrix GeneChip Human Gene 2.0 ST arrays were used to compare global gene expression between these six cell lines. A |FC|>2 FDR corrected (P<0.05) was considered in all comparatives. Genes, pathways and networks, deregulated in both CZ-resistant and D-resistant cells respect their parental cell lines, were analyzed in detail.

Results and Discussion. DU-145CZR and DR shared 61 deregulated genes in the same direction. In PC-3CZR and DR the number of common deregulated genes was 103. However, while the number of common genes with a differential expression between DU-145DR and PC-3DR was 164, only 15 genes with a differential expression were commonly deregulated in DU-145CZR and PC-3CZR. None of these genes overlapped between D- and CZ-resistant cells. Pathway and network analysis showed that genes differentially regulated in CZ-resistance were particularly involved in cytokine signaling and inflammation. In contrast, those related with D-resistance were associated with cell adhesion process and interaction with extracellular matrix. Moreover, cellular senescence and epigenetic regulation were deregulated in both models of taxanes resistance.

Conclusion. These data reveals a differential gene expression profile involved in HIPC D- and CZ-resistance, describing genes and pathways potentially responsible for overcoming D-resistance by CZ. Key genes from these networks will be validated by qRT-PCR and should be investigated further as new targets for potential therapeutic development.

No conflict of interest.

633 Aberrant ß-catenin activation drives aggressive and treatment-resistant prostate cancer

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Introduction. Prostate cancer patients with the poorest prognosis are characterised by deregulated Wnt/β-catenin signalling and loss of phosphatase and tensin homologue (PTEN) in their tumour gene expression profiles (Markert et al. PNAS, 2011). Concordantly, our clinical data demonstrate a significant correlation between low levels of PTEN and high levels of nuclear β-catenin in aggressive prostate cancer samples.

Results and discussion. To study the co-operation between PTEN loss and up-regulated β-catenin expression, we generated mice carrying a dominant allele of the β-catenin gene, with exon3 flanked by loxP sequences (Pb Ctnnb1Δ(ex3)/+), and a conditional knockout Pten allele (Pb Ptenfl/+), using the prostate-specific probasin promoter to regulate cre recombinase expression. Heterozygous loss of Pten (Pb Ptenfl/+) did not result in tumour, while mice with stabilised β-catenin (Pb Ctnnb1Δ(ex3)/+) developed prostatic intraepithelial neoplasia (PIN) and in situ carcinoma after 6 months. Combination of the two mutations (Pb Ptenfl/+ Ctnnb1Δ(ex3)/+) resulted in aggressive, invasive tumour at the same time point and poorer survival outcome compared to Pb Ctnnb1Δ(ex3)/+ mice.

We hypothesised that Pb Ctnnb1Δ(ex3)/+ tumours had longer latency due to increased nuclear Pten in PIN lesions, driven by increased ROS. NAC treatment in these mice resulted in fewer PIN lesions and less proliferation, despite a decrease in nuclear Pten, ROS and p21 levels. We also observed higher levels of cytoplasmic Pten and less pAkt. This indicates that the increased ROS in Pb Ctnnb1Δ(ex3)/+ PIN lesions drives nuclear localisation of Pten and overcomes Pten-mediated tumour suppression at the membrane. Furthermore, laser capture microdissection and immunohistochemical analysis of tumours from PbCtnnb1Δ(ex3)/+ mice showed a progressive loss of Pten expression, despite no targeted genetic alteration.

To investigate the association between Wnt/β-catenin activation and treatment-resistant prostate cancer, we used the tamoxifen-inducible Nkx3.1CreERT2 Cre-loxP system to target the Ptenfl/+ and Ctnnb1Δ(ex3)/+ alterations to the prostate (Nkx3.1CreERT2 Ptenfl/+ Ctnnb1Δ(ex3)/+). Tumour-bearing mice were treated with androgen deprivation therapy (ADT), in the form of surgical orchiectomy, and allowed to age for comparison with untreated controls. Microarray expression analysis of androgen-responsive genes in the prostate tumour tissue of hormone-naïve mice showed that β-catenin activation causes a pseudo-castrate gene expression signature. Furthermore, mice treated with ADT developed treatment-resistant tumours.

Conclusion. Our data demonstrate that activation of β-catenin in the prostate modulates Pten to drive tumourigenesis and resistance to ADT. We can apply these preclinical models of aggressive and castrate-resistant prostate cancer to study the mechanisms involved in tumour initiation and progression in this subset of prostate cancer patients.

No conflict of interest.

634 Morphological and metabolic effects detected by in vivo MRI and MRS in a D16HER2 variant of HER2-positive breast cancer model after target treatment

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Introduction. The HER2 splice variant lacking exon-16 (D16HER2) has been detected in human breast carcinomas, and has shown an increased transforming potency compared to wild-type (wt) HER2 receptors (Castiglioni et al, Endocr Relat Cancer 2006). Magnetic Resonance imaging (MRI) and Spectroscopy (MRS) have demonstrated their usefulness in tumour diagnosis, prognosis and treatment evaluation in vivo. In this work, an orthotopic model of D16HER2 variant of HER2-positive breast carcinoma in mice (Castagnoli et al, Cancer Res 2014) was treated by different target therapies and analysed by in vivo quantitative MRI and MRS analyses. Purpose of this work was to explore the possibility to non-invasively detect metabolic, morphologic and functional alterations in D16HER2 mammary carcinoma model during target treatments.

Methods. Solid tumours developed in FVB mice after intra abdominal mammary fat pad injection of 1x106 Mi6 cells in FVB mice. Tumours were treated with: 1) lapatinib 0.5 (100 mg/kg, 5 days a week); 2) phenethyl isothiocyanate 0.5 (PEITC, 1.8 mg/kg); 3) high dose proton pump inhibitor, lanzoprazole, i.p (100-50 mg/kg, 4 days a week), 4) lapatinib+lanzoprazole and 5) DMSO (as control). In vivo MRI/MRS analyses were performed by using an Agilent Inova MRI/MRS system (4.7 T) on D16HER2 mammary tumor between 68 and 88 days post injection. Quantitative MRI/MRS analyses were performed by following a quantitative protocol described in Canese et al, NMR Biomed 2012. Ex vivo MRS analyses were performed on tissue extracts at 9.4 and 16.4 T by using high resolution Bruker Avance spectrometers as described by Pisanu et al, Br J Cancer 2014.

Results and Discussion. Despite a delay in tumor growth of PEITC and Lapatinib groups, different internal composition has been detected by MRI: PEITC and lapatinib+lanzoprazole treated tumours exhibit more heterogeneity with respect to the other groups (i.e. the presence of hyper- and hypo-intense regions in the T2-weighted and diffusion-weighted images). The heterogeneity in the T2-weighted is likely due to the presence of haemorrhage (hypo-intense areas) or necrosis (hyper-intense regions) (Nardo et al, Cancer Res 2012). Furthermore, each treatment showed peculiar metabolic profile in 1H MR spectra.

Conclusion. This preliminary data show the usefulness of MRI and MRS to non-invasively detect morphological and metabolic effects which are peculiar of each therapy in our breast carcinoma model and that cannot be evaluated by measuring only tumor volume.

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635 hERG1 potassium channels expression in metastatic colorectal cancer

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Introduction. Colorectal cancer (CRC) is the fourth most common cause of death for cancer worldwide, with a 5-year survival rate higher than 60% taking into account CRC encompassing all the pathological stages (Ferlay J et al., 2012). hERG1 protein is highly expressed in colorectal adenocarcinomas with respect to hyperplastic lesions of the colon (Lastraioli E et al., 2004). In CRC cell lines, a correlation between invasive phenotype and high hERG1 levels of expression has been shown (Lastraioli E et al., 2004) and proliferation assays demonstrated that treating the cells with a specific

hERG1 blocker (E4031) reduced tumor growth (Dolderer JH et al., 2010). In CRC cell lines it was also demonstrated that hERG1 channels modulate tumor progression by switching on a VEGF-A- dependent angiogenetic pathway (Crociani O et al., 2013). In a cohort of primary non-metastatic CRC samples it was shown that hERG1 expression was associated with Glut-1 (Glucose Transporter 1), VEGF-A, CA-IX (Carbonic Anhydrase IX), and EGF-R expression (Lastraioli E et al., 2012). In a multivariate model, TNM, hERG1, and Glut-1, turned out to be prognostic factors (Lastraioli E et al., 2012). Moreover, hERG1 presence associated with Glut-1 absence represents an independent negative prognostic factor in TNM I and II colorectal adenocarcinomas (Lastraioli E et al., 2012).

Materials and Methods. A retrospective study was performed on paraffin-embedded metastatic CRC by means of immunohistochemistry with anti-hERG1 monoclonal antibody (Lastraioli E et al., 2012). Patients were followed up until disease progression or for at least 12 months. The end point of the study will be the evaluation of Progression Free Survival (PFS), as an indicator of response to therapy.

Results and Discussion. Preliminary data obtained on 25 samples showed that hERG1 is expressed in 52% of the samples with a diffused pattern of expression. For 4 samples metastatic tissue was available: from such evaluation it emerged that hERG1 protein was expressed at high levels in all the samples.

Conclusions. hERG1 channels can be proposed as tumor markers in metastatic CRC and targeted therapies might be designed to prevent resistance to therapy and treat the hardest patients.

No conflict of interest.

636 Novel chemosensitive single nucleotide polymorphism marker in metastatic colorectal cancer treated with bevacizumab

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Introduction. Bevacizumab is a humanized monoclonal antibody that binds to tumor-secreted vascular endothelial growth factor-A (VEGF)-A and inhibits tumor angiogenesis. The antibody improves survival for patients with metastatic colorectal cancer (mCRC) with chemotherapy, but no predictive markers have been identified.

Material and method. To investigate chemosensitive single nucleotide polymorphism (SNP) of mCRC, we performed exome-and RNA-sequencing using 19 patients. A clinical association analysis was carried out with the other 116 patients who had received chemotherapy to bevacizumab regimens. In vivo biodistribution studies and [18F] FDG-PET imaging were performed on mice bearing human colorectal cancers (HCT116 and HT29) xenografts after injection of bevacizumab + FOLFIRI.

Result and discussion. Five SNPs related to bevacizumab regimens sensitivity were chosen during screening. Patients homozygous for the reference alleles (GG) of PPP1R15A rs557806 exhibited a 1.6 times greater overall response rate (ORR) than those homozygous and heterozygous for the substitution allele (GC and CC; P= 0.07). In xenografted mice, HCT116 clones transfected with the G allele at PPP1R15A rs557806 were more sensitive to bevacizumab regimens than that with C allele. Tumor volume on xenografts with G allele was significantly reduced than with C allele (P= 0.004, day 13). [18F] FDG uptake decreased to 75% in HCT 116 xenograft-bearing mice with G allele, whereas [18F] FDG uptake was 42% in xenografts with C allele (P= 0.032).

Conclusion. The possibly PPP1R15A rs557806 will hopefully predict responsive patients to bevacizumab regimens, although further validation is needed in large cohorts.

No conflict of interest.

637 Optimization of novel metabolomic approach for detection of new potential biomarkers for prostate cancer

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Introduction. Biomarkers are very important for early detection of cancer, response prediction and detection of relapsing disease. Among new biomarkers for prostate cancer (PCa) diagnosis, prognosis and therapy management, molecules linked to metabolic alterations appear particularly promising. Mass spectrometry (MS) is increasingly employed for the discovery of clinical biomarkers. The aim of the present translational study was to investigate the utility of Surface Activated Chemical Ionization-Electrospray-mass spectrometry (SACI-ESI), a novel technique in chromatography-mass spectrometry which allows a reduction in chemical noise and an increase in ionization efficiency, in LC/MS analysis of metabolites in human serum samples.

Materials and Methods. First we performed MS profiles of 101 human serum samples from males candidates for prostate biopsy for PCa diagnosis enrolled at the Urology Unit of the MultiMedica Castellanza. We analyzed these samples with Electrospray Ionization (ESI) and with the new SACI-ESI technology. We then performed the analysis on serum samples from another cohort of pts (28 with PCa and 30 controls with negative biopsy) with SACI-ESI only, coupled with the NIST (National Institution of Standards and Technology) database technology.

Results. By analyzing the first cohort of patients, we demonstrated that SACI-ESI can produce MS spectra with greater sensitivity and lower noise than those obtained with the common ESI alone, increasing a number of detectable compounds able to improve disease prediction potential. In the second cohort of serum samples analyzed by using SACI-ESI approach coupled with the NIST database, we identified a group of molecules related to fatty acids metabolism, which have shown statistically significant differential expression between controls and PCa pts.

Conclusions. SACI-ESI can facilitate MS-based discovery of potential biomarkers in human serum. The SACI-NIST approach identified molecules characteristic of pathological samples thus demonstrating its potential use in the clinical setting of PCa biomarkers. The combinations of these data are preliminary and future studies will be performed using samples obtained from a serum Biobank that will be created at IRCCS-ASMN Hospital in Reggio Emilia. The samples will be analized by two systems, SACI-NIST at MultiMedica Hospital and GC-MS (Gas chromatography-Mass spectometry) at IRCCS-ASMN Hospital, in order to assess the consistency and robustness of results.

No conflict of interest.

638 Diagnostic and prognostic impact of microRNA levels in serum of high-grade serous ovarian carcinoma patients

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Introduction. Ovarian carcinoma represents the fifth leading cause of cancer deaths among women worldwide, with advanced-stage, high-grade serous (HGS-OvCa) as the most common and aggressive histotype. Current prognostic parameters are not able to adequately predict HGS-OvCa relapse and clinical course. In the literature, a lack of consensus in the normalization strategy of circulating microRNA levels exists, due to the absence of reliable reference microRNAs. In this study, we performed a global profiling of serum and tissue microRNA expression by microarrays on a cohort of HGS-OvCa patients. Our aim has been to identify HGS-OvCa specific microRNAs by comparing tissue and serum-derived microRNAs and to discover novel biomarkers associated to diagnosis, prognosis and response to chemotherapy.

Material and method. A group of 99 tumor biopsies and 110 sera were collected from HGS-OvCa stage III-IV patients. Tumor tissues and matching serum samples were collected from 76 patients. Normal sera were obtained from 19 healthy subjects. Total RNA was extracted from tissue and serum samples. Ten synthetic viral/C. elegans microRNAs were added to serum samples to allow accurate normalization. MicroRNAs were Cy3-pCp labelled and hybridized according to manufacturer's instructions (Agilent Technologies®). Preliminary microRNA level results are going to be validated using droplet digital PCR on serum samples and real-time reverse transcription-polymerase chain reaction on tissue samples. Clinical survival parameters were recorded for all patients, with the aim to find an association with microRNA profiles.

Result and discussion. Using an innovative approach of statistical analysis, circulating microRNA levels were normalized using 10 synthetic spike-in. Different levels of specific microRNAs were detected both in sera and in tissues of HGS-OvCa patients compared to healthy subjects and, more interestingly, they were able to discriminate between resistant and chemotherapy sensitive patients.

Conclusion. Our microRNA microarray results indicate that there are specific microRNAs significantly differentially expressed in HGS-OvCa tissues compared to controls. Furthermore, the application of a robust method of statistical normalization, based on the use of 10 different exogenous spike-in, allowed us to identify specific HGS-OvCa circulating microRNAs potentially characterizing treatment response.

No conflict of interest.

639 Effect of phenanthroline and bipyridine derivatives and their respective Ru(II) metal complexes on human AML cell lines

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Introduction. Most currently approved therapies against acute myeloid leukemia (AML) have serious side-effects and limited potency, necessitating the development of alternative, more potent approaches that could specifically target cancerous cells. Promising results have been reported in the field of Metallomics, in which various cancers have been targeted by compounds formed of different ligands conjugated to a metal core, one of which being ruthenium.

Materials and Methods. In this study, we sought to investigate the effects of bis-bidentate Ru(II) metal complexes, in which the ligands are phenanthroline- or bipyridine-derivatives, on five human AML cell lines (HL-60, U937, ML1, ML2 and MonoMac1).

Results and Discussion. Of the four different complexes tested {Ru-I: [Ru(II)(1,10-phenanthroline)2Cl2]; Ru-II: [Ru(II)(4,7-diphenyl-1,10-phenanthroline)2Cl2]; Ru-III: [Ru(II)(4,7-diphenyl-1,10-phenanthroline-disulfonate)2Na2]2+ and Ru-IV: [Ru(II)(2,2'-bipyridine)2Cl2]}, significant activity, on all five cell lines, was detected

only with Ru-II (IC50 values ranging from 2.42 to 9.31 μM). Of the four free ligands tested (L-I, L-II, L-III and L-IV corresponding to Ru-I, Ru-II, Ru-III and Ru-IV, respectively), significant cytotoxic activity was detected in all five cell lines, only with L-I and L-II, (IC50 values ranging from 3.12 to 4.55 μM and from 0.12 to 0.85 μM for L-I and L-II, respectively). The activity of the free ligands L-I and L-II was at least one order of magnitude higher than that of the corresponding complexes. The activity of the metal complexes correlated with the structure of the ligands with modifications of phenanthroline (L-I) that make it more hydrophobic strongly increasing the activity of the free ligand itself (L-II), as well as that of the corresponding metal complex (Ru-II). The incorporation of a negatively-charged group (sulfonate) even to the more hydrophobic 4,7-diphenyl-1,10-phenanthroline, abolishes any activity in both the free ligand (L-III) and metal complex (Ru-III), suggesting that DNA intercalation is a possible mechanism of action of these compounds. No activity was detected with the ligand-free Ru(II) metal control, indicating that the activity of these Ru(II) metal complexes is not due to the metal center alone, but to the metal coordinated to ligand.

Conclusion. We are still investigating the role of other ligands when conjugated to a ruthenium core, as well as characterizing the cellular mechanisms underlying the activity of these compounds.

No conflict of interest.

640 Megakaryocyte differentiation and proplatelet formation from long term cryopreserved CD34+ cells: From laboratory to clinic

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Introduction. Myeloablative chemotherapy and autologous peripheral blood stem-cell transplantation (APBSCT) are a widely used procedures in patients with lymphomas or myelomas with associated viral infections (HIV, HCV) or the elderly. Persistent thrombocytopenia frequently complicates APBSCT and multiple platelet (PLT) transfusions are often required. Chronic routine transfusion of apheretic or pooled allogeneic PLTs into patients increases the risk of infection and platelet refractoriness. We used a 3D silk scaffold flow bioreactor that represents the spatial organization of the bone marrow environment. In this in vitrosystem we studied the mechanisms underlying hematopoietic stem cell homing and differentiation, megakaryopoiesis and proplatelet production.

Material and Methods. CD34+ cells, derived from peripheral blood progenitors of patient undergoing APBSCT collected more then 10 years ago, were cultured and differentiated to megakaryocytes (MKs). MKs were incubated on a mixed substrate (collagen type I, VWF and fibrinogen), loaded with 8 μM FLUO 3 acetoxymethyl ester-AM, and subjected to shear (shear rate: 250 s-1) by perfusion buffer (Hepes-Tyrode buffer pH 7.4 with 2 mM Ca2+/Mg2+, 15 μg/mL VWF and 100 μg/mL fibrinogen). Ca++ dynamics were measured periodically over 20 min. MKs were also suspended in perfusion buffer, labeled with quinacrine, and perfused over the mixed substrate at 250 sec-1. MKs adhesion and platelet production were measured by confocal microscopy. Quinacrine labeled platelets generated in the 3D bioreactor were perfused in the same way as MKs. Here, thrombus formation and volume measurements were obtained by real timeconfocal microscopy and 3D reconstruction by using Volocity ® software, respectively.

Results and Discussion. Experiments run under flow conditions showed that hydrodynamic forces evoke MKs calcium mobilization from intracellular stores which activates signaling cascades and proplatelet formation.

We observed that in vitro released platelets arrest and adhere on collagen-VWF with formation of small aggregates in a manner similar to that of control normal platelets. Platelet thrombus volumes are within a range of 2-4 μm3 (35-64% of distribution) to >100 μm3 (0-12% of distribution).

Conclusion. Our research shows the potential for in vitro platelet production from long term cryopreserved hematopoietic stem cells, using a bioreactor that mimics the bone marrow environment. This may represent a promising therapeutic approach with potentially rapid clinical translation for patients with hiporigenerative thrombocytopenia in APBSC setting.

No conflict of interest.

642 IGFBP-3 influences breast cancer metastasis and experimental colitis

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Background. Insulin-like growth factor binding protein-3 (IGFBP-3) is an important carrier protein for insulin-like growth factors (IGFs) in the circulation. IGFBP-3 antagonizes the growth-promoting and anti-apoptotic activities of IGFs in experimental systems, but in certain contexts can increase IGF bioactivity, probably by increasing its half-life. While prior work revealed no major phenotype associated in IGFBP-3 knockout mice under normal conditions, we recently showed that the loss of this gene has measurable effects on breast carcinogenesis. In this study, the goal was to investigate the role of IGFBP-3 in (1) breast cancer metastasis and (2) to explore the possibility of a role in inflammation.

Materials & Methods. In the first part, wild-type and knockout IGFBP-3 mice received an IV injection of 4T1 mammary carcinoma cells and then lung nodules were counted and analyzed. In the other section, dextran sodium sulfate (DSS) was administered orally to mice of each genotypes. DSS damages the intestinal epithelial wall, subsequently resulting in an inflammatory response in the distal portions of the colon, modelling ulcerative colitis. Animal care and treatments were conducted in accordance with established guidelines and protocols approved by the Institute and McGill University's Animal Ethics Committee.

Results & Discussion. The lung metastasis assay showed significantly more and larger metastatic breast cancer nodules in the lung of IGFBP-3 knockout mice than wild-type mice. In the colitis model, knockout mice had significantly reduced colitis as measured by lower levels of pro-inflammatory cytokines, reduced weight loss, and reduced myeloperoxidase activity compared to wild-type mice. IGFBP-3 knockout mice exhibited increased colon epithelial cell proliferation leading to enhanced damage repair reduced inflammation following DSS exposure.

Conclusion. Initial reports showed that IGFBP-3 knockout mice have no major phenotype and did not examine animals in the context of specific disease processes. In the breast cancer metastasis model, we show that the loss of IGFBP-3 increases breast cancer metastasis. Our research also demonstrates the importance of IGFBP-3 in modulating IGF activity in the colon in a manner that influences severity of DSS induced colitis. Our findings justify further work to explore involvement of the IGF system in inflammatory bowel disease and the metastasis process.

No conflict of interest.

643 Expression of different biomarkers on circulating tumor cells from patients with metastatic breast cancer

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Introduction. Circulating tumor cells (CTCs) are defined as cells originating from primary and/or metastatic tumor and circulating freely in the peripheral blood of cancer patients. CTC number predicts progression-free and overall survival in metastatic breast cancer patients. CTCs can resemble tumor initiating cells. CTCs are suggested as a representative fraction of the whole cancer population that can be collected with a noninvasive procedure. Although the isolation of CTCs is difficult, they can serve as a novel, unique model for the development of personalized treatments in breast cancer patients.

Material and Method. In this ongoing study, we use an antibody-mediated enrichment method (RosetteSep®, Stem Cell Technologies) for the isolation of viable CTCs from peripheral blood of patients with locally advanced and metastatic breast cancer treated with systemic therapy. RosetteSep® consists in a tetrameric antibody complex that recognizes several antigens present on the surface of unwanted cells (i.e. CD45 and glycophorin A, present on leukocytes and red blood cells surface, respectively).When centrifuged over a density medium (Ficoll-Plaque™), the unwanted cells pellet along with RBCs, leaving the desired cells untouched. Then the aliquot of CTCs is plated on adhesion slides (ImmunoSelect®) as four separated spots and fixed with 3% paraformaldehyde. Cells are immunostained using Hoechst, anti-human CD45-Alexa 488, and either one of the following antibodies: anti-CK19, anti-Vimentin, anti-HER-2 and anti-ER, all detected using secondary antibodies conjugated with Alexa 594. MCF7, BT474, MDA-MB231 were used as control cells. Aliquots of CTCs were plated either on adherent or on low-attach dishes.

Result and discussion. We obtained an efficient enrichment of viable CTCs with a good plating efficiency after CD45 depletion. Compared with the clinical-pathological features of the primary tumor, IF of freshly isolated CTC cells showed 80% loss of expression of estrogen receptor (ER) and increased expression of vimentin (46%). CK19 and HER2 pattern were not modified.

Conclusion. The analysis of CTCs in the clinical setting is challenging and imposes multiple limitations. In our ongoing study, we provide evidence that CTC showed reduced expression of ER, and increased expression of mesenchymal markers, suggesting that an EMT transition may occur during disease progression.

No conflict of interest.

644 Dissecting the role of microenvironment-driven activation of HER2 in BC relapse

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Introduction. In breast cancer (BC), clinical and experimental evidence suggest that the wound healing response triggered by the act of surgery, promotes the survival and local re-growth of residual BC cells and modifies the growth kinetics of micrometastases. Recent clinical trials demonstrate that delivering a single dose of intraoperative radiotherapy (IORT) is efficient in preventing local recurrence formation in BC patients. Our previous studies showed that IORT alters the post-surgery microenvironment, in terms of local growth factor production and microRNAs (miR) expression, eventually counteracting the stimulatory potential of the post-surgical wound response. In particular, miR223 was the more significantly

down-modulated miR after IORT application. As we observed, miR223 targets EGF, decreasing the EGFR pathway activation. Accordingly, low miR223 expression and high EGFR phosphorylation identified HER2+ breast cancer patients with particular poor prognosis.

Material and method. We used immortalized human mammary epithelial cells (HMEC) as a model to test the roles of miR223, EGF signaling and HER2 expression and function in the wound response in the context of the breast. HER2+ BC cell lines were also used to confirm some results.

Results and discussion. To evaluate the contribution of miR223 in cells that rely or not to ligand (EGF)-dependent activation of the receptor, we transduced immortalized HMEC (expressing or not miR223) with HER2 wild type (HER2WT) or constitutively active (HER2CA). We observed that downmodulation of miR223 in HMEC (anti-miR223 HMEC) increased proliferation, both in 2D- and 3D-assays. Moreover, using HER2WT and HER2CA expressing HMEC cell silenced or not for miR223 expression, we demonstrated that miR-223 specifically dampened the activation of the ligand-responsive HER2.

Accordingly, in HER2+ BC cells miR223 overexpression cells decreased their self-renewal ability following wound fluid stimulation and increased their sensitivity to the dual EGFR/HER2 inhibitor, Lapatinib. Interestingly, Lapatinib was also able to significantly impair the self-renewal ability of HER2+ cells following wound fluids stimulation, by inhibiting STAT3 activation.

Conclusion. Our data support the possibility that peri-operative treatment of high risk HER2+ BC patients with Lapatinib could reduce the protumorigenic effects of the wound response, leading to decrease in local recurrence formation.

No conflict of interest.

645 Gastric cancer in the age of targeted agents: Identification and validation of novel therapeutic strategies through the generation of a patient-derived xenografts platform

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Introduction. Gastric cancer is the third leading cause of cancer mortality in the world. In spite of the significant therapeutic advances, the overall clinical outcome for patients with advanced gastric cancer is poor, with 5-20% 5-year survival. The only targeted therapy approved so far is trastuzumab, which has anyway given unsatisfactory results, with less than 20% of HER2+ responsive patients. Moreover, around 50% of gastric tumors bear genetic alterations affecting tyrosine kinase pathways (mainly EGFR, HER3, FGFR2 and MET pathways, besides HER2) but their clinical validation as tumor drivers is missing. The need for new therapeutic options and the possible presence of 'druggable' targets prompted us to investigate potential targeted therapies for this disease.

Material and method. Our project aims at identifying and validating novel targeted therapeutic strategies in gastric cancer, through the generation of a platform of gastric tumor patient-derived xenografts (PDXs), animal models in which tumor surgical specimens are directly transferred into mice. Upon engraftment, the tumor is split and re-implanted in a cohort of mice, allowing the simultaneous testing of different drugs on the same tumor.

Results and discussion. We established a network of 15 Italian centers for samples collection. The generation of gastric PDXs is feasible, since we got > 60% engraftment rate in mice. We have also successfully derived cell lines from engrafted tumors.

Among the tumors collected so far, we found HER2, EGFR, FGFR2, MET and KRAS amplifications. One tumor bearing MET amplification ('SG16') was used as model to validate the platform. MET amplification was validated by FISH and MET overexpression/activation was confirmed by IHC. From the SG16 PDX, we successfully derived a cell line stably growing in colture. The PDX (and the corresponding cell line) have been used to validate response to different anti-MET drugs, alone or in combinations.

The gastric PDX platform will be exploited for: 1) Validation of candidate oncogenes as relevant targets and identification of efficient therapeutic strategies 2) Identification of novel molecular targets; 3) identification of genetic predictors of response/resistance.

Conclusion. As a whole, the results of this project should provide a scientific basis for future clinical applications and guide the rational design of molecularly-oriented clinical trials for gastric cancer.

No conflict of interest.

646 Gene expression analysis of five tumor-associated markers in CTC isolated from Bulgarian metastatic breast cancer patients

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Introduction. Breast cancer is the most common malignant tumor diagnosed in women in Bulgaria. Cancer related deaths are caused mainly by metastasis. Circulating tumor cells (CTC) play a major role for metastasis formation and are known to be an important predictive and prognostic factor for aggressiveness of the tumor and its metastatic potential. CTC can be easily detected in peripheral blood after a non-invasive, 'liquid biopsy and can be used as biomarker for monitoring the disease progression and the response to therapy.

Material and method. We have analyzed the expression levels of five tumor-associated and stem cells molecular markers hMAM, CK-19, CD44, CD24 and TWIST1 by RT-qPCR in 20 patients with metastatic breast cancer before and during chemotherapy. Samples from 12 healthy women were used as negative controls. All of the included patients and controls were previously analysed for the expression of three breast cancer associated markers GA733-2, Her-2 and Muc-1 using AdnaTest BreastCancerDetect (AdnaGen) diagnostic test. Results between the two methods were compared.

Results and Discussion. Gene expression levels of CD24 were up-regulated in 20% and down-regulated in 30% of the patients compared to the healthy controls. The tumor markers CK-19 and hMAM were individually expressed in 20% and co-expressed in 25% of the patients. According to the AdnaTest results 40% of all patients tested CTC-positive before treatment. Among them 50% expressed CK19 and 62.5% expressed hMAM, respectively. CD44 and TWIST1 expression was not found in both patients and controls.

All CTC-positive patients lost their expression markers during chemotherapy according to the qualitative analysis, but one of them became positive and resistant to treatment after the sixth course. In this patient the resistance to chemotherapy correlated with constant levels of CK19 and hMAM expression during the whole period of treatment. Another patient that lost the CTC-positive status during therapy lately became positive again for CK19 and hMAM expression only. Two patients died and could not be followed. We observed a variation in detection rates comparing qualitative AdnaTest and RT-qPCR.

Conclusion. The combination of the two methods for CTC detection: AdnaGen immunomagnetic enrichment and tumor specific expression and RT-qPCR, both using multimarker strategies, could improve the sensitivity and specificity of determining the CTC status of metastatic breast cancer patients.

No conflict of interest.

647 Institution of head and neck paragangliomas cell lines and testing of drugs active on tumor metabolism

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Introduction. Head and neck paragangliomas (HN-PGLs) are rare tumors that cause important morbidity, because of their tendency to progressively infiltrate the skull base and vascular structures of the brain. At present, surgery is the only therapeutic option, but radical removal may be difficult or impossible, depending on location and stage of progression. Thus, therapeutic molecules for this tumor are highly needed. A high percentage of HN-PGLs are linked to germline mutations in succinate dehydrogenase genes (SDHx), encoding mitochondrial complex II subunits involved in the tricarboxylic acid cycle.

Materials and Methods. HN-PGLs from two patients carrying germline SDHx mutations were dissociated and cell lines for this tumor were established to test molecules with therapeutic potential. Viability in untreated and treated cells was tested by MTT. Effects of treatments on cell cycle and apoptosis were analyzed by flow cytometry and Western blot. ROS production and mitochondrial membrane potential in live cells were analyzed by flow cytometry. Clonogenic ability after treatment was examined.

Results and Discussion. Considering that both HN-PGL cell lines derived from patients with germline defects in a gene encoding for a tricarboxylic acid cycle enzyme, we tested the effects of two drugs active on tumor metabolism: GW6471, an antagonist of the PPARα nuclear receptor that controls lipid metabolism and dichloroacetate (DCA), an inhibitor of pyruvate kinase. Viability of both HN-PGL cell lines was significantly reduced by GW6471 and DCA. Flow cytometry indicated that GW6471 induced apoptosis and affected cell cycle in both HN-PGL cell lines. However, the effect of the drug on cell cycle was different in the two cell lines tested. The results of flow cytometry analyses were consistent with those obtained by Western blot. In particular, the two cell lines showed different constitutive expression of Cyclin D3 that decreased after treatment with GW6471. Clonogenic activity was drastically reduced by GW6471, which affected also ROS production and mitochondrial membrane potential.

Conclusion. The establishment of HN-PGL cell lines provides the opportunity to test new therapeutic approaches in this rare tumor. GW6471 and DCA are active in HN-PGLs, providing a rationale for further testing metabolic drugs as single agents or in combination.

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No conflict of interest.

648 Analysis of biodistribution and biological effects associated with environmental exposition to multi-walled canbon nanotubes (MWCNTs) in a murine model

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Introduction. The rapid growth in research and development involving Carbon Nanotubes (CNTs) has propelled nanotechnology to the forefront of science and technology development. Given to their peculiar physical and chemical properties, which make them ideal also for many industrial applications, CNTs are currently enployed in several production processes. This rapid and uncontrolled growth of CNT employment raises concerns about the potential risks and toxicities for public health, environment and workers associated with the manufacture and use of these new materials.

Material and Methods. Here we investigated the main routes of entry following environmental exposure to multi-wall CNTs (MWCNTs, using a novel murine model that mimick the MWCNT exposition at workplace. We traced MWCNTs localization and their possible role in inducing an innate immune response, inflammation, macrophage recruitment, and inflammatory pathologies by immunohystochemistry, trasmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Results and Discussion. Following environmental exposure of CD1 mice, we observed that MWCNTs rapidly enter and distribute in the organism, initially accumulating in lungs, intestinal tract and then the brain, later reaching other organs through the blood stream. Since recent experimental studies showed that carbon nanotubes are associated with the aggregation process of proteins related to neurodegenerative diseases, we therefore investigated whether MWCNTs are able to induce amyloid fibril production and accumulation. We found amyloid deposits in all tissues where MWCNTs aggregate, in spatial association with macrophages. Our data suggest that accumulation of MWCNTs in different organs is associated with inflammation and amyloid accumulation. Finally, in the brain, where we observed rapid accumulation and amyloid fibril deposition, exposure to MWCNTs could enhance progression of neurodegenerative and other amyloid-related diseases.

Conclusion. Our data highlight, in a novel rodent model of exposure, that multi-wall carbon nanotubes may induce macrophage recruitment, activation and amyloid deposition, causing potential damage to relevant organs.

No conflict of interest.

649 Overcoming ARV-7-driven enzalutamide resistance by docetaxel in prostate cancer cell lines

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Purpose. Docetaxel (D) has demonstrated to increase survival in both in castration sensitive (CS) and castration resistant (CR) prostate cancer. Androgen receptor (AR) variants (ARV) have been identified as important mechanisms of AR activation under androgen depletion. AR-variant 7 (ARV7) may be involved to resistance second-line hormone therapies such as enzalutamide (ENZ). D may inhibit AR transcriptional activity in a ligand-independent manner, suggesting that may be active in ARV7+ cells. Here, we studied some molecular mechanisms responsible for primary resistance in prostate cancer cell models in order to establish more effective therapeutic, sequential or simultaneous combinations of ENZ or D drugs.

Experimental design. The CS AR+/ARV7- LNCaP and CR AR+/ARV7+ 22Rv1 cell lines were treated with different time/dose of D/ENZ alone or in combination in androgen deprivation conditions. Cell viability was assayed by MTS and the interaction effect between treatments were calculated based on the combination index (CI) method (CI<1 indicated synergism, CI=1 indicated additivity, and CI>1 indicated antagonism. Expression analyses of resistance/EMT-related genes were performed by RT-PCR.

Results. In the LNCaP cell line (AR+/ARV7-), six-day treatment of ENZ reduced PSA and ARV7 expression, but has no effect on AR-FL or EMT markers in this model. Similarly, D did not affect AR-FL or ARV7 expression. The anti-tumor effect of ENZ+D was antagonisitc (CI=1.5). However, in 22Rv1 cells (AR+/ARV7+), ENZ increased ARV7 and the ARV7-regulated gene CDH2 expression. D alone reduced ARV7 but increased CDH2 gene expression but less than ENZ treatment, and the effect was more pronounced with D+ENZ combination. Notably, the combination of ENZ+D showed a synergistic anti-tumor effect (CI=0.24) and the sequential treatment demonstrated that ENZ sensitized the cells to D-mediated effects on cell viability.

Conclusions. ARV7-driven ENZ resistance may be overcome by the addition of D. The combinations of both treatments increase the effectiveness of these therapies in the 22rv1 (AR+/ARV7+) cell line. We are further studying molecular mechanisms that lead the observed synergistic effects.

No conflict of interest.

650 Plumbagin, a medicinal plant derived naphthoquinone, may restore the efficacy and reduce intolerable toxicity of prostate anticancer drug docetaxel

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Prostate cancer (PCa) is the second most common malignancy in men and the second leading cause of cancer-related deaths in the Western World. PCa first manifests as an androgen-dependent (AD) disease and can be treated with androgen-deprivation therapy. Despite the initial success of androgen ablation therapy, resistance to anti-androgen therapy manifests by progression to CRPC. CRPC is the end stage that accounts for the majority of PCa patient deaths. Docetaxel (DTX) is an approved drug for the treatment of CRPC. However, development of DTX resistance and toxicity are the major limitations in the treatment of CRPC. These limitations may be overcome by combining DTX with plumbagin (PL). PL is a quinoid constituent isolated from the root of a medicinal plant Plumbago zeylanica L. The root of Plumbago zeylanica L. has been used in Indian medicine for more than 2500 years for treatment of various ailments. We were the first to investigate PL for the prevention of the progression and metastasis of PCa. The key findings are as follows: 1) PL inhibits invasion of androgen independent (AI) PCa cells, 2) PL inhibits the growth and metastasis of AI PCa cells in an orthitopic xenograft mouse model, 3) PL treatment inhibits the spontaneous development of prostate tumor in mouse models of PCa (TRAMP and PTEN knockout mice). However, no study exists examining the effects of PL on the efficacy and the toxicity of DTX, first-line chemotherapy for control of CRPC. The mechanisms underlying the action of this microtubule-targeting drug (DTX) is not fully understood. Evidence indicates that in addition to inhibiting cell division, DTX impairs AR signaling. The microtubule network of PCa cells is critical for AR nuclear translocation and activity. Increased AR activity in CRPC is caused by cross talk of AR with multiple intracellular signaling cascades including peptide growth factors (EGF, TGFβ, IGF-1). Our results indicate that PKCη, which correlate with the aggressiveness of PCa, play roles in both AI AR activation and promotion of PCa cell survival for emergence and progression of CRPC. Plumbagin selectively inhibits expression of PKCe, PKCe-Stat3 interaction and ligand independent AR activation. Our molecular modeling results indicate that PL possibly inhibits AR activation via competing with Stat3 for interaction with AR N-terminal domain in AI C4-2 PCa cells. PL may be a potential natural agent worthy of investigation for restoring and enhancing the efficacy of prostate anticancer drug DTX for the management of CRPC (Support: NIH grant CA102431).

No conflict of interest.

651 γ-mangostin inhibits the proliferation of breast cancer cells by targeting AKR1B10

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Introduction. Aldo-keto reductase superfamily 1 member B10 (AKR1B10) is a reduced nicotinamide adenine dinucleotie (NADPH)-dependent reductase, which detoxifies cytotoxic carbonyls by reducing aliphatic aldehydes and promotes fatty acid synthesis by blocking the ubiquitin-dependent degradation of acetyl-CoA carboxylase. AKR1B10 was upregulated in breast, lung and hepatic carcinoma, suggesting its potential role as both tumor diagnostic biomarker and therapeutic target. Cumulative evidence shows AKR1B10 stimulates the proliferation of tumor cells by regulating cellular lipid metabolism. Recently it was reported what γ-mangostin is the most potent competitive inhibitor of AKR1B10, and Phe123, Trp 220, Val301 and Gln303 in AKR1B10 are important sites for the tight binding of γ-mangostin. In this study, anti-cancer efficiency of γ-mangostin was explored.

Materials and Methods. MDA-MB-231 cells with AKR1B10-positive expression, a breast cancer cell line, were cultured in 96-well plates, and treated with different doses (5, 10, 20, 50, 100 nM) of γ-mangostin for 48 hours and with 20 nM γ-mangostin for different time (12, 24, 48, 72 hours), and then the cell proliferation was detected by MTT assay. Wild AKR1B10 gene and mutational AKR1B10 gene (mAKR1B10) with its mutant forms (Phe123Ala, Trp220Tyr, Val301Leu and Gln303Ser) were transfected into MCF-7 cells with AKR1B10-negative expression, and MCF-7/AKR1B10 and MCF-7/mAKR1B10 cells were gotten by flow cytometry, and identified by western blot. The inhibition of γ-mangostin on the proliferation of MCF-7 and MCF-7/AKR1B10 and MCF-7/mAKR1B10 cells was analyzed by MTT assay after the cells were treated with 20 nM γ-mangostin for 48 hours. The anti-cancer property of γ-mangostin was examined in nude mice transplanted with MDA-MB-231 cells.

Results and discussion. γ-mangostin inhibited the proliferation of MDA-MB-231 cells in a dose and time-dependent manner. The inhibition rate of γ-mangostin on the proliferation of MCF-7/AKR1B10 cells was significantly higher than that of MCF-7 cells and MCF-7/mAKR1B10. γ-mangostin inhibited the growth of transplantable tumor derived from MDA-MB-231 cells in nude mice.

Conclusion. γ-mangostin inhibits the growth and proliferation of breast cancer cells by targeting AKR1B10, and may be a potent personalized anti-cancer drug in the patients with AKR1B10 positive expression in breast cancer tissue.

No conflict of interest.

652 Androgen receptor variant 7 as a prognostic biomarker to docetaxel response in metastatic castration resistant prostate cancer

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Introduction. Constitutively active androgen receptor (AR) isoforms have been identified as an important mechanism of AR activation under androgen depletion in metastatic castration resistant prostate cancer (mCRPC). Recently, it has been shown that the detection of AR-variant 7 (ARV7) by qRT-PCR in CTCs from mCRPC patients correlates with enzalutamide and abiraterone resistance. However, its role in docetaxel (D) response has not been defined yet. Thus, in this study, we evaluated ARV7 expression in blood as a predictor of D response in mCRPC.

Material and Method. This is a prospective observational study. Peripheral blood samples were collected before D initiation. Mononuclear cell fraction was isolated by Ficoll gradient. Total mRNA was extracted, retro-transcribed and pre-amplified to detect ARV7 by qPCR. Primary endpoint evaluated for D response was prostatic specific antigen levels (PSA). Secondary endpoints were PSA progression-free survival (PSA-PFS), clinical/radiologic PFS (PFS) and overall survival (OS). PSA-PFS, PFS and OS curves were estimated by the Kaplan-Meier method evaluated by log-rank test.

Results and Discussion. Among the 50 enrolled patients, 84% had detectable levels of ARV7, considered as ARV7+. Median clinical/radiologic PFS were 8.1 vs 6.7 months for ARV7+ and ARV7- patients, respectively (HR: 0.39, 95% CI: 0.1601-0.989; P<0.05). Despite no significant differences were found, PSA response and OS were more favorable in ARV7+ patients (61% of D-responders for ARV7+ vs 42.9% for ARV7- patients; median OS for ARV7+ was 26.9 months vs 17.3 months for ARV7- patients, HR: 0.382, 95% CI: 0.1377-1.06; P=0.06). No significant differences were found in PSA-PFS (HR: 0.4896, 95% CI: 0.198-1.211).

Conclusion. ARV7 mRNA in peripheral blood correlates with longer time to radiological/clinical progression to D. Further studies should be performed to validate these results.

No conflict of interest.

653 Involvement of the proto-oncogene mertk receptor tyrosine kinase in the hepatic fibrogenic process

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Introduction. Fibrosis and cirrhosis are major risk factor for hepatocarcinogenesis. Hepatic stellate cells (HSC) are the major cell type involved in the liver fibrosis. Following liver damage, HSC undergo a process of phenotypic transition, leading to increased proliferation and migration, a shift towards production of fibrillar matrix components and increased expression of pro-inflammatory cytokines. MERTK is a member of TAM receptor tyrosine kinase family with oncogenic properties and is often over-expressed or activated in various malignancies. MERTK promotes antiapoptotic and prosurvival actions, activating both ERK1/2 and Akt. Several studies reported that MERTK mediates signaling pathways that lead to antiinflammatory cytokine production as well as enhanced proliferation, migration and invasion.

MERTK variants have been associated with fibrosis severity in genome wide association studies in chronic hepatitis C. In particular, the variant rs4374383 G>A of MERTK gene has been associated with reduced liver fibrosis progression, but currently there is no experimental evidence on the possible involvement of MERTK in the modulation of the hepatic fibrogenic process.

Material and Methods. Primary Human HSC were isolated from normal human livers and cultured on plastic. Cell migration was evaluated in modified Boyden Chambers. C57BL6/J mice were treated with CCl_4 (0,5 ml/Kg twice I.P. in olive oil) for 6 weeks to induce liver fibrosis. Balb/C mice were fed with a methionine choline-deficient (MCD) diet for 8 weeks. Intrahepatic gene expression was assayed by quantitative real time PCR

Results. Using immunoprecipitation we found that MERTK is expressed in human HSC. Stimulation of Human HSC with GAS6, a MERTK ligand, resulted in a time-dependent activation of ERK1/2. Moreover, we observed an enhancement of HSC migration comparable with the one observed in response to FBS stimulation. In two well-established experimental murine models of fibrosis (chronic CCl_4 and MCD diet), we observed a significant increase of expression of MERTK in treated mice compared to control groups. Finally analysis of the MERTK level in liver specimens from patients affected with NASH presenting different degree of liver fibrosis, showed that MERTK expression was higher in patients with more severe fibrosis.

Conclusions. The results of this study indicate that MERTK could play an role in the process of hepatic fibrosis. .

No conflict of interest.

654 Acquired resistance to vemurafenib in BRAF V600E mutant melanoma: BRAF translocations and rapid clonal dynamics

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Introduction. Although malignant melanomas harboring BRAF V600E mutations are quite sensitive to treatment with BRAF inhibitors, ultimately disease progression occurs. Several mechanisms of acquired resistance to vemurafenib have been reported, although no clear mechanism seems to predominate. We now report a case where acquired resistance to vemurafenib was associated with selection for a novel AGAP3-BRAF fusion protein in a BRAF V600E mutant cancer.

Material and Methods. Tumor blocks from biopsies obtained at multiple timepoints in the treatment of a patient with BRAF V600E mutation were subjected to targeted sequencing of a panel of cancer related genes (FoundationOne). The cDNA from in-frame predicted rearrangement of AGAP3 and BRAF was synthesized and introduced into UACC903 human melanoma cell lines harboring BRAF V600E. Cell lines were treated with a BRAF inhibitor, a MEK inhibitor or combination and assayed for cell growth by colony formation assay, and for activation of MEK by western blotting. PDX models were generated from surgical specimens and grown in NGS mice.

Results. Multiple tumor samples were analyzed by targeted sequencing in a patient with BRAF V600E mutant melanoma. Patient had an initial dramatic response to vemurafenib followed by clear progression at several sites including an axillary mass. Biopsy and sequencing of the progressing lesion revealed the presence of a novel in-frame AGAP3-BRAF re-arrangement. Introduction of the AGAP3-BRAF fusion into UACC903 melanoma cells resulted in resistance to vemurafenib, but continued sensitivity to MEK inhibitors. The patient was treated with the combination of dabrafenib and trametinib and had a brief clinical response. Ultimately patient had all targeted therapy discontinued and was treated with pembrolizumab with initial mixed response followed by progression. Analysis of the resected axillary metastasis revealed that this tumor, growing in the absence of BRAF or MEK inhibitors, now showed only the presence of a BRAF V600E mutation. PDX models were generated and their characterization will be presented.

Conclusion. BRAF translocations are a novel mechanism of acquired resistance to vemurafenib. Cells harboring both AGAP3-BRAF translocation and BRAF V600E may only be more fit than parental BRAF V600E cells in the setting of active treatment with vemurafenib. Rapid clonal dynamics under selection may underlie the temporal evolution of dominant clones over time in malignant melanoma.

No conflict of interest.

655 Development of diffuse intrinsic pontine glioma preclinical models at Hospital Sant Joan de Deu Barcelona

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Introduction. There is a strong need for preclinical research using accurate diffuse intrinsic pontine glioma (DIPG) models to understand the biological reasons underlying the dramatic therapeutic failure in this disease and to characterize the activity of innovative treatments. Thus, we have recently established a fully translational research program at our institution Hospital Sant Joan de Deu Barcelona to develop preclinical DIPG models from patient biospies.

Material and Method. The technical approach to develop our models is based on the recently published experience by the Stanford group. We have evaluated in vitro and in vivo the activity of treatments (such as irinotecan and cisplatin) recently used at our institution in a clinical trial for DIPG patients. We have studied several blood-brain barrier (BBB) markers in the developed DIPG xenografts.

Results and Discussion. We have developed 6 in vitro models named after the codes HSJD-DIPG-007, -008, -011, -012, -013 and -014. Our first model, HSJD-DIPG-007, was developed from autopsy samples of one patient treated with only 3 doses of irinotecan-cisplatin and no previous radiotherapy. The other 5 models were developed from biopsies at diagnosis. All the models share the K27M mutation of the H3.3 histone and the model HSJD-DIPG-007 contains a mutation in the gene ACVR1. HSJD-DIPG-007 tumorspheres are positive for nestin and contain a significant fraction of cells (25%) that self-renew under stem cell culture conditions (currently in passage 58; p58). Injection of such cells in the brainstem of Nod.Scid mice and athymic nude rats has generated a consistent xenograft model with 100% take rate and median survival time of 55 days in Nod.Scid mice (p39 cells). Diffuse, infiltrative tumors corresponding to WHO grade III gliomas (anaplastic astrocytoma) grow homogeneously in the pons and invade the cerebellum. The tumor is highly mytotic, brain structures are conserved and neurons remain entrapped between the tumor cells. In vitro, we observe a remarkable activity of treatments such as SN-38 (the active metabolite of irinotecan) and cisplatin. However, such activity is not replicated in vivo, which reproduces the clinical outcome of the patients. BBB markers (claudins 3 and 5, occludin, BCRP) are fully conserved in our DIPG models and might explain lack of response to treatments in vivo.

Conclusions. We have generated a powerful preclinical resource to study DIPG from the genetic, biological and functional perspectives.

No conflict of interest.

656 Activity of dasatinib in a patient-derived xenograft of alveolar rhabdomyosarcoma

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Introduction. Recent preclinical studies show that the inhibition of the SRC family kinase (SFK) with dasatinib suppresses the growth of alveolar rhabdomyosarcoma (aRMS) cell lines in vitro and in vivo, by a mechanism likely related to the overexpression of CRKL in the cell lines and its interaction with SFK, specifically with the YES kinase. Such cell line data should be complemented with activity data in patient derived xenografts (PDX). In this study we have evaluated the activity of dasatinib in a model of aRMS PDX established at Hospital Sant Joan de Deu Barcelona.

Material and Method. Tumor cells were isolated from a pleural effusion of a 13 year old patient with a second relapse of aRMS. Cells were diluted in matrigel and injected in the flank of one Nod.Scid mouse. A fraction of the cells was transferred to 96 well plates to study dasatinib activity (MTS assay). The subcutaneous tumor (HSJD-aRMS-002 model) grew in 15 weeks and it was expanded to 12 mice in double flank for further in vivo studies. Two groups of mice were included: dasatinib (50 mg/kg, twice daily (5 day on, 2 off) x 4 weeks) and control (oral vehicle), with 7 and 5 mice each, with tumor volumes at the start of treatment in the range 0.2 to 0.5 cm3. Individual survival was defined as the time to achieve 2 cm3 tumors. Immunohistochemistry (IHC) studies were performed to detect CRKL and YES in solid tumor tissue FFPE samples. Western blot was used to detect YES in tumor cell lysates.

Results and Discussion. Primary tumor cells were sensitive to dasatinib, with an IC50 of 30 nmol/L (20-45; 95% confidence interval). The oral administration of dasatinib inhibited tumor growth significantly, although it did not induce tumor regression, consistent with previous preclinical studies in aRMS cell lines. Median survival of the dasatinib group was 79 days, as compared to 54 days for controls (P=0.0045; log-rank test). CRKL and YES were positive in the tumor tissue and YES expression decreased upon in vitro treatment with dasatinib.

Conclusions. Our data supports the activity of dasatinib in aRMS and the suitability to identify determinants of drug response using PDX models.

No conflict of interest.

657 Targeting oncogenic pathways and HIF-1a in colon tumors by combination of irinotecan and mTOR inhibitors of new generation

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Introduction. In sporadic colorectal cancer, activation of PI3K/Akt and Ras/MAPK pathways is commonly involved in the pathogenesis of the disease. Downstream of these pathways, mammalian target of rapamycin (mTOR) is acting via two effectors (mTORC1 and mTORC2) and regulates protein synthesis and survival. In vitroand in vivo studies have shown that 1st generation mTOR inhibitor, rapamycin and its analogs, targeting mTORC1 complex alone, had limited antitumor activity. Moreover, colon tumors are highly hypoxic. Overexpression of Hypoxia Inducible Factors 1α (HIF1α) is associated with tumor aggressiveness and is an independent poor prognostic factor. Interestingly, irinotecan, a topoisomerase I inhibitor, used as a chemotherapeutic agent in colon cancer, has recently been identified as a potent HIF-1α protein accumulation inhibitor. We hypothesize that targeting mTOR/HIF-1α axis by a dual mTORC1/C2 inhibitor AZD8055 will sensitize colon cancer cells to low doses of irinotecan, thereby improving treatment response with limited toxic side effects.

Methods. AZD8055 and rapamycin's effect on colon cancer cell proliferation was assessed by crystal violet method. The efficacy on mTOR axis inhibition was analyzed by Western-Blot for the phosphorylation of mTOR effectors. The effect of combination was evaluated on cell proliferation and migration. Combination index (CI) and dose reduction index (DRI) were calculated with CompuSyn software. In vivo sensitivity of two xenografted human colon tumor (derived from stage III and IV patients) to irinotecan alone or in combination with AZD8055 was evaluated in tumor-bearing mice treated with low concentrations of drugs.

Results. Our results show that rapamycin induces a partial inhibition of colon cancer proliferation (40%) whereas AZD8055 allows a much greater inhibition (80%). AZD8055 efficiently inhibits PI3K/mTOR axis, decreasing AKT, S6RP and 4E-BP1 phosphorylation via an inhibition of mTORC1/C2 complex. In contrast, rapamycin is unable to sustain 4EBP1 dephosphorylation and reactive Akt pathway. CI reveals synergistic effects on proliferation for drugs combination in both Caco2 and HCT116 cell lines. DRI values indicate that combination reduced the median effective dose of each agent. Moreover, the combination is effective to control cell migration. In vivo studies show that the drug combination results in a complete and sustained suppression of tumor growth whereas neither AZD8055 nor irinotecan is able to prevent increase in tumor volume.

Conclusion. In different colon cancer cell lines, AZD8055, as compared to rapamycin, is more efficient to inhibit mTOR axis. In addition, AZD8055 sensitizes colon cancer cell lines to irinotecan in vitro and in vivo. These preliminary results are encouraging and could improve the treatment response of patients with advanced stages of the disease or those harboring tumors with a molecular profile detrimental to the use of conventional regimens or targeted therapies.

No conflict of interest.

658 MCT1 is crucial in metabolic adaptation of uterine cervix carcinomas to microenvironment - driven by STAT3:FOXM1

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Uterine cervix cancer is the second most common malignancy in women worldwide with human papillomavirus (HPV) has the main etiologic factor. There are two main histological variants, squamous cell carcinomas (SCC) and adenocarcinomas (AC), which resemble the cell morphology of exocervix and endocervix, respectively. Cancer metabolism is an emerging cancer hallmark, being conditioned by microenvironment. As the uterine cervix homeostasis is largely dependent on the levels of lactate, we hypothesized that lactate plays a role in uterine cervix cancer progression.

Using in vitro (SiHa SCC and HeLa AC cell lines) and BALA-c/SCID xenograft tumor models, we demonstrated that lactate metabolic profiles are linked to the histological cell type, with SSC cells, being predominantly lactate consumers and AC cells lactate producers. MCT1 is a key element, allowing cells to consume lactate and its expression is in vitro regulated by the presence of lactate through FOXM1 activated by STAT3 pathway. In vivo models showed that SCC (SiHa) tumors express MCT1 and are dependent on lactate to grow, whereas AC (HeLa) express both MCT1 and MCT4, having a high growth capacity. By immunohistochemical analysis of tissue micro arrays (TMA), we observed that MCT1 expression has a significant association with SCC tumors and metastatic AC, whereas MCT4 expression concomitantly increases from in situ SCC to invasive SCC and is significantly associated with AC tumors.

Our study reinforces the role of microenvironment in the metabolic adaptation of cancer cells. In particular, MCT1 is a key element in the selection of uterine cervix cancer cells and disease progression. Hence, MCT1 may be a suitable therapeutic target in uterine cervix SCC and AC, however validation studies are needed.

No conflict of interest.

660 In vitro study of CNS metastasis in human acute lymphoblastic leukemia cell line

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Introduction. Central nervous system (CNS) metastasis, defined as the spread of cancer cells from the original tumor to the CNS, are a frequent and devastating complication of cancer associated with high morbidity and poor prognosis 1, 2. The reasons underlying why tumor cells have an increased predilection for the nervous system and the mechanisms that underlie CNS invasion by tumor cells are still poorly understood 3. Since the study of CNS and its disorders using human subjects is limited, of animal models are important tools to investigate these disease 4.

Methodology. To study the tumor progression and the importance of tumor's microenvironment it was established a leukemia murine model. Mice were inoculated in the tail vein with cells from 697 cell line (in vitro model of Human Pre-B cell leukemia) transduced with a luciferase-green fluorescence protein reporter gene. Mice where maintained with two types of diets: normal and fat diet (1.2% cholesterol). Afterwards, leukemia cells were isolated from the places where the tumor cells appeared: skin, bone marrow and brain. Different approaches, including metabolomics, gene and protein expression, were used to study the differences between these cell lines isolated from mice.

Results and discussion. The metabolic analysis by NMR spectroscopy of cell extracts of these lineages revealed that the type of diet affects more the cell metabolism than the localization of the tumor. The gene and protein expression analysis indicated that GLUT1 and LDHA may play a role in the in vivo selection of tumor cells and SGLT1 expression was increased in the cells from mice exposed to fat diet. Additionally, results suggest that gluconeogenesis may be one of the pathways involved in the tumor progression in mice exposed to a diet rich in cholesterol. These results support that cholesterol may contribute to cancer progression and development.

Conclusions. Overall, this study may contribute in understanding the pathophysiology of this disease, including the site-specific properties of tumor cells and the CNS microenvironment.

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No conflict of interest.

661 Cholangiocarcinoma (CCA) express chemokine receptor CXCR7: Important role of CXCR7 in mediating CXCL12 induced CCA cells chemotaxis and survival

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Background/Aims. Migration, invasion and metastasis of CCA cells are dependent on signals generated by stromal cells, including myofibroblastic hepatic stellate cells (HSC). We recently showed that HSC produce CXCL12, a chemokine which binds two different receptors, CXCR4 and CXCR7. CXCR7 has been shown to act either as a scavenger of CXCL12, or to start intracellular signaling alone or in cooperation with CXCR4. The purpose of the present study was to investigate the expression and function of CXCR7 in CCA cells (HuCCT-1).

Methods. CXCL12 expression and signaling pathways were investigated by Western blotting. Knock-down of CXCR7 and beta arrestin-2 was performed by siRNA. Migration of CCA cells was assayed using modified Boyden chambers, and cell survival was evaluated by MTT. Immuno-histochemistry was conducted to evaluate the expression of CXCR7 in human specimens of CCA. The association between CXCR7 and beta-arrestin-2 and between CXCR7 and CXCR4 was evaluated by immunoprecipitation and Western blotting.

Results. CXCR7 was expressed by CCA cells in human tissues and by CCA cell lines. CCX733, a specific antagonist of CXCR7, inhibited CXCL12-induced HuCCT-1 migration and survival, without affecting CXCL12-induced phosphorylation of ERK1/2 and AKT. Similarly, in CXCR7-depleted HuCCT-1 cells, the ability of CXCL12 to induce cell migration was reduced. CXCR7 binds to beta-arrestin-2, and knock-down of this molecule was associated with reduced ability of CXCL12 to induce cell migration. In contrast, pretreatment with pertussin toxin, a Gi inhibitor, affected CXCL12-induced cell survival but not CXCL12-mediated chemotaxis. Co-precipitation experiments indicate that upon CXCL12 stimulation, CXCR7 is associated with CXCR4.

Conclusions. CCA cells express CXCR7 in vivo and in vitro. Activation of CXCR7 is involved in CXCL12-induced CCA migration and survival via activation of different signaling pathways. Interaction between CXCR7 and CXCR4 may be relevant for effective downstream signaling.

No conflict of interest.

662 A novel small-molecule IAP antagonist, AZD5582, draws Mcl-1 down-regulation for induction of apoptosis through targeting of cIAP1 and XIAP in human pancreatic cancer

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Inhibitor of apoptosis proteins (IAPs) plays an important role in controlling cancer cell survival. IAPs have therefore attracted considerable attention as potential targets in anticancer therapy. In this study, we investigated the anti-tumor effect of AZD5582, a novel small-molecule IAP inhibitor, in human pancreatic cancer cells. Treating human pancreatic cancer cells with AZD5582 differentially induced apoptosis, dependent on the expression of ACORE-A and p-XIAP. Moreover, the knockdown of endogenous ACORE-A or XIAP via RNA interference in pancreatic cancer cells, which are resistant to AZD5582, resulted in increased sensitivity to AZD5582, whereas ectopically expressing ACORE-A or XIAP led to resistance to AZD5582. Additionally, AZD5582 targeted cIAP1 to induce TNF-a-induced apoptosis. More importantly, AZD5582 induced a decrease of Mcl-1 protein, a member of the Bcl-2 family, but not that of Bcl-2 and Bcl-xL. Interestingly, ectopically expressing XIAP and cIAP1 inhibited the AZD5582-induced decrease of Mcl-1 protein, which suggests that AZD5582 elicits Mcl-1 decrease for apoptosis induction by targeting of XIAP and cIAP1. Taken together, these results indicate that sensitivity to AZD5582 is determined by ACORE-A-inducible XIAP phosphorylation and by targeting cIAP1. Furthermore, Mcl-1 in pancreatic cancer may act as a potent marker to analyze the therapeutic effects of AZD5582.

No conflict of interest.

663 ACORE-F serves as a response marker for the treatment of human hepatocellular carcinoma (HCC) with a MET inhibitor

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The MET receptor tyrosine kinase, the receptor for hepatocyte growth factor (HGF), has been implicated in cancer growth, invasion, migration, angiogenesis, and metastasis in a broad variety of human cancers, including hepatocellular carcinoma (HCC). Recently, MET was suggested to be a potential target for the personalized treatment of HCC with an active HGF/MET signaling pathway. However, the mechanisms of resistance to MET inhibitors need to be elucidated in order to provide effective treatment. Here, we demonstrated that HCC cells exhibit different sensitivities to the MET inhibitor PHA665752, depending on the ACORE-F. Treatment of cells expressing both ACORE-F and phospho-MET with PHA665752 did not cause growth inhibition and cell death, whereas treatment with ACORE-F inhibitor, resulted in decreased colony formation and the cleavage of caspase-3. Moreover, silencing of ACORE-F by RNA interference of HCC cells expressing ACORE-F and phospho-MET overcame the resistance to the PHA665752 treatment. Furthermore, treatment of primary cancer cells from patients with HCC expressing both ACORE-F and phospho-MET with PHA665752 did not induce cell death, whereas ACORE-F treatment induced cell death through the cleavage of caspase-3. In addition, treatment of cells resistant to PHA665752 with ACORE-F abrogated the activation of downstream effectors of cell growth, proliferation, and survival. Based on our findings, we concluded that the ACORE-F pathway is critical for HCC survival and that targeting this pathway with ACORE-F inhibitor may be beneficial for the treatment of patients with HCC expressing ACORE-F and phospho-MET.

No conflict of interest.

664 Ovarian cancer cells are auxotrophic for arginine and sensitive to human recombinant arginase I [HuArgI(Co)-PEG5000]-induced arginine depletion

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Introduction. Arginine is a non-essential amino acid synthesized from L-citrulline and catalyzed by argininosuccinate synthetase. Some tumor cells, due to down-regulation of argininosuccinate synthetase, are unable to synthesize arginine and are, therefore, auxotrophic for arginine. Subsequently, targeting extracellular arginine for degradation in the absence of argininosuccinate synthetase induces cell death in arginine auxotrophs, rendering arginine depletion a potential therapeutic method for arginine auxotrophic tumors. HuArgI(Co)-PEG5000, a pegylated recombinant human arginase I, has shown promising results in several tumor types that display low levels of argininosuccinate synthetase such as acute lymphoblastic T cell leukemia (T-ALL), acute myeloid leukemia (AML), hepatocellular carcinoma (HCC) and glioblastoma multiforme (GBM).

Materials and Methods. In this study, we sought to investigate potential arginine auxotrophy in ovarian cancer cell lines (Caov-3, Sk-ov-3 and SW626) and attempt to target them using HuArgI (Co)-PEG5000-induced arginine depletion. Furthermore, we investigate the contribution of autophagy to arginine depletion-induced cell death using the autophagy inhibitor, chloroquine (CQ).

Results and Discussion. All three cell lines tested were auxotrophic for arginine and sensitive to HuArgI (Co)-PEG5000-induced arginine depletion with IC50 values ranging from 95 to 410 pM and percent cell death at highest concentration of approximately 90%. Addition of L-citrulline led to the rescue of SW626 and Caov-3 cells, indicating partial arginine auxotrophy in these cell lines, while Sk-ov-3 cells were not rescued by L-citrulline at the highest concentration used (11.4 mM) indicating complete arginine auxotrophy of this cell line. Inhibition of autophagy by CQ increased the sensitivity of SW626 cells to HuArgI (Co)-PEG5000-induced arginine depletion, with the IC50 of HuArgI (Co)-PEG5000 decreasing from 240 pM to 13 pM in the presence of CQ, indicating that autophagy is activated and it plays a protective role following arginine depletion in ovarian cancer cells.

Conclusion. We have shown that ovarian cancer cells are either partially or completely auxotrophic for arginine and can be effectively targeted using arginine deprivation. Further investigation of the tumor selectivity of this targeting and its mechanisms in ovarian cancer cells is currently underway.

No conflict of interest.

Poster Session: Translational Research II

665 Methylation status of histone 3 lysine can predict the prognosis of glioblastoma patients with methylated MGMT gene promoter

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Introduction. In this study, we primarily investigated the role of H3K4 methyltransferase (MLL4) and H3K27 demethylase (UTX) in progression-free survival (PFS) and overall survival (OS) of glioblastoma patients. Additionally, we examined the prognostic factors predicting PFS and OS of these patients.

Materials and Methods. The medical records of 76 patients with glioblastoma aged 18-70 years, as newly diagnosed and histologically proven from January 2000 to June 2013 at our hospital, were reviewed retrospectively. Clinical data included patient gender and age at time of surgery, WHO performance status, extent of surgery, RPA class, type of postoperative adjuvant treatment, duration of follow-up, and date of recurrence and death.

Immunohistochemical staining was performed on archived paraffin-embedded tissues obtained by surgical resection for MLL4 and UTX. The methylation status of MGMT gene promoter was determined retrospectively by methylation-specific PCR analysis.

To determine the cut-off value of immunoreactivity, we performed receiver operating characteristic (ROC) curve analysis of the MLL4 and UTX to predict the likelihood of overall survival.

Results. During follow-up period (mean duration of 27.5 months, ranged from 4.1 to 43.5 months), 8 patients survived and 68 patients died. There were 25 patients (32.9%) who was <50 years, and 47 male (61.8%) patients. In terms of WHO performance status, 30 patients (39.5%) had a score 0, 38 patients (50.0%) had a score 1, and 8 patients (10.5%) had a score 2. Ten patients (13.2%) underwent biopsy, 30 patients (39.5%) did subtotal resections, and 36 patients (47.4%) did gross total resections.

Seventeen patients (22.4%) were included in RPA class III, 43 patients (56.6%) in RPA class IV, and 16 patients (21.0%) in class V. MGMT gene promoter was methylated in 49 patients (64.5%) and unmethylated in 27 (35.5%). Thirty-five patients (46.1%) received nitrosurea-based chemotherapy before or after radiotherapy and 41 patients (53.9%) received concurrent chemoradiotherapy with temozolomide as the 1st line adjuvant treatment. In terms of immunoreactivity of UTX-MLL4 complex, overexpression was found in 42 samples (55.3%) and underexpression in 34 samples (44.7%).

Median PFS was 9.2 months (95% CI of 6.8-11.6 months). Extent of surgery and methylation status of MGMT gene promoter was associated with PFS in multivariate analysis of factors predicting PFS. Median OS was 18.6 months (95% CI of 16.3-20.9 months). Age of patients, performance status, extent of surgery, RPA class, methylation status of MGMT gene promoter, and immunoreactivity of UTX-MLL4 complex were associated with OS in multivariate analysis of factors predicting OS. Interestingly, in the patients with unmethylated MGMT gene promoter, immunoreactivity of UTX-MLL4 was not associated with OS (p=0.350). However, in the patients with methylated MGMT gene promoter, immunoreactivity was strongly associated with OS (p<0.001). Even patients with underexpression of UTX-MLL4 did not have statistical difference in OS between methylated MGMT gene promoter (p=0.589) and unmethylated MGMT gene promoter (p=0.838).

Conclusion. This study suggests that overexpression of UTX-MLL4 influenced on the better outcome of glioma patients with methylated MGMT gene promoter. This result can explain the reason that some patients even with methylated MGMT gene promoter cannot show good response to concurrent chemoradiotherapy with temozolomide and cannot survive longer than those with unmethylated MGMT gene promoter. Therefore, the immunoreactivity of UTX-MLL4 can be useful for predicting the response of concurrent chemoradiotherapy with temozolomide in the patients with methylated MGMT gene promoter

No conflict of interest.

666 Mechanisms of resistance to venetoclax (ABT-199) and strategies to circumvent it in leukemia and lymphoma cell lines

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Introduction. Venetoclax is a first-in-class, orally bioavailable BCL-2 selective inhibitor that has shown convincing clinical activity in chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL) without causing dose-limiting thrombocytopenia that is associated with the BCL-2/BCL-XL inhibitor navitoclax (ABT-263). As with any targeted anti-cancer therapy, it is important to identify the potential mechanisms of resistance, not only to inform patient selection but also to develop strategies to circumvent it as it emerges.

Materials and Methods. Venetoclax-resistant variants were generated from several venetoclax-sensitive leukemia and lymphoma cell lines by increasing their exposure in a stepwise manner over several weeks. Cell viability was determined by Cell Titer Glo assay following treatment for 24 h in medium containing 10% FBS. Synergy was assessed using Bliss independence model. Western blots, Affymetrix U133A 2.0 gene expression arrays, and Affymetrix SNP 6.0 arrays were used to determine protein and mRNA levels and DNA copy number, respectively.

Results and Discussion. Compared to the parental cell lines, the variants were 10 to >100-fold less sensitive to venetoclax and cross-resistant to navitoclax. Gene and protein expression analyses revealed multiple alterations in the expression of BCL-2 family members in the resistant variants; however, not all of the changes were universal. Alterations included changes in the levels of the target protein BCL-2, reduction of the pro-apoptotic proteins BAX, BIM or NOXA, or increases in the anti-apoptotic proteins BCL-XL or MCL-1. In some but not all cases, the changes to gene and protein expression levels were accompanied by changes in DNA copy number. Pre-clinical models indicate that both BCL-XL and MCL-1 expression can contribute to venetoclax resistance. Strong synergy was observed with venetoclax in combination with the BCL-XL or MCL-1 selective inhibitors A-1155463 and A-1208746, respectively, in resistant variants that overexpressed either BCL-XL or MCL-1. Strong synergy was observed also when venetoclax was combined with the CDK-9 inhibitor A-1592668, likely due to the rapid loss of the short lived protein MCL-1 following transcriptional inhibition.

Conclusions. Overall our data indicate that there are distinct mechanisms of resistance to venetoclax in hematologic malignancies. Inhibiting BCL-XL or MCL-1 can overcome venetoclax resistance in specific cell lines. These data have important implications for both patient selection and developing strategies to overcome resistance to venetoclax.

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Conflict of interest: Ownership: AbbVie Inc.

667 FOXM1 targets XIAP and Survivin to modulate breast cancer survival and chemoresistance

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Introduction. Breast cancer is the leading cause of deaths throughout the world. Drug resistance is a major hurdle for successful treatment of breast cancer. Overexpression of FOXM1 transcription factor and Survivin and XIAP antiapoptotic proteins is a common feature of breast tumors and associates to poor clinical outcome. FOXM1 transcription factor can transcriptionally regulate genes involved in DNA repair, metastasis, cell invasion and migration. However, little is known about the role of FOXM1 in cell survival and the gene targets involved. This work aimed to investigate the regulation of Survivin and XIAP by FOXM1 in breast cancer and how it would impact drug resistance and prognosis.

Material and Method. The human breast cancer cell lines MCF-7 and MDA-MB-231, as well as MCF-7 TaxR and MCF-7 EpiR, derived from MCF-7 and resistant to taxanes and anthracyclines, respectively, were used as models for this study. Cell culture, MTT assay, flow cytometry, immunoblotting, real-time PCR, plasmidial transfection, siRNA and chromatin immunoprecipitation were performed. We also analysed FOXM1, Survivin and XIAP expression by immunohistochemistry in breast cancer patients.

Results and Discussion. Our results show that FOXM1-overexpressing cells displayed an apoptosis-resistant phenotype towards doxorubicin and docetaxel, which associates with upregulation of XIAP and Survivin antiapoptotic genes. Conversely, FOXM1 knockdown results in XIAP and Survivin downregulation as well as decreased binding of FOXM1 to the promoter regions of XIAP and Survivin. We also demonstrated that FOXM1 can regulate XIAP through forkhead responsive elements on its promoter. Consistently, FOXM1, XIAP and Survivin levels are higher in drug-resistant cell lines when compared to their sensitive counterparts. In agreement, FOXM1 expression was significantly associated with Survivin and XIAP expression in samples from breast carcinoma patients. Importantly, patients co-expressing FOXM1, Survivin and nuclear XIAP had significantly worst overall survival, further confirming the biological relevance of regulation of Survivin and XIAP by FOXM1.

Conclusion. Collectively, our findings suggest that overexpression of FOXM1, XIAP and Survivin promotes drug resistance and is associated with poor clinical outcome in breast cancer patients.

No conflict of interest.

668 Chemically-modified microRNA-205 inhibits the growth of melanoma cells in vitro and its clinical trial in canine melanomas

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Abstract. We recently reported that microRNA (miR)-205-5p is downregulated and acts as a tumor suppressor in human melanoma cells. Previously, for clinical application, we added aromatic benzene-pyridine (BP-type) analogs to the 3'-overhang region of the RNA-strand and changed the sequences of the passenger strand in the miR-143 duplex. Here, we demonstrated the anti-tumor effect in vitro and in vivo of miR-205 that was also chemically modified by BP and had altered passenger sequence. In vitro experiments, transfection with the synthetic miR-205 (miR-205BP/S3) significantly inhibited the growth of human melanoma A2058 and Mewo cells. Exogenous miR-205BP/S3 suppressed the protein expression levels of E2F1,Bcl-2 and VEGF, which are validated targets of miR-205-5p, as did Pre-miR-205-5p. Based on the results of a luciferase activity assay, miR-205BP/S3 directly targeted E2F1and Bcl-2, as did Pre-miR-205-5p. However, miR-205BP/S3 was much more resistant to RNase than Pre-miR-205-5p in fetal bovine serum and to RNase in mice xenografted with human melanoma tissues. Additionally, the intratumoral injection of miR-205BP/S3 exhibited a significant anti-tumor effect compared with the case of control miRNA or Pre-miR-205-5p in human melanoma A2058 cell-xenografted mice. These findings indicate that miR-205BP/S3 is a possible promising therapeutic modality for melanoma. Now, we are trying a clinical study of miR-205BP/S3 in canine melanomas.

No conflict of interest.

669 Gain-of-function nature of human ALK (Anaplastic lymphoma kinase) Y1278S mutation found in neuroblastoma favors a new perspective to the ALK activation mechanisms

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Introduction. Neuroblastoma is the most common solid extracranial childhood cancer. ALK point mutations are described in both sporadic and germline forms of this disease. However, the activation mechanism of this receptor in terms of oncogenic signaling remains unclear. The structure of ALK kinase domain has previously

been reported and Y1278 of ALK has been described to be the most important tyrosine phosphorylated on the YRASYY motif in the activation loop for activity and transformation of the fusion product NPM-ALK. It has also been reported that Y1278 makes crucial interaction with C1097 to keep the inactive conformation of the kinase.

However, the report of two neuroblastoma patients harboring Y1278S mutation led us to investigate this residue further. In our study, we constructed mutations at Y1278, C1097, Y1282 and Y1283 amino acid positions in the full length ALK receptor. We show that Y1278 may not be as crucial for ALK's activation in full length receptor as has been shown in case of NPM-ALK fusion protein. We further speculate that Y1283 in the activation loop plays a significant role in the receptor's activation.

Material and Method. We have primarily worked with different cell culture based systems together with Drosophila as a model organism to characterize ALK mutations. We have performed biochemical analysis, IC50 determination, kinase assay and phosphotyrosine profiling to further exemplify our study.

Results and Discussion. We were able to characterize Y1278S as a true gain-of-function nature in both cell culture and Drosophila. Moreover, we were able to show that Y1278A mutation was similar to an inducible wildtype ligand dependent receptor. This further indicated that Y1278 may not be as crucial in the activation of full length receptor ALK as it has been in case of NPM-ALK. Also, C1097A, C1097S, C1097K mutations showed wildtype like characteristics that further suggested that the interaction between Y1278-C1097 may not be completely responsible for keeping the kinase inactive. Additionally, we investigated the Y1282 and Y1283 in the activation loop. Y1282F mutation resulted in a wild type like phenotype. Y1283F on the other hand rendered the kinase dead. Through this, we predict that Y1283 may be imperative for the ALK full length receptor's activation.

Conclusion. Thus, we propose that the activation mechanism of ALK full length receptor works differently from the fusion protein NPM-ALK. We speculate that Y1283 is preferentially phosphorylated in the YRASYY motif in the activation loop. In addition to that, the interaction between Y1278 and C1097 may not be accountable in keeping the inactive conformation of the kinase. From a clinical point of view, in our studies we also showed that the mutation Y1278S can be blocked using ALK inhibitor crizotinib.

No conflict of interest.

670 Targeting a novel MYCN mediator, PA2G4, as a therapeutic approach for the treatment of neuroblastoma

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Introduction. MYCN is amplified in approximately 25% of primary neuroblastoma tumours, and is associated with advance disease stage and poor prognosis. Here we identified Proliferation-associated protein 2G4 (PA2G4) as a novel binding partner for MYCN. PA2G4 is a transcriptional regulator and capable of interacting with DNA, RNA and protein. Its long isoform has an oncogenic function.

Material and Method. Using a panel of MYCN amplified and MYCN non-amplified human neuroblastoma cell lines and patient tumour samples, we analysed the expression of PA2G4 by real-time PCR and Western blotting. We also used the chromatin immunoprecipitation, GST-pull down assays with MYCN deletion mutants, molecular modelling studies and siRNA knockdown/overexpression to determine the structure and biological relationships between MYCN, PA2G4 and its chemical inhibitor, WS6.

Results and Discussion. We analysed the expression of PA2G4 in a panel in 11 human neuroblastoma cell lines and 477 patient tumour samples, we found that high expression of PA2G4 was a strong independent clinical predicator of poor survival, and positively correlated with MYCN expression and gene amplification. PA2G4 mRNA was expressed 8-fold higher in ganglia from TH-MYCN transgenic homozygote, compared to wild-type mice. Using GST-pull down assays with MYCN deletion mutants, we showed that MYCN via Myc Box4 binds to PA2G4. Chromatin immunoprecipitation showed MYCN binds to PA2G4 promoter to activate its transcription. Overexpression of PA2G4 increased MYCN protein stability by increasing pERK and MYCN protein phosphorylation at Serine 62, thus creating a positive forward feedback loop that may be essential for maintaining mutual high expression. Knockdown of PA2G4 markedly reduced MYCN protein level and downregulated pMDM2 and pAKT, leading to an increase in p53 expression. Importantly, the expression of MYCN was required for the effect of PA2G4 on colony formation. A small molecule inhibitor of PA2G4, WS6, significantly decreased neuroblastoma cell growth, as well as PA2G4 and MYCN protein levels. Molecular modelling studies revealed that PA2G4 has two possible docking sites for WS6, implicating WS6 may directly bind to PA2G4 protein and has strong therapeutic potential for the treatment of neuroblastoma.

Conclusion.PA2G4 is a novel MYCN-binding protein and a therapeutic target which increases MYCN protein stability and acts as an oncogenic co-factor in a forward feedback expression loop with MYCN.

No conflict of interest.

671 KITENIN/ErbB4-c-Jun axis confers resistance to cetuximab in colorectal cancer

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Introduction. Approximately 20% of metastatic colorectal cancer (CRC) patients who show no response to anti-EGFR targeted therapies do not harbor mutations in KRAS, BRAF, and PIK3CA, nor loss of PTEN expression. Previously, we identified KITENIN (KAI1 C-terminal interacting tetraspanin) as a metastasis-enhancing gene and found it to be highly expressed in sporadic CRC tissues. We recently found that EGF further increases invasiveness of KITENIN-transfected CRC cells via KITENIN/ErbB4-c-Jun axis. Here we investigated whether this novel EGF function endows CRC cells expressing higher KITENIN with enhanced survival in response to cetuximab.

Material and Method. We examined the susceptibility of various CRC cells to increasing doses of cetuximab and analyzed the expression levels of KITENIN in resected tumor tissues obtained from metastatic CRC patients before cetuximab treatment.

Results and Discussion. HCT116 and Caco2 cells expressing higher KITENIN were more resistant to cetuximab than were DLD1 and SW620 cells expressing lower KITENIN. The highly KITENIN-expressing Caco2 cells, a KRAS/BRAF wild-type cell line, were resistant to cetuximab, whereas KITENIN-knockdown Caco2 cells showed increased sensitivity. In DLD1 and HCT116 cells, mutant KRAS/BRAF cell lines, highly KITENIN-expressing DLD1 cells were resistant to cetuximab, whereas KITENIN-knockdown HCT116 cells showed sensitivity to cetuximab. The immunohistochemical expression of KITENIN was great in tumor tissues from metastatic CRC patients who showed progression of disease (PD) at initial stages after treatment with cetuximab, compared to those who exhibited a partial response (PR). These results indicated that KITENIN/ErbB4-c-Jun axis could be a molecular basis for conferring resistance to anti-EGFR agents in CRC tissues in which KITENIN is highly expressed.

Conclusion. We propose lower KITENIN levels in resected tumor tissues from metastatic CRC patients as a useful marker for identifying patients who would benefit from anti-EGFR targeted therapies, together with 'quadruple negative' genotype.

No conflict of interest.

672 The cytotoxicity of nutraceuticals in both the free and liposomal bound form against K562 cancer cells

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Cancer deaths are projected to double worldwide over the next two decades. Our mission is to develop more effective cancer treatments with minimal side effects that also lend themselves to prevention as well as treatment of the disease. . An accurate, fast assay system is described that reveals the ability of chemically defined natural products, such as Curcumin, Genistein, Resveratrol, Artemisinin, Acemannan, Ashwagandha and Vitamin C, that can destroy cancer cells in vitro. K562 Erythroleukemic cells were added to 48 well plates in 500ul portions [105 cells/ml], cultured 48 h. and exposed to the various compounds for up to 72 h. All compounds were dissolved in DMSO and diluted sufficiently to prevent solvent effects. In addition, Curcumin and Vitamin C were encapsulated into 5nm diameter liposomes (NutraSpheres™) and compared to the 'free' molecules in culture. A unique cell viability stain, which allowed the rapid staining of dead cells by membrane penetration using propidium iodide, was used to measure the cell viability of the surviving cells by flow cytometry. The control cultures were greater than 95% viable during and at the end of the experiments and were used as a baseline to measure the degree of killing. All samples were run as 3-6 replicates and SD determined. Curcumin showed complete cell destruction (zero detected cells out of about 7x105 cells in the control wells) at 25 µmol/well (LD50 = 12.5µmol) as early as 20 h. in culture. Cell death by alteration of the cancer cell membranes could be seen within 40 s of exposure to Curcumin. In contrast, Genistein, Resveratrol and Artemisinin all showed a slower increase in cellular destruction from 0.5-70 h. of incubation, suggesting the possibility of apoptosis being involved. Acemannan and Ashwagandha both showed 80-90% cytotoxicity at 48 h. at 50-70 µg per culture. Vitamin C, up to 10mmol, did not affect cancer cell viability. However, the NutraSphere Vitamin C (5 nm diameter-60mg/50ul) showed an LD50 = 133 µmol ±11 SD, which was greater than a 75 fold increase in cytotoxicity. Furthermore, the NutraSphere Curcumin (5 nm diameter-25mg/50µl) had an LD50 = 49.3 nmol ±8.3 SD (n=8) and represented over a 254 fold increase in the ability of Curcumin to destroy cancer cells. Our clinical goal is to develop water soluble mixtures of NutraSpheres with high bioavailability (>90%) and without degradation in the stomach and intestines for the prevention and treatment of cancer.

No conflict of interest.

673 Expression of tNASP in prostate cancer: Opportunities for a novel diagnostic and prognostic biomarker

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Introduction. Nuclear auto-antigenic sperm protein (NASP) is a histone chaperone and a facilitator of chromatin assembly. NASP is expressed as two splice variants:

tNASP, specific to the testis and embryonal tissues, and sNASP, expressed in all somatic cells. Interestingly, we and others have shown that tNASP acts as a tumor-associated antigen, as it is present in transformed cell lines and cancer cells in addition to its typical testicular expression. Moreover, tNASP is known to be an extremely auto-immunogenic protein. While in cancer-free patients it is sequestered in an immunologically-privileged compartment behind the blood-testis barrier, aberrant expression of tNASP protein in cancer tissues induces a robust humoral immune response.

These findings, along with the lack of reliable clinical markers for prostate cancer (PC), led us to investigate the utility of tNASP as a potential diagnostic and prognostic marker for this disease. We hypothesized that cancer-specific expression of tNASP may serve as a tissue-based marker of PC, and that auto-antibodies produced against this tNASP may be detected as a serum-based marker of disease.

Material and Method. Surgical specimens of prostate tissues and sera were received from Roswell Park Cancer Institute, Fox Chase Cancer Institute, and University of North Carolina at Chapel Hill School of Medicine. Tissue microarrays and histological sections were immunohistochemically probed with an anti-NASP antibody (specific to both sNASP and tNASP), affinity purified anti-tNASP antibody, and hematoxylin-eosin. Sera from prostate cancer patients and healthy prostate patients were tested for the presence of antibodies against tNASP using ELISA with a recombinant tNASP fragment as bait.

Results and Discussion. Expression of tNASP was detected in 76% of prostate specimens from patients with androgen-dependent prostate cancer (AD PC), 43.8% of castration recurrent prostate cancer (CR PC), 29.2% of benign prostatic hypertrophy (BPH), and 5.3% of normal prostates. Combined detection of sNASP and tNASP was 88.2% for AD PC, 48.7% for CR PC, 46.7% for BPH, and 7.1% for normal prostates. ELISA demonstrated markedly elevated concentrations of anti-tNASP antibodies in the sera of PC patients versus healthy prostate patients.

Conclusion. This study demonstrates the utility of tNASP protein as a tissue-based diagnostic and prognostic biomarker of PC, and detection of anti-tNASP antibodies as a serum-based biomarker of PC.

No conflict of interest.

674 Organoid cultures of human colon adenocarcinoma for modelling of drug response

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Cell lines derived from patient tumours have contributed tremendously to our understanding of cancer biology and therapeutic drug response. However, cancer cell lines grown in 2D in vitro culture have several limitations including failing to represent the heterogeneity of cancer. The derivation from clinical specimens of epithelial organoid models grown in 3D culture could transform the preclinical cancer setting by better reflecting the biology of the tumour of origin, and by providing more predictive models of patient responses to cancer therapies.Using a collection of 20 organoid cultures derived from colon cancer patients, we show that they reflect the genetic landscape of driver mutations and copy number alterations present in the primary tumour from which they were derived. Gene expression analysis confirms that the major colorectal clinical subtypes are represented in this organoid panel. Furthermore, we developed a high-throughput screening assay to correlate drug sensitivity with the genetic footprint of organoid cultures. Screening of 80 clinical and pre-clinical compounds confirmed the clinical finding that KRAS mutant tumours are insensitive to EGFR-targeted agents, and TP53 mutant tumours are resistant to the MDM2 inhibitor nutlin-3a. We also observe exclusive activity of porcupine inhibitors in the RNF43 mutated setting, and differential sensitivity to the AKT1/2 inhibitor MK-2206 and the pan-HER inhibitor AZD8931, drugs of key clinical interest. By carrying out further analyses, we aim to identify novel therapeutic strategies for colon cancer together with molecular biomarkers to direct their clinical use.

No conflict of interest.

676 Integrin-linked kinase is a key mediator of stromal cell-enhanced resistance of CML stem/progenitor cells to tyrosine kinase inhibitors

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Introduction. The human bone marrow (BM) compartment consists of a multi-functional network of cells that interact with hematopoietic stem cells. Evidence suggests that this microenvironment likely adds to the resistance of chronic myeloid leukemia (CML) stem cells to tyrosine kinase inhibitor (TKI) therapy. Integrin-linked kinase (ILK) is a serine/threonine kinase, an important constituent of focal adhesions and a key mediator of multiple signaling pathways.

Material and Method. Biological and molecular functions of ILK, including response/resistance of leukemic stem cells (LSCs) to TKI and an ILK inhibitor, were investigated.

Results and Discussion. We have demonstrated for the first time that expression of ILK and its downstream targets are highly upregulated in pre-treatment CD34+ CML cells (n=6) compared to normal BM controls (n=3, $p<0.05$), using RNA-seq analysis, with confirmation by qRT-PCR analysis of CD34+ cells from an additional 28 CML patients and 9 normal adults ($p<0.05$). ILK expression was much higher in CML LSCs (lin-CD34+CD38-) than progenitors (lin-CD34+CD38+) or mature cells (lin+CD34-, $p<0.01$). ILK transcripts and intracellular protein levels were elevated in CD34+ CML cells, but not in CD34- cells, in response to TKI treatment, especially in the presence of BM stromal cells. TKIs in combination with a pre-clinically validated ILK inhibitor (QLT0267) significantly reduced colony yields compared to a single agent or combination of TKIs, and this enhanced cell killing was most pronounced on cells from TKI nonresponders (n=6; $p<0.01$). Long-term culture-initiating cell assays showed that CML LSCs co-cultured with stromal cells were more significantly eliminated by combination treatments. Mechanistically, the combination treatments enhanced apoptosis of CD34+ cells and CFSE tracking of cell division showed that QLT0267 targets quiescent LSCs from TKI-nonresponders ($p<0.05$). The combination also enhanced apoptosis of LSCs by abolishing the protective effect of BM stromal cells with TKI alone. Combination treatments of TKI and QLT0267 displayed strong synergy (CI value <0.3). Importantly, QLT0267 (up to 10 μM) was not toxic to normal CD34+ BM cells.

Conclusion.ILK thus emerges as an attractive druggable target and the enhanced and selective effect obtained by dual inhibition of ILK and BCR-ABL, particularly in the presence of protective stromal cells to mimic the BM microenvironment, may offer an important new therapeutic possibility for CML.

No conflict of interest.

677 The ratio of ErbB4:ErbB3 predicts preclinical activity of the ErbB3-directed therapeutic MM-121

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Introduction. MM-121 is a fully human ErbB3-directed antibody designed to inhibit tumor cell survival and reverse resistance to standard-of-care therapeutics by blocking heregulin (HRG1)-driven ErbB3 activation. Both pre-clinical and clinical studies of MM-121 implicate the level of HRG expression as a predictive biomarker of MM-121 activity. HRG, however, is known to potently activate two distinct cell surface receptors: ErbB3 and ErbB4. Like ErbB3, ErbB4 has the ability to form dimers with other ErbB receptors upon ligand binding and activates many of the same downstream signaling pathways as ErbB3. We therefore investigated the potential role of ErbB4 as a mediator of resistance to MM-121.

Materials and Methods. We characterized signaling patterns and phenotypic responses to HRG and MM-121 in-vitro in a diverse panel of cell lines spanning a range of ErbB3 and ErbB4 expression levels. Using RNAi and cDNA overexpression, we explored the causal relationship between ErbB4 expression and MM-121 sensitivity/resistance in-vitro and in mouse xenograft models.

Results and Discussion. Our data show that in cells with a low ErbB4:ErbB3 ratio, HRG activates downstream signaling pathways and cell proliferation through ErbB3-containing dimers, which can be inhibited by MM-121. In contrast, in cells with a high ErbB4:ErbB3 ratio, HRG induces signaling and cell proliferation through ErbB4-containing receptor dimers, which MM-121 does not block. We also demonstrate that MM-121-responsive cell lines and tumor models can be converted to non-responders and vice-versa by modulation of ErbB4 levels alone. Finally, by analyzing MM-121 response patterns across a large panel of cell line models, we show that the linear ratio of ErbB4:ErbB3 protein levels better predicts MM-121 sensitivity than multiple alternative metrics.

Conclusion. Taken together, our data highlight the ErbB4/ErbB3 ratio as a potential predictor of the activity of MM-121 and potentially other ErbB3-directed therapeutics as well.

No conflict of interest.

678 Gene electrotransfer of canine interleukin 12 into canine melanoma cell lines

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Introduction. Gene electrotransfer (GET) of interleukin 12 (IL-12) had already proved to give good results when treating tumors in human and veterinary clinical trials. So far, plasmids used in veterinary clinical studies encoded human or feline IL-12 and ampicillin resistance gene, which is not recommend by the regulatory agencies to be used in clinical trials. Therefore, the aim of the current study was to construct the plasmid encoding canine IL-12 with kanamycin antibiotic resistance gene suitable for clinical use in veterinary oncology.

Materials and Methods. The plasmid carrying the sequence for canine IL-12 and kanamycin antibiotic resistance was constructed using standard molecular biology techniques of restriction and ligation. Its activity was evaluated in vitro using GET. Canine and human malignant melanoma cell lines were transfected with plasmid encoding enhanced green fluorescence protein (EGFP) at different electric pulse parameter conditions to determine the transfection efficiency and cell survival. IL-12 expression at the most suitable conditions for GET of plasmid encoding canine IL-12 was determined at mRNA level with qRT-PCR and at protein level with ELISpot assay.

Results and Discussion. The plasmid with canine IL-12 and kanamycin antibiotic resistance gene was successfully constructed. The validation of newly constructed plasmid was carried out on canine malignant melanoma cells which have not been used in GET studies so far and on human malignant melanoma cells. The results showed that the plasmid has similar expression of IL-12 compared to plasmid with human IL-12 and ampicillin antibiotic gene, which was previously used in clinical studies in dogs' mastocytomas.

Conclusions. Based on the canine origin, and high expression level demonstrated in the two canine malignant melanoma cell lines, the newly constructed plasmid represents a promising therapeutic plasmid for further clinical studies with IL-12 GET treatment in canine spontaneous tumors. In addition, the constructed plasmid also meets the criteria of regulatory agencies.

No conflict of interest.

679 Antitumor efficacy of the sequential treatment of Vinorelbine and Gefitinib in Non-Small Cell Lung Cancer cell lines: In vitro and in vivo Results.

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Introduction. Gefitinib (GEF) is a reversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (EGFR-TKI) indicated for the treatment of advanced non-small cell lung cancer (NSCLC) with activating mutations of EGFR. Preclinical studies suggest that the combination of antitumor agents with EGFR-TKIs may increase the cytotoxic activity albeit influenced by the type of drug and the order of administration. The purpose of the present study was to investigate in vitro and in vivo the antitumor efficacy of different combinations of GEF and vinorelbine (VNB) in NSCLC cells, poorly responsive to EGFR-TKIs, as a rationale to be potentially exploited in the clinical setting.

Material and Method. The human NSCLC cell lines A549 and H1975, exhibiting wild- type and EGFR mutation (L858R-T790M) respectively, were used to evaluate the antiproliferative effects of three different treatment schedules: GEF followed by VNB, VNB followed by GEF and the two drugs given individually or concurrently. Cell viability was assessed by the MTT assay (short-term). In addition, the efficacy of repeated weekly VNB treatment along with sequential or continuous GEF administration for 21 days was investigated by cell count (long-term). Expression of the un-phosphorylated and phosphorylated (p) forms of EGFR and its downstream effectors AKT and ERK1/2 were investigated by western blot. The in vivo effects of single or combined treatments were studied in a CD1-Nude mouse model by subcutaneous injection of the H1975 cells. Cancer growth was determined by measurements of tumor volume every other day. Moreover, the 18F-fluorodeoxyglycose uptake (SUV) and cancer glucose consumption was estimated by means of a dynamic micro Positron Emission Tomography scans. After framing rate optimization, pixelwise maps of cancer glucose consumption (MRGlu) were determined.

Results and Discussion. In vitro short and long-term experiments demonstrated that the sequence of VNB followed by GEF was significantly more active in inhibiting the cell growth than GEF followed by VNB or the concurrent administration of the two drugs, especially in the EGFR-mutated H1975. Western blot indicated that the increased cytotoxic effect of the VNB and GEF sequence was accompanied by inhibition of p-EGFR, p-AKT and p-ERK1/2 expression levels, mainly in the H1975 cells. Moreover, the increased antitumor efficacy of the sequence VNB followed by GEF was also confirmed in vivo by a significant inhibition of the H1975 tumor growth ($p<0.0001$) that was paralleled by a corresponding decrease in cancer glucose consumption ($p<0.05$).

Conclusion. These preclinical findings in NSCLC cell lines, poorly responsive to reversible EGFR-TKIs, showed increased sensitivity to the sequential schedule of VNB followed by GEF in vitro and in vivo as compared with single agent treatment or the reverse scheme, supporting a possible application to a clinical trial.

No conflict of interest.

680 The role of circulating free DNA and circulating tumor cells in advanced non-small cell lung cancer patients

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Introduction. NSCLC is the leading cause of cancer-related death worldwide and the 5-year survival rate is very poor (~15%), mainly due to late diagnosis. Circulating free DNA (cfDNA) and circulating tumor cells (CTC) have been recently associated with metastatic and aggressive disease in various cancers including non-small cell lung cancer (NSCLC), and might represent non-invasive prognostic markers. The aim of the present study was to assess whether the variation of cfDNA and CTC might predict the objective response, progression-free survival (PFS) and overall survival (OS) in patients with advanced NSCLC receiving 1st line platinum-based chemotherapy (CHT).

Material and method. Peripheral blood samples and computed tomography (CT)-scans were obtained at baseline and after every two CHT cycles from 70 patients affected by NSCLC treated with 1st line CHT; CTC were isolated by size using a filtration-based device (ScreenCell®) and characterized and enumerated through H&E staining and immunofluorescence; whereas cfDNA was quantified by qPCR. Radiological responses were assessed by CT-scans according to RECIST v. 1.1.

Result and discussion. Patients' characteristics: median age 67 (44-80); 69% males, 31% females; non-squamous: 90%, squamous: 10%; no patient harbored activating mutations of EGFR or ALK translocation. Median cfDNA at baseline: 76.8 copy number, median CTC at baseline: 14/3ml blood. Median PFS and OS were 5.0 and 7.5 months, respectively. Baseline cfDNA quantification significantly predicted PFS (1st vs. 4th quartile: 7 vs. 3 months) and OS (1st vs. 4th quartile: 8 vs. 5 months), whereas baseline CTC count alone did not predict outcome. The cfDNA decrease/increase after 2 CHT cycles was able to predict OS (-30% vs. +30%: 12 vs. 9.5 months), the same trend for OS was observed using CTC variation (-30% vs. +30%: 8 vs. 5 months).

Interestingly, in patients with high number of baseline CTC (>25/3 ml), their variation (-30% vs. +30%) significantly predicted prognosis in terms of PFS (5.5 vs. 1.0 months) and OS (9 vs. 2.0 months).

Conclusion. Although the evaluation of cfDNA and CTC did not anticipate CT-scan result, it could be used as additional non-invasive approach of disease monitoring; in particular, CTC count was reported as a reliable prognostic marker in the subset of patients with high baseline CTC number. ClinicalTrials.gov n° NCT02055144

No conflict of interest.

682 Steroidal and non-steroidal third-generation aromatase inhibitors induce pain-like symptoms via TRPA1

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Use of aromatase inhibitors (AIs), exemestane, letrozole and anastrozole, for breast cancer therapy is associated with severe pain symptoms, the underlying mechanism of which is unknown. The electrophilic nature of AIs suggests that they may target the transient receptor potential ankyrin 1 (TRPA1) channel, a major pathway in pain transmission and neurogenic inflammation. By using pharmacological or genetic tools, we found that AIs evoke TRPA1-mediated calcium response and current in rodent dorsal root ganglion neurons and in human cells expressing the recombinant or native channel. In mice, AIs produce acute nociception, which is exaggerated by pre-exposure to proalgesic stimuli, and, by releasing sensory neuropeptides, neurogenic inflammation in peripheral tissues. AIs also evoke mechanical allodynia and decreased grip strength, which do not undergo desensitization upon prolonged AI administration. These effects are markedly attenuated by TRPA1 pharmacological blockade or in TRPA1-deficient mice. The ability of AIs to activate sensory nerve terminals, via the TRPA1 channel may be responsible for the inflammatory and painful side effects related to AIs therapy. Present findings suggest that a TRPA1 antagonist could be useful in the treatment of patients with painful states evoked by AIs.

No conflict of interest.

683 Dogs with naturally occurring cancers as new predictive models for human pathology: A fast and relevant way to optimize clinical development

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Context. Most novel drug candidates entering clinical trials fail to reach approval, largely because preclinical models used in development do not provide adequate information about their efficacy or toxicity. That's why, more predictive models of efficacy in oncology are needed. The study of novel drug candidates in dogs with naturally occurring tumors allows drug assessment in neoplasms sharing many fundamental features with its human counterparts, and thus provides an opportunity to answer questions guiding the cancer drug development path in ways not possible in more conventional models.

Project. Metastatic melanoma is one of the most aggressive cutaneous tumors in humans. It constitutes 4 to 11% of cutaneous malignancies causing 80% of skin cancer deaths. In dogs, melanomas are one of the most frequently diagnosed malignancies of the oral cavity and are characterized by focal infiltration, recurrence, and metastasis to regional lymph nodes and less frequently to lungs and other organs. These cancers account for 7% of all malignant tumors in dogs and share the same sites as humans. Moreover, the strong homologies in clinical presentation, morphology, and overall biology between humans and dogs, make companion animals a good model to investigate tumor process from aetiology to tailored treatments. Many challenges have been identified recently, among which, immune escape and tumor dormancy. This is for example the case in the PDL-1/PD-1 interaction axis and its importance in melanoma, inducing immune escape and invasion through cytotoxic T lymphocyte blockade.

Material & Method. A surgically removed, oral malignant melanoma tissue sample from a 14 year-old female Yorkshire was treated enzymatically, in order to isolate tumor cells.Two groups of SCID/ Nude mice, aged 12 weeks were subcutaneously injected with cancer cells and tumor growth was monitored every 3 - 4 days. Mice were euthanized when tumors reached 2000mm3 volume, tumors were then harvested and cell extracted and cultured in flasks. We are now investigating chromosomal copy number variation mutations among isolated melanoma cell lines using comparative genome hybridization array study. Cancer stem cells are also being identified through a functionalized test battery (sphere formation, exclusion assay)

Results. We recently developed two canine xenograft melanoma mouse models and canine derived melanoma cell lines, expressing PDL-1 protein at their surface. Also, mutations, correlated with clinical outcomes are being studied. Finally, we isolated and are now identifying tumor-initiating cells capable of self-renewal and differentiation through functionalized test battery. **Conclusion.**we obtained canine melanoma cell lines expressing immunoevasion markers and among which, a population of stem cells have been identified. This results make a step forward in the validation of canine spontaneous tumors as good model to better drug development and give rise to new hope in cancer research

No conflict of interest.

684 Red pigment from saw palmetto: A natural product for potential alternative cancer treatment

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Introduction. various natural products from saw palmetto extract were sold in the world. Saw palmetto extract is an extract of the fruit of Serenoa repens (Bartram) J.K.Small, an edible plant in the southeastern United States. It has been used in traditional, complementary and alternative medicine as a tonic, or an expectorant and antiseptic to treat various ill conditions, especial urinary and reproductive system problems and cancer supportive care. However, there are discrepancy in some studies. The different results may come from different products. Saw palmetto extract is rich in fatty acids and phytosterols. As functional food, NYG, a new natural product isolated from red pigment of Saw palmetto has received positive response in late stage patients with various cancers in Japan, so it is necessary to investigate the effect and mechanism of NYG by scientific research. Objective: To explore the anti-tumor potential of red pigment fraction from Saw Palmetto Extract (NYG) on human hepatocellular carcinoma (HCC).

Material and Method. three products named NYG1, NYG4 and NYG7 were used to evaluate the tumor inhibitory effect of NYGs by in vitro and in vivo HCC models. Xenograft model of HCC, cell migration and blood vessel formation models were used to evaluate the inhibitory effect of NYGs in tumor growth and invasion. The mRNA and protein expression related to HCC after NYGs treatment were analyzed by PCR and western blot.

Result and discussion. Significant tumor inhibition of NYGs in dose-dependent manner in vitro and in vivo HCC models was observed, while NYGSs has no inhibitory effect on the cell viability by MTT assy. NYGs also showed minimal effect on normal hepatic cells, which may suggest the NYGSs safety on patients. These results suggested that the inhibitory effect of NYGSs in tumor growth and invasion may be caused by anti-angiogenesis effect of NYGSs.

Conclusion.NYGs has no cytotoxic effect on HCC cell lines, but it can inhibit cancer growth in in vivo model and cancer blood formation suggesting that NYGs may be used for alternative cancer treatment by inhibiting neovascularization. The study was supported by grants from the University of Hong Kong (Project code: 104002889), Wong's Donation (Project code: 207040314) and Gaia Family Trust (project code: 200007008).

Keywords: NYG; Hepatocellular carcinoma; Tumor inhibition; Angiogenesis; Xenograft model

No conflict of interest.

685 Ondansetron modulates the multidrug resistance phenotype in preclinical model of brain tumors

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Introduction. The cure rate of malignant brain tumors lags behind the success obtained in other areas of hematology-oncology. This depends also on limited diffusion of chemotherapy agents, as anthracyclines, within the brain tissue due to multidrug resistance (MDR) mechanisms which hamper their delivery across the Blood-Brain Barrier (BBB). A wide range of factors influences the drug uptake into the brain. One of the underlying mechanisms of BBB-mediated drug resistance is the over-expression of influx and efflux proteins, as P-glycoprotein (ABCB1/P-gp/MDR1). Many chemotherapy agents and other drugs are substrates of P-gp, whose ATP-dependent active transport at the BBB is considered one of the most relevant mechanisms of tumor resistance.

Material and Method. We have recently performed a study to assess whether Ondansetron (Ond) enhances Doxorubicin (Dox) cytotoxicity in cell lines interfering with P-gp and it is able to increase Dox concentration in rat brain tissue. Dox is the most widely administered of the anthracyclines, although its clinical application suffers from poor efficacy due to MDR; Ond is commonly used during chemotherapy as a potent antiemetic drug. Both Ond and Dox are specific substrates of P-gp efflux pump. The effect of Ond and Ond/Dox exposure on the MDR phenotype was studied in drug sensitive and MDR expressing clones from cancer (P5 and P1(0.5), respectively) and not cancer cells (PSI-2 and PN1A, respectively) and in two glioblastoma cell lines (A172, U87MG).

In order to assess whether Ond is an effective drug in allowing Dox accumulation within the brain, male adult Wistar rats were pretreated with Ond before injection of Dox. Quantitative analysis of Dox was performed by LC-MS/MS mass spectrometry.

Results and Discussion. The data showed that Ond is able to modulate the MDR phenotype in cancer and not cancer cells. Accordingly to our in vitro experiments, Ond is an effective 'door-keeper' in allowing accumulation of Dox within the brain. This effect is most likely due to the fact that both Ond and Dox compete for P-gp mediated transport at the BBB level.

Conclusion. Our investigations on preclinical models demonstrate the therapeutic efficacy of pharmacological treatment in malignant brain tumors by safe and temporary BBB permeabilization. All these data pave the way to new approaches to enhance the delivery and, as a result, the efficacy of drugs which are usually cleared by the BBB into the brain.

No conflict of interest.

686 Value of the prognostic biomarkers related to TP53 pathway after >10 years in a cohort of 61 locally advanced head and neck cancers

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Introduction. Chemo-radiotherapy (CRT) cisplatin-based is a curative regimen in a subset of patients (pts) with locally advanced head and neck squamous cell carcinomas (LAHNSCCs), but with considerable toxicity. Thus, additional biomarkers are required to optimise treatments.

Pts carrying TP53 mutations are associated with decreased Overall Survival (OS) and Progression Free Survival (PFS). Our aim was to reconsider 61 LAHNSCC pts (median follow-up: 4.3 yrs; range 0.1-16.8) that were characterised both for mutations and SNP72 in TP53 gene and clinical history, under the light of the new available prognostic markers, including MDM2 SNP309, p16 and EGFR.

Material and Method. We updated the follow-up of 61 LAHNSCC pts described >10 yrs ago. We analysed by direct sequencing mutations and SNP72 in TP53 and SNP309 in MDM2 genes. When mutations occurred in heterozygote SNP72 pts (72RP), we identified the allele carrying the mutation, in order to split mutant-72R or mutant-72P pts. p16 and EGFR expressions were analysed by IHC. TP53, EGFR, p16 and MDM2 were correlated to OS and PFS using the Kaplan-Meier method and the log-rank test to evaluate significance.

Results and Discussion. Clinical outcome in 61 LAHNSCC pts was influenced by TP53 SNP72.

In mutated TP53 pts, both OS and PFS were significantly longer in pts carrying mutations in the 72P than in the 72R allele: 3/8 vs 26/29 dead pts and median survival not reached vs 2.8 yrs after 15 yrs in 72P and 72R pts respectively (p=0.006).

Wt TP53 pts showed an intermediate behaviour with 21/24 dead, while the OS was longer for 72RR and 72RP pts (median OS=6 and 5.42 yrs respectively) compared to 72PP ones (median OS=0.6 yrs, p=0.005).

At the time of analysis, 34 of the 61 pts had progressed and the mutated 72P carriers showed a higher PFS than the 72R ones: 2/8 vs 16/29 progressed pts and median PFS of 15.6 vs 2.1 yrs in 72P and 72R pts respectively (p=0.028).

Wt TP53 pts showed an intermediate behaviour with 13/24 progressions and median survival of 6 yrs.

Median OS was significantly shorter in pts with MDM2 SNP309 genotypes GG or GT, compared to TT (2.2 vs 8.1 yrs; p=0.002). p16 and EGFR curves don't discriminate statistically.

Conclusion. The predictive utility of the TP53 SNP72 may be of value in the selection of LAHNSCC pts for CRT. However its validity in clinical practise has to be related with TP53 mutational status. Thus the analysis of MDM2 SNP309 might be favoured as clinical biomarker in this subset of pts.

No conflict of interest.

687 In vivo efficacy evaluation of doxorubicin on tumor regression on a xenograft mice model pre-treated with morphine

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Introduction. The main trouble for the treatment of Central Nervous System (CNS) tumors is the structure and activity of the Blood-Brain-Barrier (BBB), an anatomic and metabolic barrier between blood and brain. BBB is characterized by the presence of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP, ABCG2), two Multi Drug Resistance (MDR) efflux transporters that work in tandem on the BBB and limit the spread of anticancer agents within the brain, as Doxorubicin (Dox), .

Recently, we have demonstrated that morphine allows an accumulation of Dox within the rat brain by LC-MS/MS mass spectrometry, interfering with the permeability of the BBB.

The aim of the current proposal is to investigate the influence of morphine on the efficacy of Dox treatment in an orthotopic xenograft mice model of brain tumor.

Material and Method. Nude fox1nu mice have been micro-injected into the striatum with U87MG-luc cell line to establish an experimental brain tumor.

Following intracranial tumor injection bioluminescence levels of mice brains have been immediately acquired for baseline data and on a weekly basis thereafter. Once imaging studies had confirmed tumor engraftment, animals have been treated with morphine ,Dox or their combination for up to 2-3 weeks. Control mice have received an equivalent volume of saline once a week for 2-3 weeks. Tumor growth and weight have been carried out weekly.

Results and Discussion. Our preliminary results demonstrated significant differences between groups treated with Dox or Dox plus morphine compared with control group. The data showed a considerable tumor volume inhibition in both these groups; however, the treatment with Dox plus morphine seemed to decrease the invasiveness of xenografted U87MG-luc cells in nude mice brains.

Further studies are needed to determine the optimum dosing regimen, as well as defining the effect of morphine on Dox delivery into the brain.

Conclusion. This study may offer the opportunity to explore the role of the morphine on the delivery of active but currently inapplicable drugs for the treatment of brain tumors or other debilitating neurological diseases.

No conflict of interest.

688 Hereditary genetic polymorphisms of suppressor of cytokine signaling 5 (SOCS5) gene predicts prognosis of patients with esophageal squamous cell carcinoma

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Introduction. The family of suppressor of cytokine signaling (SOCS) proteins represents one of the key mechanisms regulating signaling derived from cytokines and growth factors, and plays important anti-inflammatory and tumor suppressive roles. Esophageal squamous cell carcinoma (ESCC) is a deadly disease with poor prognosis. The effect of single nucleotide polymorphisms (SNPs) of SOCS genes on the prognosis of ESCC has not been investigated. We thus investigate the correlation between SOCS SNPs and prognosis of patients with ESCC.

Material and Methods. We analyzed 15 germline SNPs, including 4 SNPs at SOCS1, 3 SNPs at SOCS3, 5 SNPs at SOCS5, and 3 SNPs at CISH. A total of 632 patients diagnosed with ESCC were enrolled in the study and randomly assigned to a training set (n=268) or replication set (n=364). The genotypes of these SNPs were analyzed from the buffycoat DNA of these ESCC patients by Sequenom MassARRAY platform and iPLEX gold chemistry, and correlated with overall survival or progression-free survival by multivariate Cox regression analysis adjusted for for age, gender, stage, tumor location, surgical status and CCRT. 3 SNPs at SOCS5, including SOCS5:rs3814039, SOCS5: rs3738890, and SOCS5: rs3768720 were further correlated with metastasis in patients by multiple logistic regression analysis.

Results. In the training group, there were 8 SNPs, including 2 at SOCS1, 1 at SOCS3, 3 at SOCS5 and 2 at CISH, significantly or borderline significantly correlated with overall (OS) or progression-free survival (PFS). The correlation can only be repeated in 3 SNPs at SOCS5, the SOCS5:rs3814039, SOCS5: rs3738890, and SOCS5: rs3768720, in replication group (rs3814039, CC vs. GG, HR[95 % CI]=1.33[0.92-1.92] , p=0.127 for OS, HR[95 % CI]=1.45[1.03-2.06] , p=0.034 for PFS; rs3738890, GG vs. CC, HR[95 % CI]=1.34 [0.93-1.93] , p=0.119 for OS, HR[95 % CI]=1.41 [1.00-2.00] , p=0.051 for PFS; rs3768720, CC vs. AA, HR[95 % CI]=1.37 [0.95-1.96] , p=0.092 for OS, HR[95 % CI]=1.53 [1.08-2.16] , p=0.016 for PFS). The prognostic relevance were all statistically significant in the combine group which combining the subjects in training and replication sets (rs3814039, CC vs. GG, p=0.032 for OS and p=0.009 for PFS; rs3738890, GG vs. CC, p=0.016 for OS and p=0.008 for PFS; rs3768720, CC vs. AA, , p=0.005 for OS and p=0.002 for PFS). Furthermore, SOCS5: rs3768720 significant correlated with the risk of 2-year distant metastasis (OR[95 % CI]=2.25 [1.10-4.60] , p=0.027). The Kaplan-Meier survival curves were constructed for both OS and PFS by the genotypes of prognostically-relevant SNPs. Both the OS and PFS durations differed significantly between patients with different genotypes of SOCS5:rs3814039, SOCS5: rs3738890,and SOCS5: rs3768720 in total subjects.

Conclusions. Hereditary genetic polymorphisms of SOCS5 gene can serve as prognostic predictors of patients with ESCC. Because SOCS5 target EGF/EGFR and IL-6 signaling which are highly correlated with the prognosis of ESCC, the SNPs at SOCS5 might regulate the expression of SOCS5 to effect on downstream signaling and lead to the different clinical outcome of the patients.

No conflict of interest.

689 Radiolabeled click-chemistry derived non-peptide RGD peptidomimetics for SPECT molecular imaging of melanoma

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Introduction. The role of integrins in the regulation of angiogenesis is an essential feature in tumor progression and metastatic dissemination. In recent years, many efforts have been dedicated to integrins as selective targets in molecular imaging. αvβ3 Integrin-targeted radiotracers are putatively able to reveal the presence of a tumor mass and to monitor the growth and diffusion of tumor cells. In this view, we identified a class of click chemistry derived peptidomimetics antagonist to avb3 integrin which showed high in vitro affinity to αvβ3 receptors, inhibition of human melanoma cell adhesion to Arg-Gly-Asp-(RGD) containing substrata, and, labelled with 125-I, functioned as efficient molecular imaging biomarkers in pre-clinical model of avb3 expressing xenografts. In this study we used pre-clinical Single Photon Emission Computed Tomography (SPECT) for in vivo imaging of 125I-labelled compounds. High resolution SPECT studies are proven to be essential for developing new imaging probes for diagnostic (124I) and therapeutic (131I) approaches.

Material and methods. Solid-phase assays. Cytofluorimetric analysis. Inhibition of adhesion assay. Pre-clinical imaging with a mCT-PET-SPECT FLEX™ Triumph™(Trifoil-Imaging, Northridge, CA).

Results and discussion. Cold iodinated RGD-peptidomimetics were first selected for their in vitro binding competition activity and their capacity to inhibit adhesion of human melanoma cells to RGD substrates. Three of the compounds differing for the Arg mimetic portion were selected for preclinical studies. The compound 125I-3 showed a reduced renal elimination kinetics as compared to compound 125I-2, and a prolonged persistence in the circulatory system. Compound 125I-4 showed a faster time-dependent increase of the activity in the tumor mass, also higher as compared to compound 125I-3. Differences in the metabolic patterns of excretion between compound 125I-2 and the other two were related to the more lipophilic arginine mimetic portion and less basic behavior of the molecule. Displacement experiments confirmed the the specific binding activity of the two radiolabeled compounds.

Conclusions. Here we present two promising RGD peptidomimetic markers for molecular imaging in early detection of cancer lesion

No conflict of interest.

690 Chemical genetics uncovers a vulnerability of breast cancer cells harbouring a highly frequent GATA3 mutation

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Background. Breast cancer is the most common cancer in women, causing more than 500,000 deaths per year. Despite existing targeted therapy strategies, it is still challenging to target 'undruggable' cancer genes and to predict drug sensitivity and therapy outcome due to the genetic heterogeneity of breast tumours, with only three genes (PIK3CA, TP53, GATA3) being mutated in >10% of breast cancer patients. Thus, additional approaches towards personalized medicine are urgently needed.

Methods. We employ a systematic approach to investigate functional interactions between genes that are mutated in breast cancer and a panel of drugs to reveal synthetic lethal (or resistant) relationships. We use a model of isogenic cell lines that differ only in a defined genetic aberration (i.e. overexpression of an oncogene or knock-down of a tumour suppressor) to quantitatively assess the cellular fitness in response to drug treatments.

Results. We have screened a drug library of about 100 small molecules (approved anti-cancer drugs, tool compounds, or compounds targeting epigenetic modifier proteins) to identify novel sensitivity/resistance mechanisms. Interestingly, we discovered that cells expressing a frequently found mutant of the transcription factor GATA3 are hypersensitive to the specific inhibition of a chromatin-modifying enzyme. This gene-drug interaction is specific to mutant GATA3 and not observed with the wild-type protein. The observed hypersensitivity is due to increased apoptosis upon drug treatment in GATA3mut cells. We have obtained mechanistic insight into the impact of this so far uncharacterized mutation by affinity purification and mass spectrometry, yielding a number of specific interactors for either the wild-type or mutant form. We are currently further investigating the mechanism of this gene-drug interaction by biochemical and transcriptomic analysis, aiming at a better understanding of the biologic relevance of this highly frequent breast cancer mutation.

Conclusions. Our systematic drug-gene interaction study revealed a novel vulnerability of breast cancer cells harbouring a highly frequent mutation in GATA3, potentially leading to a novel therapeutic avenue in an important subgroup of breast cancer patients.

No conflict of interest.

693 Assessing the biological role of miR-211 family in a zebrafish melanoma model

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Introduction. We have recently found that miR-211 family, which is composed by miR-204 and miR-211, can affect the biological properties of melanoma cells, both in terms of differentiation and in terms of survival. In order to increase the relevance of these in vitro data, we take advantage of the MiniCoopR melanoma model system in zebrafish [1].

Material and Method. Analogously to the mouse [2], in zebrafish the melanocytic lineage-restricted expression of BRAFV600E in a p53 null background induces the formation of invasive melanomas, which resemble those found in humans. In the recently developed Tg(mitfa:BRAFV600E);p53-/-;mitfa-/-transgenic zebrafish line, melanocytes (and hence melanomas) are suppressed because of a mutation in the endogenous melanocytic lineage-specific mitfa gene, but can be both rescued if one-cell stage embryos are microinjected with the miniCoopR vector, which expresses a rescuing minigene composed of the promoter, ORF and 3'-untranslated region

of the wt zebrafish mitfa. Furthermore, the miniCoopR vector is compatible with the Gateway® recombination system. This means that it can be used to restrict the expression of a gene of interest to the rescued melanocytes under the control of a mitfa promoter and hence to assess if the concomitant presence of that gene alters the tumorigenic properties of BRAFV600E in the p53 null background [1, 3].

Results and Discussion. Our aim is to adapt the miniCoopR system and test if the modulation of the level of miR-204/211 (overexpression or inhibition through sponging constructs [4]) affects melanoma formation in zebrafish.

In particular, we have already subcloned into the miniCoopR vector both a sponging construct for miR-204 and a non-targeting sponging construct that will be used as negative control. We have also started with the injection of 100-200 embryos per experimental condition. All the experiments are performed in our zebrafish facility, in compliance with the Italian and the European regulation. Successfully injected fishes will be monitored for up to 30 weeks and will be scored weekly for the presence of visible tumors, so that melanoma-free survival curves can be generated.

Conclusion. This extremely specific animal model will allow us to establish if our miRNA family has a biological role in vivo and will be instrumental for the identification of the molecular targets through which this role is exerted.

No conflict of interest.

695 Tracking in vivo CXCR4 expressing human cancer cells with a near infrared labelled-CXCR4 cyclic peptide antagonist Peptide R

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Introduction. CXCL12/CXCR4 plays a major role in tumor invasion, proliferation and metastasis. Early cancer detection, praecox relapse and monitoring therapy efficacy will benefit from effective imaging **Methods.** A new family of CXCR4 antagonist cyclic peptides was recently described. Three Peptides R, S and I were identified as the most promising CXCR4 antagonist as they inhibited CXCR4 in vitro and in vivo (1). To realize a suitable probe for molecular imaging that will selectively target CXCR4 expressing tumors, a Peptide R antagonist based-(NIR)fluorescent probe was developed.

Material and Method: Peptide R was conjugated with NIR fluorescent VIVOTAG-S750. In vitro binding was evaluated in B16-huCXCR4 murine melanoma, PES43 human melanoma and FB-1 human anaplastic thyroid cancer cells expressing respectively high and low CXCR4 level. Cells were grown on coverslips and incubated with Peptide R-VIVOTAG-S750 at 100 nM concentration. The fluorescence signal was observed by confocal microscope. In vivo studies were conducted in nude mice injected with B16-huCXCR4 versus FB-1 cells. Peptide R-VIVOTAG-S750 vs VIVOTAG-S750 alone was injected after 10-20 days. After 45 min the lungs and nodules were explanted. Tumor-tissue slides were incubated with DAPI and monoclonal anti-huCXCR4 (Alexa Fluor 488) and imaged with fluorescence microscopy (Axio Scope.A1 Zeiss).

Result and discussion. In vitro results showed that Peptide R-VIVOTAG-S750 binds distinctly B16-huCXCR4 and PES43 cells but not FB-1 cells, negative/low CXCR4 expressing cells. In vivo data showed that Peptide R-VIVOTAG-S750 specifically labeled B16-huCXCR4 derived lung metastases. Similarly, Peptide R-VIVOTAG-S750 specifically targeted B16-huCXCR4 subcutaneous xenograft, while FB-1 subcutaneous xenograft showed no fluorescent signals when injected with Peptide R-VIVOTAG-S750. Thus Peptide R-VIVOTAG-S750 discriminated B16-huCXCR4 from FB-1 tumor cells, proving its applicability in the detection of CXCR4 expressing cells. Ongoing experiments are visualizing labeled Peptide R distribution in a model of B16-huCXCR4 lung metastases and subcutaneous nodules through Xenogen IVIS system.

Conclusion.A novel probe for CXCR4 receptor optical imaging was developed conjugating the newly developed CXCR4 antagonist, Peptide R, with the in vivo tracer VIVOTAG-S750.

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No conflict of interest.

696 Targeting Aurora kinases and cyclin-dependent kinase 2 (CDK2) in osteosarcoma

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Introduction. Osteosarcoma (OS) is the most common malignant bone tumour for which conventional neoadjuvant chemotherapy treatments have reached a survival plateau with a 60-65% long term survival probability. The major cause of treatment failure in OS is the development of drug resistance, mainly due to the overexpression of ABCB1.

To improve the present clinical results, novel tailored regimens are necessary. Targeting protein kinases emerged as a new promising therapeutic strategy in several human tumours. However, it has to be taken into account that some anti-kinase drugs may be substrates of ABCB1 and, therefore, the potential clinical usefulness of these agents must be estimated also in relation to the status of this transporter in tumour cells.

Aurora kinases and cyclin-dependent kinase 2 (CDK2) are key regulators of the cell cycle and represent new promising therapeutic targets in several human tumours. In this study, we analysed the clinical and biological impact of these kinases in OS, in order to elucidate their activity in both drug sensitive and drug resistant ABCB1-overexpressing tumour cells.

Material and Method. Clinical and biological relevance of Aurora kinases A-B and CDK2 were assessed, respectively, on OS clinical samples and by silencing these genes with specific siRNA in human OS cell lines. In vitro efficacy of Aurora kinases- and CDK2-targeting drugs was evaluated on a panel of drug-sensitive and -resistant human OS cell lines.

Results and Discussion. In OS patients, Aurora kinases A-B and CDK2 overexpression at diagnosis appeared to be associated with a trend toward a worse clinical outcome. Human OS cell lines growth and survival proved to be partially dependent from Aurora kinases A-B and CDK2 expression. Drugs targeting these kinases (VX-680, ZM447439, and Roscovitine) showed remarkable in vitro activity on these cells. Anti-Aurora kinases drugs (VX-680 and ZM447439) showed a decreased activity in doxorubicin-resistant, ABCB1-overexpressing cells, but were able to sensitise cells to doxorubicin when conbined with this drug. Roscovitine effectiveness did not prove to be dependent from ABCB1, being similarly active in both sensitive and resistant cell lines.

Conclusion. Aurora kinases and CDK2 may represent new candidate therapeutic targets for OS and agents targeting these kinases might be considered as possible adjuvants to conventional chemotherapeutics.

No conflict of interest.

697 Changes in linc-RNA expression associated with the outcome of stage I epithelial ovarian cancer

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Introduction. Epithelial Ovarian Cancer (EOC) is the fifth most frequent cause of cancer death in women. EOC stage I represents only 10% of EOC and it is generally characterized by a good prognosis, however about 20% of patients relapse. Previous experiments performed by our group showed that EOC stage I patients with poor prognosis had alterations at transcriptional level. We investigated long intergenic non-coding RNAs (linc-RNAs), a new class of RNA involved in transcriptional regulation, to explore a possible correlation between their expression in Stage I EOC and patients' clinical outcome.

Materials and Methods. 208 snap-frozen tumor biopsies of stage I EOC patients (45 of which relapsed) with median follow up of 9 years were obtained from three independent Italian tumor tissue collections. In the selected samples the three grades and the four cellular histotypes of EOC are homogenously represented. Microarray and qRT-PCR were used to identify and validate lincRNA expression. Results were correlated, in multivariate analysis, with overall survival (OS) and progression-free survival (PFS). In vitro models were exploited to investigate the localization of selected linc-RNAs using qRT-PCR. Digital Droplet PCR (Bio-Rad®) was employed to evaluate copy number of PVT1, a well-known linc-RNA which results from our work.

Results. A lincRNA based signature associated to relapse was identified by microarray analysis. Signature validation by qRT-PCR revealed that three out of 14 selected lincRNAs were independently associated with poor outcome (p-value < 0.05 in OS and/or in PFS) in multivariate analysis: linc-SERTAD2, linc-SOX4 and linc-MYC (PVT1). Increased expression of the three linc-RNAs was associated with poor patient outcome ($p<0.05$). In vitro studies showed that the selected lincRNAs predominantly had a nuclear localization in all examined six ovarian carcinoma cell lines, thus suggesting they have a key role in transcriptional regulation.

No association was reported between overexpression of PVT1 and gene copy number variation.

Conclusion. Our results represent the first evidence that the expression levels of specific linc-RNAs are strongly associated with prognosis of stage I EOC patients, confirming that mechanisms controlling transcriptional regulation are deregulated in patients who relapse. Should these results be confirmed in further studies they could provide a means to improve patients stratification at time of diagnosis.

No conflict of interest.

698 Profiling cancer gene mutations in longitudinal epithelial ovarian cancer biopsies by targeted next-generation sequencing: A retrospective study

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Background. The vast majority of stage III-IV epithelial ovarian cancer (EOC) patients relapse after initial response to platinum-based chemotherapy, and develop resistance. The genomic features involved in drug resistance are still unknown. To go further in detail we investigated the mutational profile of genes involved in pathways related to drug sensitivity in a cohort of matched tumors taken at first (Ft-S), and second surgery (Sd-S).

Patients and methods. 33 matched biopsies taken at Ft-S and Sd-S were selected from 'Pandora' tumor tissue collection. DNA libraries for 65 genes were generated using TruSeq Custom Amplicon kit and sequenced on MiSeq (Illumina). Data were analyzed according to established techniques using a high performance cluster computing platform (Cloud4CARE project) and independently validated.

Results. A total of 2270 somatic mutations were identified (89.85% SNPs and 8.19% indels, 1.92% unknown). Homologous recombination genes and TP53 were mutated in the majority of Ft-S, while ATM, ATR, TOP2A and TOP2B were mutated in the entire dataset. Only 2% of mutations were conserved between matched Ft-S and Sd-S. Mutations detected at second surgery clustered patients in two groups characterized by different mutational profiles in the homologous recombination, PI3K, miRNA biogenesis and signal transduction..

Conclusions. Our results on a selection of genes involved in key processes of tumor growth and drug resistance, show a low level of concordance between Ft-S and Sd-S, suggesting the importance of future longitudinal analysis for the clinical management of relapsed EOC.

No conflict of interest.

699 Multi-modal nanomedicine for glioblastoma

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Introduction. Glioblastoma multiforme (GBM) is an aggressive primary neoplasm of the brain that exhibit notable refractivity to standard treatment regimens. Recent large-scale molecular profiling has revealed deregulated molecular networks as potential targets for therapeutic development. MicroRNAs (miRNAs) are non-coding RNA molecules which act as post-transcriptional regulators of specific messenger RNA transcripts. miRNAs play major roles in normal developmental processes, and their deregulation significantly contributes to various aspects of carcinogenesis. Nevertheless, in vivo delivery of small interfering RNA (siRNA) and miRNA remains a crucial challenge for their therapeutic success. siRNAs and miRNAs on their own are not taken-up by most mammalian cells in a way that preserves their activity.

Results and Discussion. In order to circumvent these limitations, we developed a cationic carrier system, which can strongly improve its stability, intracellular trafficking and silencing efficacy. Polyglycerol (PG)-Amine, a water-soluble polyglycerol-based hyperbranched polymer accumulates in the tumor microenvironment due to the enhanced permeability and retention (EPR) effect, and therefore, represents an ideal nanocarrier for antitumor oligonucleotides.

Using our novel nanocarrier, we have studied the expression targets and functional effects of miR-34a in several human glioblastoma cell lines and human tissue samples. miR-34a levels inversely correlated to their target gene levels measured in the same cell lines or tissue. Transient transfection of PG-NH2-miR-34a polyplex into glioblastoma cells strongly inhibited cell proliferation, cell cycle progression, and cell migration. Consequently, we performed an in vivo experiment and achieved a significant tumor growth inhibition following treatment with PG-NH2-miR-34a polyplex in a human glioblastoma mouse model. We further characterized the synergistic effect of combining PG-NH2-miR-34a polyplex with chemotherapy and achieved promising results.

Conclusions. Together, our findings show that PG-NH2 efficiently delivers anticancer miRNAs to glioblastoma cells and suppresses brain tumor growth. These results suggest that our polyplex could serve as a potential nanomedicine for glioblastoma.

No conflict of interest.

700 Valproic Acid potentiates the anticancer activity of capecitabine in vitro and in vivo breast cancer models by inducing thymidine phosphorylase expression

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Introduction. Capecitabine, commonly used in different settings for metastatic breast cancer, is a prodrug that take advantage of the tumor elevated levels of thymidine phosphorylase (TP), a key enzyme for its conversion to 5-florouracil (5FU). We have previously demonstrated that the histone deacetylase-inhibitor (HDACi) vorinostat induces synergistic antitumor effects in combination with capecitabine by upregulating, in vitro and in vivo, in colorectal cancer cells TP mRNA and protein expression. Valproic acid (VPA) has been widely used as an anti-epileptic drug and has recently been reported to act as a class I HDACi. Based on these data we studied the effects of several HDACis, including VPA, to potentiate the anticancer activity of capecitabine in breast cancer cells.

Methods. In vitro antiproliferative effects of HDACis and/or capecitabine-metabolite 5'-deoxy-5-fluorouridine (5'-DFUR), which requires the presence of TP to be converted into 5FU, were performed by sulforhodamine B colorimetric assay. Drugs synergism was determined by calcusyn software. Proteins and transcripts expression were detected by western blotting and real-time PCR, respectively. Apoptosis was measured by FACS analysis. siRNAs were used for transient knockdown of HDAC isoforms and shRNA for stable knockdown of TP. In vivo experiments were performed on MCF-7 xenografts in female NOD/SCID previously injected with 17β-estradiol pellet and treated with VPA i.p. (200 mg/Kg) and/or capecitabine per os (359 mg/Kg) 5 days/week for 3 weeks.

Results. HDACis, including VPA, induced dose and time-dependent upregulation of TP transcript and protein in breast cancer cells but not in non-tumorigenic MCF-10A cell line. By using siRNA or isoform specific HDACis we showed that HDAC-3 is the main isoform whose inhibition is involved in TP modulation. We then demonstrated synergistic/additive antiproliferative and pro-apoptotic interaction between 5'-DFUR and several HDACis, including VPA. TP knockdown experiments highlighted the crucial role of TP in the synergism observed. Synergistic antitumor effect of VPA and capecitabine was also demonstrated in vivo in MCF7 breast cancer xenografts, but not in xenografts from MCF7 TP-knocked cells, confirming in vitro data.

Conclusion. Overall, this study suggests that the combination of an HDACi, such as VPA, and capecitabine, is an innovative antitumor strategy and warrants further clinical evaluation for the treatment of metastatic breast cancer.

No conflict of interest.

701 Derivation and characterization of cancer-initiating cells representative of metastatic colorectal cancer genetic heterogeneity

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Introduction. Metastatic colorectal cancer (CRC) remains largely incurable. The efficacy of innovative agents such as EGFR antibodies is limited by primary resistance conferred by genetic lesions. CRC contains a tumorigenic cell hierarchy sustained by a subpopulation of cancer-initiating cells (CC-ICs), in which the genetic lesions accumulate to drive progression, determine the response to therapy, and cause subclonal selection under therapeutic pressure. We thus set out to isolate an ample panel of CC-IC representative of the genetic heterogeneity of metastatic CRC, in order to obtain reliable in vitro models to study therapeutic responses.

Material and method. From a previously set-up platform of patient-derived xenografts of metastatic CRC (xenopatients), CC-IC were systematically selected in culture (xenospheres), and analysed for their ability to self-renew in vitro, and to generate secondary tumors that phenocopy the tumors of origin. Xenospheres were analyzed for their mutational profile, and for their response to the EGFR antibody cetuximab, and compared with the original xenopatients.

Result and discussion. 38 independent xenospheres were isolated. 18 xenospheres harbor genetic lesions known to confer primary resistance to cetuximab, either a frequent or a rare KRAS mutation, or a PIK3CA, or a BRAF mutation. 20 xenospheres (52%) are 'quandruple-wt', as they display intact KRAS, NRAS, BRAF or PIK3CA genes, but include 6 xenospheres expressing high levels of IGF2, 2 with ERBB2 amplification or mutation, and 2 with mutations in the EGFR extracellular domain, features that were recently shown to reduce cetuximab sensitivity in xenopatients. The effect of cetuximab on xenosphere proliferation and survival was measured in vitro, showing a strong correlation between lack of therapeutic effect and genetic alterations leading to constitutive activation of the RAS pathway. Conversely, xenospheres without RAS constitutive activation were strongly dependent on EGFR, and susceptible to cetuximab inhibition. However, these xenospheres were also sensitive to other growth factors that compensate for EGFR inhibition, and are amenable to systematic screening of microenvironmental factors that can confer cetuximab resistance.

Conclusion. We generated a panel of CC-ICs representative of the most common genetic determinants and therapeutic response of metastatic CRC to EGFR inhibition, which can be exploited to identify mechanisms of resistance and new therapeutic targets.

No conflict of interest.

702 Choline metabolism is an early predictor of EGFR-mediated survival in NSCLC

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Introduction. Aberrant choline and phospholipid metabolism is a hallmark of tumour progression: however little mechanistic evidence links choline kinase alpha (ChoKa) to oncogenic signalling. We analysed the role of de novo lipid synthesis in NSCLC by breaking down choline metabolism in three independent steps, namely influx, phosphorylation and efflux.

Materials and Methods. ChoKa endogenous expression levels were evaluated against EGFR status and intracellular choline uptake was measured and correlated with ChoKa activity. Choline metabolism was evaluated in three steps following stimulation: choline influx, phosphorylation and efflux were measured in cell culture. Rapid modulation of this cholinic phenotype was measured in response to four FDA approved drugs (erlotinib, cisplatin, pemetrexed, paclitaxel) and a ChoKa specific inhibitor (MN58b). Choline influx and phosphorylation were further evaluated in vivo by PET imaging using the tracer [18F]-D4-Choline. Also, stable isotope labelling by amino acids in cell culture (SILAC) analysis was performed.

Results. Results showed that choline uptake was dependent on ChoKa expression levels while inhibition of ChoKa decreased choline uptake. We found that high intracellular choline uptake could be induced as a response to nutrient deprivation and this effect was reversed through second messenger signalling or growth factor stimulation. Furthermore, a decrease in choline metabolism within three hours of treatment was dependent on the concentration of drug dosage and could be used as an early predictor of survival, evaluated at 72 hours post treatment. Tracer kinetics were diagnostic of choline kinase expression and sensitive to therapeutic dosing of cisplatin or choline kinase inhibitor. Furthermoer, the proteomic and phosphoproteomic signature of the choline kinase inhibitor MN58b against Erlotinib in mutant EGFR cells showed significant overlap.

Conclusions. EGFR signal transduction activation decreased choline uptake whereas pharmacological inhibition of ChoKa affected the EGFR signalling pathway. We found correspondence between ChoKa and EGFR inhibition providing the first mechanistic evidence that Choline Kinase and lipid metabolism are effectors of the EGFR signalling cascade in NSCLC.

No conflict of interest.

703 Clinical significance of Anoctamin-1 gene at 11q13 in laryngeal tumorigenesis

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Introduction. The calcium-activated chloride channel Anoctamin-1 (ANO1) has emerged as a candidate to drive 11q13 amplification in head and neck squamous cell carcinomas (HNSCC), thereby contributing to disease progression and metastatic dissemination. This study investigates for the first time the clinical significance of ANO1 in early stages of laryngeal tumorigenesis and malignant transformation.

Material and Method. ANO1 gene amplification and protein expression were respectively evaluated by real-time PCR and immunohistochemistry in paraffin-embedded tissue specimens from 35 patients with laryngeal premalignancies. In addition, ANO1 expression was analyzed in 57 patients with laryngeal squamous cell carcinoma. Correlations with clinicopathologic parameters and laryngeal cancer risk were also investigated.

Results and Discussion. ANO1 gene amplification was frequently detected along the different stages of laryngeal tumorigenesis from mild to severe dysplasias (ranging 50-75%). In contrast, ANO1 expression was only found in severe dysplasia/CIS and at a lower frequency (37%, 7/19 cases). Patients with lesions harboring ANO1 gene amplification showed a higher laryngeal cancer risk (HR = 3.62; 95% CI 0.79-16.57; P = 0.097; Cox regression), compared to patients with ANO1 expression-positive lesions (HR = 1.65; 95% CI 0.44-6.24; P = 0.46). ANO1 expression in laryngeal squamous cell carcinomas was infrequent (11%, 6/57 cases), and showed no significant correlations with patients outcome.

Conclusion. ANO1 gene amplification, but not ANO1 expression, occurred frequently in early stages of laryngeal tumorigenesis and, more importantly, showed potential in predicting laryngeal cancer risk. These data suggest that other genes at 11q13 could contribute to the clinical phenotype.

No conflict of interest.

704 The role of nitric oxide in generation and maintenance of cancer stem cells: New therapeutic opportunities in cancer

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Introduction. - Various studies have revealed a nitric oxide (NO)-induced poor outcome phenotype in cancer. However, the mechanisms responsible for the effects of NO in tumor biology and disease progression remain poorly understood. Many cancers, including breast and colon cancer, are driven by a subpopulation of cells that display stem cell properties, known as cancer stem cells (CSCs), which mediate metastasis and contribute to tumor relapse. It is therefore essential to investigate the role of NO in the generation and/or maintenance of these cell subpopulations.

Materials and Methods. - NO levels in human cancer cells were modulated using NO-scavengers or NO-donors and CSCs subpopulations were evaluated using tumorospheres formation assays, tumor spheroid growth and xenograft experiments. Cell cycle analysis was performed using flow cytometry and the activity of stemness-related signaling pathways was assessed by inmunoblotting.

Results and discussion. The pre-treatment of colon (HCT-116, Caco-2, DLD1) or breast (MCF-7, BT-474) cancer cells with the NO scavenger carboxy-PTIO (c-PTIO) markedly inhibited their subsequent ability to form tumorospheres in suspension. Also, NO depletion altered their capacity to growth as tumor spheroids when cultured in matrigel. This indicates that the selective removal of NO produced by tumor cells can abolish the ability of self-renewal in colon and breast cancer CSCs. Furthermore the effect was NO-specific, since the use of NO donors (DETANONOate and CSNO) eradicated the anti-CSC activity of c-PTIO. The NO scavenging pre-treatment downregulated Notch, Bmi-1 and wnt/beta-catenin signaling pathways, which are known to be involved in the generation and expansion of breast and colon CSCs. The depletion of endogenous NO in breast cancer cells by the heterologous expression of the bacterial NO-consuming enzyme flavohemoglobin also impaired tumorosphere formation and the growth of tumor spheroids. Furthermore, tumor cell xenograft experiments showed that c-PTIO pre-treatment of colon cancer cells markedly reduced the size tumors formed compared to control cells.

Conclusions. NO exert autocrine and/or paracrine effects on stem cell signaling pathways which are essential for the generation and maintenance of CSCs subpopulations in tumors and may constitute a therapeutic target which can be efficiently blocked by selective elimination of this molecule. Funded by MINECO-ISCIII: PI13/00553.

No conflict of interest.

705 Targeting CXCR4 with new cyclic peptide antagonists potentiates conventional chemotherapy in in vivo colorectal cancer

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Introduction. The prognostic role of CXCL12/CXCR4–CXCR7 was previously shown in colorectal cancer (CRC) (D'Alterio 2014, Int.J.Cancer: 135, 379–390). Being the CXCR4-CXCL12-CXCR7 axis overexpressed, functional and affecting prognosis in CRC, this is an ideal setting for clinical development of CXCR4 antagonists. We recently patented (IT Patent M12010A000096; PCT WO2011/092575 A1) a new family of CXCR4 antagonists (Portella, PLoS One. 2013; 8(9): e74548). Aim of the present study is to evaluate the effect of Peptide R, a new CXCR4 antagonist peptide, in the development and metastatic dissemination in in vitro andin vivo models of colorectal cancer.

Materials and Methods. The cytotoxic activity of 5-fluorouracil (5FU) oxaliplatin (OX) and Pep R was measured by the SRB assay in human CRC cell lines HCT116, SW620 and HT29. In vivo Athymic Nude-Foxn1nu/Foxn1+ mice, 7-10 weeks old (6 mice/group) were subcutaneously injected with HCT116 (2*106). Tumor growth was followed by bi-weekly measurement of tumor diameters by caliper and by ultrasound imaging. Differences between groups were considered significant at P<0.05.

Results and discussion. Cytotoxic effects of chemotherapy agents commonly used for the treatment of CRC in combination with CXCR4 antagonist Peptide R were evaluated in human colon cancer cells HCT116, SW620 and HT29. In vitro Peptide R revealed no toxicity. HCT116 human subcutaneous xenograft (>=50mm3) were treated with 1) Peptide R [5mg/Kg daily 5days week for 2week]; 2) 5FU [30 mg/Kg daily 5days week for first week]+OX [4.2 mg/kg once week every 5 days, for 2week]; 3) 5FU [30 mg/Kg]+OX [4.2 mg/kg]+Pep R [5mg/Kg] i.p. for 2 weeks. Chemotherapy treatment reduced growth of the subcutaneous nodule, also Peptide R alone inhibited primary tumor growth suggesting that the Peptide R acts also on tumor microenvironment. Moreover combination treatment with Peptide R and chemotherapy dramatically reduced primary tumor growth suggesting that Peptide R targeted CXCR4 expressing primary tumor cells and fibroblasts, endothelial, mesenchymal and inflammatory cells.

Conclusions. Concomitant chemotherapy plus CXCR4 new antagonist Peptide R synergizes in in vivo model of human colon cancer. Ongoing studies are characterizing KRAS mutational status and EMT as possible mechanisms of CXCR4 antagonist activity.

No conflict of interest.

706 Novel insight into Notum and glypicans regulation in a colorectal cancer mouse model

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Introduction. A connection between colorectal cancer (CRC), Wnt signaling pathway activation and mutations in the Kras and Apc genes is well known, although a full elucidation of the underlying molecular regulation of the Wnt/βcatenin pathway and other biological functions involved in CRC pathogenesis is still needed. Here, the well known AOM/DSS cancer model, achieved by the administration of azoxymethane (AOM) with dextran sulfate sodium (DSS) in Balb/c mice, was used to perform a genome-wide expression profiling in order to identify potential novel biomarkers related to CRC progression to propose for clinical use.

Material and method. 30 Balb/c mice were divided in 4 groups: 1) AOM/DSS treated, 2) AOM treated, 3) DSS treated, 4) untreated. 20 weeks after the start of the AOM/DSS protocol, 6 mice/group were sacrificed. RNA was isolated from each sample using Trizol®. Genome-wide expression microarray was performed using MouseWG-6v2.0 Expression BeadChip (Illumina, Inc, CA). Microarray data were evaluated by state of the art statistics tools. IPA7 (www.ingenuity.com) was used for Gene Ontology (GO) analysis. Histopathological analysis and immunohistochemistry (IHC) followed standard procedures. 6 AOM/DSS treated mice were investigated at 5th week for the early colon lesions.

Results and discussion. We identified 2036 differentially expressed genes in AOM/DSS induced tumors, including 1092 upregulated and 944 downregulated genes. GO analyses revealed that the most altered genes were significantly involved in the activation of the Wnt/βcatenin pathway. Interestingly, the enhanced expression of genes that encode Wnt antagonists was observed, which may be a negative feedback in response to activated Wnt/βcatenin signaling. Particularly, we found an enhanced expression of NOTUM and GLYPICAN-1 (GLY1) and a reduced expression of GLYPICAN-3 (GLY3). Moreover, the IHC revealed a positive correlation of NOTUM overexpression with intracellular βcatenin staining and a correlation with glypicans expression. We showed an inverse correlation between molecular expression and protein distribution between Notum and GLY3. Conversely, GLY1 showed a very similar expression pattern to Notum in late lesions, although a positive staining was observed in 90% of early lesions.

Conclusion. We provide the first demonstration of significant changes in NOTUM, GLY1 and GLY3 gene expression in the CRC development and we suggest their deeper investigation to be proposed as new biomarkers.

No conflict of interest

707 Phenethyl isothiocyanate hampers tumor growth and progression of d16HER2-positive breast cancer

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Background. Almost 90% of HER2-positive breast cancers (BCs) express the aggressive HER2 splice variant lacking exon 16 (d16HER2). This deletion promotes the generation of stable and active d16HER2 homodimers. Comparison of the tumorigenic potential of the human d16HER2 and WTHER2 genes in the corresponding transgenic mouse models revealed a significantly shorter tumor latency period and a higher tumor incidence in the d16HER2 line, suggesting a potential enrichment of this variant in HER2-positive breast cancer-initiating cells (BCICs). Based on reports that cruciferous vegetable-derived compounds such as isothiocyanates (ITCs) mediate strong anti-tumorigenic effects of numerous oncotypes and target BCICs, we tested whether the ITC phenethyl isothiocyanate (PEITC) impairs d16HER2-positive tumor growth and progression in transgenic and orthotopic models and affects mammosphere-forming efficiency (MFE%) in d16HER2- and WTHER2-positive BC cell lines.

Methods. Therapeutic activity of PEITC and Trastuzumab (T) was evaluated in parallel in d16HER2 transgenic and orthotopically implanted FVB mice injected into the mammary fat pad with MI6 mammary tumor cells derived from a d16HER2 spontaneous transgenic lesion. Anti-tumor effects of PEITC were assessed in orthotopic tumors by magnetic resonance imaging (MRI). PEITC activity was also compared to that of T by Western blot and immunohistochemistry analyses. MFE% was evaluated in MI6 and WTHER2_1 mammary tumor cells, derived from a spontaneous WTHER2 transgenic lesion, in the presence or absence of PEITC.

Results. In transgenic mice, PEITC treatment led to a significant reduction in mammary tumor incidence ($p=0.0210$) compared to controls and, in the orthotopic model, effectively suppressed d16HER2-driven tumor growth ($p<0.001$) to the same extent as T. MRI indicated hemorrhagic and non-hemorrhagic necrosis after PEITC treatment, confirming its therapeutic activity. In PEITC-treated lesions, downregulation of d16HER2 was coupled with a massive increase in mesenchymal-like tumor cells. PEITC significantly reduced MFE% as compared to T only in MI6 cells, with no effect of either agent on WTHER2_1 mammospheres.

Conclusion. Our results provide evidence that PEITC mediates a significant anti-tumor effect in in vivo d16HER2-positive models, most likely targeting BCICs.

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No conflict of interest.

708 Photo-acoustic and sonoporation methods: Synergic improvement in tumoral treatment

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Introduction. Photoacoustic imaging (PAI) demonstrated a wide potential to obtain simultaneously structural, functional and molecular information. Liposomes are clinically used as nanocarriers for many drugs, including chemotherapeutics as theranostic agents. The aim of this work was to assess changes in tumor vascular dynamics with the use of PAI, followed by the systemic injection of theranostic liposomes, and to show the differences on nanosystem's flows with and without sonoporation treatments applied on tumoral masses and compare the behavior of tumoral necrotic zones.

Material and method. Ten Balb/C bearing a subcutaneous syngeneic breast cancer (TS/A) were enrolled in the study: five of them immediately after liposomes injection (t=0) have been exposed to sonoporation treatment. Sonoporation was carried out by using a custom system with non-focused 1 MHz transducer and low acoustic intensity with an insonation time of 40 sec. The mice were injected i.v. with 200 µl of liposomes (130 nm diameter). PEGylated liposomes were labelled with near-infrared cyanine dye in order to pick up the PAI signal. The image acquisition with VEVO LAZR system (Fujifilm VisualSonics Inc.), equipped with a highly sensitive photo-acoustic transducer at a frequency of 21 MHz, consisted in: 1) application of sequential laser pulses at 750 and 850 nm to obtain PAI of the tissue vasculature: this method allowed the quantification of Oxygen saturation (sO2) and total hemoglobin (HbT) 2) scanning tumour in the range 680-970 nm to get a whole spectra profile. The maximum liposomes flow value, , measured between 1 and 90 minutes after injection, was quantified by the Optical Flow Matlab code, after using Unmixing Tool software onboard the VeVO-LARZ 2100.

% increase	sO2 TUMOR AREA	sO2 NECROTIC TUMOR AREA	HbT TUMOR AREA	HbT NECROTIC TUMOR AREA	Optical FLOW TOTAL TUMOR AREA (deg/s)
WITHOUT sonoporation	45 %	89 %	30 %	57 %	55.23
WITH sonoporation	68 %	120 %	45%	73%	83.50

Results. Conclusion. The results show a great improvement in all the values only when sonoporation treatment was applied.

Liposomes flow has increased of 51.2% in the tumoral mass and increasing values sO2, HbT in necrotic portion are observed. Each result is the average of five values. Non-invasive ultrasound sonoporation as tumor treatment method causing transient cell membranes permeabilization and PAI, as growing bioimaging modality, represent a fundamental response to the clinical application to strengthen effectiveness of cancer treatment.

No conflict of interest.

709 Development of small molecule activators of protein phosphatase 2A (SMAPs) for the treatment of high-grade serous ovarian and uterine cancer

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Introduction. More than 70,000 women in the United States are estimated to be diagnosed with ovarian or endometrial cancer in 2014. It is estimated that about 15,000 and 8,000 women are expected to die each year from ovarian and endometrial cancers, respectively. Unlike, low-grade ovarian or endometrial cancer, high-grade subtypes of these cancers (HGSOC and UPSC) follow an aggressive clinical course and are difficult to treat, as responses to traditional cytotoxic chemotherapy regimens remain poor. Though both are derived from different organs, they share many clinical and molecular features that drive disease progression, thus we hypothesize that therapeutic activation of phosphatases which suppress multiple oncogenic signaling proteins altered in both HGSCO and UPSC simultaneously, will result in broader and more durable clinical responses. This approach of testing the same drug in two molecularly similar cancers may facilitate the discovery of effective, targeted treatments, and uncover that drugs found to work against one type of cancer may also work to fight other cancer types that share similar genotypes. This project is pursuing newly-created re-engineered pharmaceutic compounds, Small Molecule Activators of Protein Phosphatase 2A (SMAPS), that produce an enzyme activation of

a major serine/threonine phosphatases thus coordinately inactivating multiple oncogenic signaling pathways altered in UPSC and HGSOC. PP2A accounts for the majority of cellular Serine/Threonine phosphatase activity in cancer cells and inhibits many signaling cascades critical to UPSC and HGSOC cancer growth and progression, including PI3K-AKT, MAPK, MYC and p53 signaling.

Material & Methods.MTT, Cell Cycle Analysis, and Colony Forming Assays using commercially available and patient-derived human EMCA and HGSCO cell lines were performed to assess the activity of SMAPs in vitro. Protein expression after treatment with SMAPs was performed to assess affects on apoptosis (cleaved parp), DNA damage (H2Ax) and ERK and AKT signaling. Patient-derived mouse xenografts (PDX) were established using primary tumor implants into athymic nude mice to test in vivo oral activity of SMAPs versus intraperitoneal cisplatin and oral placebo controls.

Results. Our preliminary data has uncovered that SMAPs are highly effective at decreasing cellular viability and inducing apoptotic cell death in both established and primary HGSOC and UPSC cell lines as well as in patient-derived xenografts (PDX) models of the diseases through coordinate inhibition of ERK and AKT signaling pathway. Treatment of EMCA cells with SMAPs resulted in decreased cellular proliferation and survival. Oral treatment with SMAPs led to either suppressed tumor growth or tumor regression, which resulted in increased survival. This increase in survival was achieved with minimal toxicity, when compared to placebo and platinum controls.

Conclusion. Our findings suggest that SMAPs may represent a novel therapeutic option for the treatment of advanced or recurrent EMCA and HGSOC.

No conflict of interest.

710 Novel therapeutic approaches for human malignant mesothelioma

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Introduction. Human malignant mesothelioma (MM) is an aggressive and highly lethal cancer that has been linked to asbestos and erionite exposure. MM causes about 3,200 deaths per year in the US and more than 100,000 deaths per year worldwide. We previously found that high-mobility group box-1 protein (HMGB1), a prototypic damage-associated molecular pattern (DAMP), plays a critical role in the early events of malignant transformation of primary human mesothelial cells and MM development. More recently we showed that most MM cells secrete high amounts of HMGB1, and that HMGB1 supports the malignant phenotype by promoting proliferation and invasiveness of MM cells. Therefore, MM cells are 'addicted' to HMGB1 for the growth and progression.

Materials and Methods. BoxA and ethyl pyruvate (EP) were used to inhibit the activities of HMGB1. BoxA is a specific HMGB1 antagonist. EP is the ethyl ester of pyruvic acid, known to suppress oxygen radical formation and HMGB1 secretion. Cell proliferation, migration, invasion, wound healing were performed according to standard procedures. Colony formation was measured by soft agar assay. Tumor growth in vivo was examined by using xenografted mice.

Results and Discussion. We demonstrated that BoxA and EP significantly impaired motility, migration, invasion, proliferation and anchorage-independent colony formation of MM cells in cell culture. Both BoxA and EP inhibited the activity of the pro-inflammatory transcription factor NF-κB, as well as reduced HMGB1 serum levels and tumor growth in severe-combined immuno-deficient (SCID) mice xeno-transplanted with human MM cells. We also found that BoxA, a specific inhibitor of HMGB1, dramatically extends survival of xenografted SCID mice.

Conclusion. Taken together, our results provide a preclinical proof-of-principle that inhibition of HMGB1 activity using either BoxA or EP offers novel therapeutic approaches for MM treatment. Given the emerging importance of HMGB1 and its tumor-promoting functions in many cancer types, our investigation is poised to provide broadly applicable information.

No conflict of interest.

711 Validation of drug resistant murine tumor models to facilitate development of new anticancer therapeutics

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Despite the expanding successful development of new anti-cancer drugs, all cancer therapeutics, either conventional cytotoxic chemo- or targeted therapeutics, are limited by the drug resistance. Resistance may be divided into two broad categories: intrinsic/de novo or acquired, involving various mechanisms. Certain patients are de novo resistant to a specific therapy, while others initially responded, but eventually recurred with resistant tumors that no longer respond to the original therapy. Even with the newly developed immunotherapies that promise to have long lasting tumor inhibitory effect in patients, a large percentage of patients still do not respond to the therapy. To overcome the resistance to the existing drugs, effective new strategies, e.g. next-generation selective inhibitors, rational drug combinations, and new drug delivery or formulation designs, are developed and under active preclinical and clinical investigations. To enable a more informative selection of resistant models for in vivo preclinical drug evaluation, here we set out to identify a series of animal models, which are intrinsically resistant to either chemotherapy, targeted therapy, or immunotherapy, from more than 200 CrownBio validated cell line xenograft and syngeneic tumor models. On another hand, we also validated a panel of acquired drug resistant models, covering the resistance to Doxorubicin, Imatinib, Elotinib, Quizartinib, Crizotinib or Androgen ablation (castration), etc. The possible mechanisms of some resistance were discussed. In conclusion, these resistant models provide a valuable platform for the development of new strategies to overcome the drug resistance.

No conflict of interest.

712 Overcoming primary and acquired resistance to ALK inhibitors in ALK translocation-positive non-small cell lung cancer cells: Rationale to combine metformin

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Background. Crizotinib is an effective tyrosine kinase inhibitor (TKI) against non-small cell lung cancers (NSCLCs) with anaplastic lymphoma receptor tyrosine kinase (ALK) translocation. Recently, the second generation ALK inhibitors, including alectinib (CH5424802) and ceritinib (LDK378), are actively investigated in clinical trials and show strong anti-tumor effect both in crizotinib-naïve and refractory NSCLC patients with ALK translocation. Unfortunately, the efficacy of these ALK inhibiting TKIs is limited due to primary and acquired resistance to these agents. Metformin is a widely used antidiabetic drug and shows promising anti-tumor effect in several cancers. Here, we decribe a therapeutic synergism between ALK inhibitors and metformin.

Material and Methods. The following NSCLC cells were evaluated: ALK translocation-positive (ALK+) and crizotinib-sensitive H2228 and H3122, ALK+ crizotinib-resistant SNU2535 and ALK translocation-negative H226. Over several months, ALK+ cells were chronically exposed to ALK inhibitors to acquire resistance to these agents. We assessed response of these NSCLC cells to crizotinib, alectinib and ceritinib alone or in combination with metformin by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay and anchorage independent growth in soft agar. Combination indices (CIs) were calculated by CalcusynTM (Biosoft, Cambridge, UK). Western blots were performed to assess biochemical changes, especially those related with ALK signaling components.

Results. The combination of ALK inhibitors with metformin induced a strong anti-proliferative effect only in ALK+ cell lines (CI of crizotinib and metformin: 0.31 – 0.79, CI of alectinib and metformin: 0.40 – 0.86, CI of ceritinib and metformin: 0.38 – 0.65). Alectinib and ceritinib effectively inhibited growth of crizotinib-resistant SNU2535, while crizotinib did not. Notably, synergistic effects of these ALK inhibitors and metformin were also shown in SNU2535 cells. Compared to ALK inhibitor alone, the combination of ALK inhibitor plus metformin resulted in decreased phosphorylation of ALK and downstream targets, such as AKT or ERK, both in crizotinib-sensitive and resistant ALK+ cells. Cells with acquired resistance also responded if metformin was given in addition to ALK inhibitors.

Conclusion. Collectively, these data support that ALK inhibitors and metformin are synergistic in ALK+ NSCLC cells. Resistance to crizotinib as well as alectinib and ceritinib might be overcome by addition of metformin.

No conflict of interest.

713 The ErbB3-targeting antibody MM-121 (seribantumab) reverses heregulin-drivenresistance to multiple chemotherapies on tumor cell growth

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Introduction. Heregulin-mediated activation of the human epidermal growth factor receptor 3 (HER3/ErbB3) is required for the growth and survival of many epithelial cancers. This signaling pathway is also emerging as a mechanism of resistance to targeted agents and chemotherapies. MM-121 (seribantumab) is an investigational human monoclonal anti-ErbB3 antibody that has previously been shown to effectively block ligand-dependent activation of ErbB3 in a range of tumors, and has demonstrated clinical activity in biomarker positive patients in several Phase II trials. The purpose of this study was to examine in three indications of interest, the ability of heregulin to induce resistance to standard chemotherapies and the reversal of this effect by MM-121. Such systematic evaluation of different combinations can serve as a guide for the future clinical development of MM-121.

Material and Methods. To assess the effect of heregulin and MM-121 on chemotherapies in cancer cells, we conducted a high throughput proliferation screen in 3D cultures. A panel of 60 cell lines of relevant clinical indications (ovarian, breast and lung cancer) was selected and tested for the sensitivity to respective standard-of-care chemotherapies in the absence or presence of exogenously added heregulin. Using these data, we analyzed the rescuing capacity of heregulin and the MM-121 combination's sensitivity, and selected representative combinations for in vivo models.

Results and Discussion. We show that in a large panel of cancer cell lines the presence of heregulin can induce resistance to multiple chemotherapies with very different mechanisms of action. The combination of MM-121 with any one of these chemotherapies can reverse the heregulin-meditated rescue and provide an additive treatment effect at therapeutically relevant doses achieved in the clinic. These results were further validated in xenograft mouse models of all three indications, using representative chemotherapies and doses. In addition, biomarker analysis revealed that ErbB3 receptor levels largely determine responsiveness to heregulin and MM-121.

Conclusions. MM-121 is an anti-ErbB3 antibody designed to block ligand-mediated

signaling, and currently in clinical development. The results presented here demonstrate the role of heregulin in reducing the sensitivity of tumors to standard-of-care chemotherapies, and the effect of ErbB3 pathway inhibition across indications.

No conflict of interest.

714 ETV4 overexpression cooperates with Pten deletion for prostate cancer progression in mice

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Introduction. Half of cases of prostate cancer carry a chromosomal translocation involving one of the genes of the ETS family (ERG, ETV1, ETV4, ETV5, FLI1). Each translocation results in the deregulated expression of an ETS protein in the prostate that, likely, plays a direct role in prostate cancer pathogenesis. We have showed that in prostate cell lines ETV4 overexpression increases migration and invasion, and also proliferation, cell cycle progression, anchorage-independent growth, tumor growth in xenograft model. Here we have investigated the effects of ETV4 expression in mouse prostate.

Material and methods. The truncated form of ETV4, present in patients, has been cloned under the control of a modified rat probasin gene promoter that drives prostate specific expression. By using this construct we have obtained two different transgenic mouse lines with ETV4 specific prostate expression (ETV4 mice). The tissues of these mice have been analyzed by H&E staining and immunohistochemistry.

Result and discussion. 6 month-old ETV4 mice did not present prostate lesions, whereas low grade prostatic intraepithelial neoplasia (LGPIN) lesions have been found in about half of 10 month-old mice (10 of 18 line C, 4 of 11 line E) and in none of control mice. Since Pten loss is common in prostate cancer and it is often associated with ETS translocations, we have investigated the interaction of ETV4 expression with Pten loss by crossing our ETV4 mice with PTEN+/– mice. Analyzing the progeny of this cross at 7 months of age we found LGPIN in 33% of ETV4 mice and in 50% of Pten+/- mice, whereas we found multifocal high grade PIN (HGPIN) lesions in all Pten+/-,ETV4 mice (n=9). In addition, at least one LGPIN was found in all 2 month-old Pten+/-,ETV4. Thus, Pten+/–,ETV4 developed early neoplastic lesions whose number and severity increased with age: in fact, HGPIN progressed to locally invasive adenocarcinoma in 12.5% of 7 month-old mice and in 50% of 10 month-old mice.

Conclusion. These results suggest that ETV4 over-expression is sufficient to initiate neoplastic transformation and cooperates with Pten loss in promoting prostate cancer progression. Thus, also in vivo, ETV4 induces most and perhaps all of the features that make a tumor. Now we are investigating the molecular mechanisms through which ETV4 cooperates with Pten loss to promote cancer progression. These mouse models, in addition to provide insight into prostate cancer biology, will be useful to test the effectiveness of experimental therapeutic approaches.

No conflict of interest.

715 The Spindle Assembly Checkpoint (SAC) is required for the activity of the novel anti-cancer agent BAL101553 (prodrug of BAL27862)

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Introduction. BAL101553 is a highly soluble prodrug of BAL27862, a novel, small molecule, microtubule (MT)-depolymerizing agent with a broad in vitro anti-proliferative activity against human tumor lines refractory to standard MT-targeting agents. The prodrug has anti-cancer activity in diverse tumor models when given orally or IV, alone or in combination therapy, and is currently in Phase 2a clinical evaluation in advanced cancer patients.

Materials and Methods. To evaluate BAL27862-resistance mechanisms, tumor lines were selected in vitro by increasing drug levels. Anti-proliferative activity was analyzed using Crystal Violet assay. siRNA transfection for 24h was followed by drug treatment (50nM) for 48h. Mitotic index was evaluated after 18h BAL27862 treatment by p-Histone H3 staining or flow cytometry. SAC complex formation (50nM BAL27862, 18h) was assessed by BubR1 immunoprecipitation (IP) and immunoblotting for SAC components.

Results and Discussion. The SAC ensures cell cycle arrest upon spindle disruption, promoting tumor cell death after prolonged mitotic arrest. SAC formation involves phosphorylation and assembly of a protein complex, of which BubR1 and Mad2 are essential components. Using siRNA approaches, BubR1 and Mad2 were shown to be required for BAL27862 anti-proliferative activity in a panel of sensitive tumor lines e.g. control siRNA-treated H460 lung cancer cells entered a cell death program following BAL27862 treatment (~45% cell death vs. starting cell number) a response attenuated by BubR1 and Mad2 downregulation (71% & 62% proliferation resp. vs. DMSO controls). The mitotic arrest associated with BAL27862 treatment was also absent after BubR1 or Mad2 downregulation. To investigate whether BAL27862 induces the formation of the SAC, BubR1 IPs from sensitive tumor lines were analyzed for SAC components. In all cases, complex formation was observed, consistent with a concomitant mitotic accumulation. Strikingly, in variants of the same lines selected for BAL27862 resistance, no SAC formation or mitotic accumulation was observed under the same conditions. Moreover, BAL27862-induced protein mobility shifts, consistent with activating phosphorylation of SAC components, were also absent in the resistant variants.

Conclusion. The activity of BAL101553 as a tumor checkpoint controller suggests that an intact SAC may predict tumor response. Exploratory patient stratification biomarkers are currently being clinically evaluated.

Conflict of interest: Corporate-sponsored research: Employee of Basilea Pharmaceutica International Ltd
Other substantive relationships: Basilea Pharmaceutica International Ltd Shares

716 Oncogenic TBX2 confers cisplatin resistance in breast cancer and melanoma by promoting DNA repair

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Introduction. The emergence of drug resistant tumours which are able to escape cell death poses a major problem in the treatment of cancers. Tumours develop resistance to DNA damaging chemotherapeutic agents by acquiring the ability to repair their DNA. Combination therapies that induce DNA damage and which disrupt the DNA damage repair process may therefore prove to be more effective against such tumours. The developmentally important transcription factor TBX2 has been suggested as a novel anti-cancer drug target as it is overexpressed in several cancers and possesses strong anti-senescence functions in melanoma and pro-proliferative functions in breast cancer and rhabdomyosarcoma cells. Ectopic expression of TBX2 also results in cancer cell resistance to the DNA damaging chemotherapeutic drugs, cisplatin and doxorubicin. However, whether endogenous TBX2 confers cisplatin resistance in TBX2-driven cancers and the mechanism(s) by which this may occur have not been reported. This study explores this in melanoma and breast cancer cells.

Material and Method. To determine the role of TBX2 in cisplatin resistance the MCF-7 breast cancer and 501-melanoma cells in which TBX2 was knocked down (shTBX2) and shcontrol cells were treated with cisplatin and assessed for: (1) cell viability using clonogenic survival assays, (2) cell cycle distribution by flow cytometry, (3) DNA damage and mitotic catastrophe by immunocytochemistry/western blotting with antibodies to γ-H2AX and cyclin B1; (4) apoptosis by PARP cleavage and (5) the DNA damage response by comparing the levels of p-ATM, p-CHK2, p53 and p21 by western blotting.

Results and Discussion. The ATM-CHK2-p53 signalling cascade has been shown to regulate cisplatin-induced DNA damage repair and is thus an attractive pathway to disrupt in order to increase the efficacy of this drug. We show that knocking down TBX2 in melanoma and breast cancer cells sensitises the cells to cisplatin by disrupting the ATM-CHK2-p53 signalling pathway. Cell cycle analyses demonstrate that when TBX2 is knocked down there is an abrogation of an S-phase arrest but a robust G2/M arrest that correlates with a reduction in p-CHK2 and p53 levels. This prevents DNA repair resulting in TBX2 deficient cells entering mitosis with damaged DNA and consequently undergoing mitotic catastrophe.

Conclusion. This study provides compelling evidence that targeting TBX2 in combination with chemotherapeutic drugs such as cisplatin could improve their efficacy and that it may be a viable treatment of TBX2-driven cancers.

Poster Session: Tumour Immunology I

718 xCT: A new cancer stem cell immunotherapeutic target for breast cancer

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The several unsuccessful treatments in metastatic cancers might miss cancer stem cells (CSC), a sub-population of cells with a critical role in cancer. By contrast, a vaccine-elicited immune response against CSC might be particularly effective. The identification of oncoantigens (OA) expressed by CSC may provide new targets for effective anticancer vaccines.

To this purpose, the transcription profile of the ErbB2+ TUBO mammary cancer cell line was compared with that of TUBO cells grown under specific conditions to generate CSC-enriched mammospheres.

Integrating the data obtained with meta-analyses of seven independent human breast tumor data sets we identified new CSC mammary OA that were validated both in vitro and in vivo.

Among the identified OA, we focused on xCT, a channel that supports glutathione synthesis regulating the intracellular redox balance. We observed that xCT expression increases in TUBO and 4T1 derived mammospheres; its silencing, obtained with specific siRNA, or incubating mammospheres with its inhibitor sulfasalazine (2), significantly reduces TUBO cells ability to generate mammospheres. In vivo xCT inhibition impaired CSC ability to generate lung metastases derived both from an intravenous injection of TUBO-derived mammospheres and from subcutaneous 4T1 tumors; furthermore, this effect was improved when combined with the cytotoxic drug doxorubicin. Moreover, the anti xCT vaccination of mice bearing mammosphere-derived tumors both from TUBO and 4T1 cells reduced the speed of tumor growth.

In conclusion, this study provides a genomic characterization of mammary CSC and identifies fresh target for new and potentially effective anticancer vaccines, thus providing a new tool for the design of combined therapeutic approaches that efficaciously target both CSC and more differentiated cells in breast cancers, leading to both cancer treatment and prevention of metastases and recurrence.

No conflict of interest.

719 Tumor-infiltrating lymphocytes and cancer associated fibroblasts crosstalk in prostate cancer progression

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Introduction. Leukocyte infiltration plays a role in controlling tumor development and is now considered one of the hallmarks of cancer. During the early stages of tumour onset, tumor-infiltrating T lymphocytes (TIL) with an effector phenotype (CD4+ Th1, CD8+, NK cells) counteract tumour growth through the recognition and rejection of malignant cells, a process referred to as immunosurveillance or immunoediting. However, at later stages of tumor progression TILs are reported to acquire functional defects, entering a state of reversible anergy. This generates an immunosuppressive microenvironment that can hinders the anti-tumour immunity. Tumor microenvironment has been proposed to modulate the function of tumour-associated T cells. The aim of our study is to investigate the role of cancer associated fibroblasts (CAFs) in immune modulation during prostate carcinoma (PCa) progression.

Methods. Fibroblasts were extracted from aggressive prostate carcinoma (CAFs) or from benign prostate hyperplasia (HPFs), while T cells were isolated from blood sample and intratumoral region of PCa-bearing patients. The quantitative determination of cytokines was performed with a bead-based multiplex immunoassay. T cells proliferation was measured by [3H] thymidine incorporation. Invasion assay was performed by matrigel coated-Boyden chambers.

Results. Multiplex cytokine analysis revealed that co-colture of CD4(+) T cell clones (TCC) with CAFs increased secretion of several inflammatory mediators, including interleukin 6 (IL-6), IL-8, G-CSF, CXCL10 and Treg-recruiting chemokines (CCL5, MCP-1). Basic fibroblast growth factor (bFGF) production was also significantly enhanced and a significant increase of both IL-5, a Th2-specific cytokines and EOTAXIN, a Th2-recruiting chemokine, was observed. By contrast, while CAFs are directly able to foster PCa invasiveness, TCC are unable to directly affect PCa behavior. Interestingly, CAFs are able to rescue the TCC-induced inhibition of PCa cell migration, resulting in a net enhancement of cancer cell invasiveness. Notably, TCC proliferation was not affected by co-colture with irradiated CAFs or PCa cells.

Conclusion. Our preliminary data indicate that tumour recruited-T cells cooperate with resident CAFs in generating a pro-inflammatory environment and suggest a role in recruiting a pro-tumoral subset of T cells, ultimately leading to immunosuppressive effects.

No conflict of interest.

720 Norepinephrine establishes immunosuppressive network in human melanoma microenvironment

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Introduction. Stress related factors, such as catecholamines, can impact on immunological response and cancer progression through β-adrenoreceptors (βARs) activation. The exact role of the immune system on stress-induced cancer progression has not been well elucidated. In many cancers, malignant progression is accompanied by profound immune suppression that arises from the ability of tumors to subvert normal immune regulation to their advantage. The tumor microenvironment can prevent cytotoxic T cells expansion by promoting production of proinflammatory cytokines and growth factors, leading to the accumulation of suppressive cell populations (e.g. regulatory T cells -Treg) that inhibit instead of promoting immunity.

Material and methods. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy human donors (Meyer Hospital) by centrifugation using a Ficoll Histopaque solution; these cells were exposed to hypoxia and/or Norepinephrine (NE) and then labeled with fluorescent antibodies. Analysis of natural killer (NK) citotoxicity was assessed by FACS analysis through the identification of CD107a in CD16/CD56 positive cells. Treg cells were identified by positivity of CD4 and CD25.

Results and Discussion. Our results show that that stress related factors like NE create an immunosuppressive environment by coordinating a network of events both in cancer and immune cells, such as NK and Treg. Particularly, NE reduces citotoxicity of NK cells both by direct stimulation of PBMCs and by cocolturing NE -prestimulated A375-Melanoma cells with lymphocytes, but that this effect is reverted by administration of specific β-blockers. Moreover, NE and hypoxic conditions increase expansion of Treg cells both in tumor microenvironment and in peripheral blood cells. Furthermore, NE increases immunosuppressive environment by reducing the action of interleukin 15 (IL-15), an established inductor of NK activity. However, lymphocytes combined stimulation with IL-15 and β3-blockers restores and increases NK activity, as demonstrated by dramatic reduction of A375-Melanoma cells viability.

Conclusion. Our work provides a new concept that indicates stress induction by NEas strongdown-regulator of the immune response against tumor.

No conflict of interest.

721 The pro angiogenic phenotype and function of tumor infiltrating (TINKs) and tumor associated (TANKs) Natural Killer cells in colorectal-cancer

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Introduction. Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide and a prevailing cause of cancer-related mortality. The presence of immune cells within tumors contributes to the carcinogenic and metastatic process, since their polarization toward anti-tumor or pro-tumor and pro-angiogenic state can determine the difference between tumor eradication/dormancy and clinically relevant oncologic disease.

Natural Killer (NK) cells are effector lymphocytes of the innate immune system that could potentially control tumour growth and dissemination by their cytotoxic activity and Th1 cytokine production. We recently discovered that tumour infiltrating (TINKs) and tumour associated (TANKs) NKs in non small cell lung cancer (NSCLC), in addition to functional anergy, show a pro-angiogenic phenotype and functions. This phenotype is quite similar to that of decidual NK (dNK) cells, an immunosuppressive, pro-angiogenic NK subset participating in decidual vascularization and spiral artery formation.

Materials and Methods. We investigated phenotype, function and subset distribution of NKs in tumor, adjacent normal tissue and peripheral blood and the correlation with pro-angiogenic factor production, i.e. VEGF, PlGF and IL-8/CXCL8 from patients CRC. Multiparametric flow cytometry was applied to assess NK surface antigen expression as far as cytokine profile, while functional studies (MTT, morphogenesis and chemotaxis assay) were used to directly study CRC TINK pro-angiogenic functions on human umbilical vein endothelial cells (HUVECs).

Results and Discussion. We found that CD56brightCD16- NK cells predominate in both the tumors and adjacent tissues derived from CRC samples and they were able to express higher levels of CD9 and CD49a decidual-markers as compared from those derived from peripheral blood either from CRC patients and healthy donors. Moreover, CD56brightCD16- NKs from CRC samples were able to release substantial amounts of pro-angiogenic factors, including VEGF, PlGF and IL-8 and exert low cytotoxic activities. Finally supernatants derived from CRC TINKs induced HUVE cell proliferation, migration and migration and capillary-like structure formation.

Conclusion. Our data clearly suggest that NK cells in CRC are switched to a pro-angiogenic phenotype, thus placing NK cells as a new player in inflammation and pro-angiogenic activity for tumor development and progression.

No conflict of interest.

722 Cancer immunotherapy diagnostic panel

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Introduction. There is ample evidence that development of novel prognostic and predictive biomarkers is a critical step for selecting patients predisposed to respond to existing and novel immunotherapy treatments and their combinations. The aim of the current studies was to develop the Cancer Immunotherapy 500 (CI500) assay to dissect cancer immunosurveillance mechanisms and discover novel prognostic and predictive immune response gene signatures.

Material and method. We have developed the CI500 assay to quantitatively profile expression, copy number, and mutation level of 500 key cancer genes using multiplex PCR amplification from both total RNA and genomic DNA followed by HT sequencing. The built-in internal calibration standards allow calibration and adjusting of digital HT sequencing data depending on the level of intrinsic noise and quality of samples. The CI500 assay provides quantitative expression data of 500 key cancer immune-related genes with 1,000-fold dynamic range and sensitivity down to 100 cells in whole lysate and isolated cell fractions from frozen xenograft clinical samples.

Results and discussion. The CI500 panel includes 15 experimentally validated core gene signatures which predict efficacy of immunotherapy in several cancer types, including melanoma, colorectal, breast, and lung cancers. Furthermore, we expanded the core signatures by developing an immunotherapy computational functional interaction network model used for predicting key nodes in pathways specific for antigen presentation and recognition, inhibition, activation and motility of immune cells, adhesion, and apoptosis of cancer cells. The CI500 panel also includes a set of genes specific for detection in the tumor microenvironment of activated immune cells of adaptive and innate immunity and a set of housekeeping genes with constant expression between different cancer types. Data will be presented which demonstrate the performance and utility of the CI500 assay in profiling of key mechanisms of breast cancer tumors used to escape from immunosurveillance.

Conclusion. Immunoprofiling of the tumor microenvironment with the CI500 gene panel will enable researchers to discover prognostic and predictive immune response biomarker signatures. The predictive signatures have the potential to stratify cancer patients for responses to the growing number of immunotherapeutic treatments

No conflict of interest.

723 Efficacy of a cancer vaccine against ALK-rearranged lung tumors

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Introduction. Non-Small Cell Lung Cancers (NSCLC) harboring Anaplastic Lymphoma Kinase (ALK) gene rearrangements are successfully treated with first and next-generation ALK tyrosine kinase inhibitors (TKIs) for up to 18 months. However, almost inevitably tumors relapse due to the development of TKI resistance. Here, we provide pre-clinical evidence for the efficacy of a specific immunotherapy in ALK-rearranged NSCLC based on an ALK vaccine.

Materials and Methods. We developed an orthotopic mouse model of ALK-positive lung cancer by transducing EML4-ALK in the BALB/c murine lung cancer cell line ASB-XIV. We also generated transgenic mice ectopically expressing human EML4-ALK or TFG-ALK under the lung specific SP-C promoter. For DNA vaccination, we electroporated 50 ug of plasmid ALK-DNA in the femoral muscle of anesthetized mice for a total of 3 immunization. To evaluate the generation of an immune response, we performed in vivo citotoxicity assay with CSFE-labelled cells. Tumor growth and progression were monitored overtime by Magnetic Resonance Imaging (MRI). To combine ALK vaccine and ALK inhibition, we vaccinated and treated mice with crizotinb (100mg/kg) for 2 weeks. For PD-1/PD-L1 blockade, we vaccinated mice and injected anti-PD-1 (0.2mg) i.p. every 3 days for a total of 5 injections. Immunohistochemistry was performed on different specimens with specific antibodies.

Results and Discussion. The ALK vaccine induced a strong, specific immune response in both grafted or primary mouse models of ALK-rearranged lung tumors. ALK vaccine elicited a potent anti-tumor cytotoxic response primarily mediated by CD8+ T cells, significantly reduced tumor growth and extended survival of vaccinated mice. ALK vaccine maintained its efficacy when combined with crizotinib and significantly delayed tumor relapses after crizotinib suspension. It was also effective against all the tested EML4-ALK mutants that are selected during TKI resistance in NSCLC patients. Finally, we show that oncogenic ALK induces PD-L1 expression on the surface of NSCLC cells. ALK vaccine efficacy decreased in the presence of high PD-L1 expression on tumor cells but was readily restored by an anti-PD-1 antibody.

Conclusion. The ALK vaccine generates a strong and specific tumor immune response that might represent an attractive treatment for ALK-rearranged NSCLC in combination with ALK TKIs and/or immunotherapy.

No conflict of interest.

724 Mutated variant of HER2 as a target for immunotherapy in breast cancer

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Introduction. Overexpression of HER2 oncogene in breast tumors has been associated with a more aggressive disease. Trastuzumab, a HER2-specific monoclonal antibody, has emerged as an important intervention for patients with HER2-positive tumors, but a number of concerns, including resistance, considerable costs and side effects, make active immunotherapies a desirable alternative approach. We focus our attention on the HER2 oncogenic variant lacking exon 16 (d16HER2) because of its high frequency in primary and metastatic tumors and its correlation with trastuzumab-resistance. From the immunologic perspective, mutations can create neoantigens that are not subject to central immune tolerance.Due to these characteristics, d16HER2 represent a suitable target for immunotherapy in breast cancer.

Materials and Methods. Dendritic cells (DCs), generated from patients with HER2-overxpressing tumors, were transfected with DNA plasmid coding for wild type HER2 (HER2-DCs) or for d16HER2 (d16-DCs) and used to activate autologous T cells. Activation was evaluated by flow cytometry, ELISpot and cytotoxic assays. In order to assess the effect of stimulation on regulatory T cells (Treg) activation, purified Treg were cultured with transfected DCs. Treg activation was defined as capability to degrade ATP and suppress T cell proliferation.

Results and Discussion. we observed that HER2-DCs were completely inefficient in eliciting an antitumor response in T cells from breast cancer patients, while d16-DCs were able to mount a specific immune response. Mutated determinants are potentially immunogenic because they are not controlled by central tolerance. In fact, Treg stimulated with HER2-DCs displayed higher suppressive activity on the proliferation of autologous T cells and higher capacity to degrade ATP compared to control DCs. By contrast, d16-DCs did not affect the functionality of Treg.

Conclusion. Cancer vaccines based on self antigen can activate immune tolerance mechanisms hence suppress antitumor response. Vaccination with mutated variants of oncoantigen, as in the case of d16HER2, seems to be more promising compared to wild type proteins in eliciting antitumor response in patients. This finding represent an important point that should be considered to design cancer vaccines in the future.

No conflict of interest.

725 Novel anti-tumor effects of Dkk1 neutralization via targeting ß-catenin in myeloid suppressor cells

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Introduction. In the bone metastasis field the mutual interaction between osteoclasts (OC) and cancer cells perpetuates tumor growth in bone. Despite the successful therapeutic use of anti-resorptive agents in mice with bone metastases, the anti-tumor effects of OC inhibitors in patients remain controversial. We have previously shown that the anti-tumor efficacy of OC inhibitors is reduced in mice with decreased T cells. We have also found that expansion of myeloid suppressor cells (MDSC), potent inhibitors of T cells responses, supports bone metastatic dissemination regardless of the OC status. Thus, we hypothesized that targeting MDSC could prevent tumor growth in bone by restoring anti-tumor T cell responses.

Material and Methods. As tumor models we used Lewis Lung Carcinoma, B16 melanoma or PyMT breast cancer cell lines injected subcutaneously or intra-cardiacally into mice or peripheral blood mononuclear cells from cancer patients. Percentage of MDSC was measured by FACS and signaling pathways activated in these cells were analyzed by western blot.

Results. We find increased percentage of MDSC during tumor progression to bone. Despite being more numerous, MDSC from tumor bearing mice or cancer patients have reduced β-catenin levels. Consistent with a functional role for β-catenin in MDSC accumulation, mice with deletion of β-catenin in the myeloid lineage show greater MDSC expansion and enhanced tumor burden. Conversely, constitutive activation of β-catenin in myeloid cells protects from tumor growth and reduces MDSC numbers and functionality. To determine how β-catenin is regulated in MDSC during tumor progression, we measured the levels of Dkk1, an inhibitor of the Wnt/β-catenin pathway. DKK1 is upregulated in mice with subcutaneous (s.c) tumors or with bone metastases. Neutralization of DKK1 significantly decreases s.c. tumor growth by targeting MDSC and restoring anti-tumor T cell responses. Importantly, the anti-tumor effects of DKK1 neutralization are lost in mice lacking β-catenin in myeloid cells. Finally, we tested whether anti-DKK1Ab in combination with chemotherapy could prevent bone metastatic dissemination. While chemotherapy alone has limited anti-tumor effects, the combination of chemotherapy with anti-DKK1Ab significantly reduces tumor growth in bone by limiting MDSC expansion.

Conclusion. Downregulation of β-catenin induces MDSC expansion during tumor progression. Targeting β-catenin in MDSC via DKK1 neutralization should be considered for patients with bone metastasis refractory to conventional therapies.

Conflict of interest: Other substantive relationships: Amgen provided the anti-Dkk1 Ab

726 Characterisation of novel selective and dual-acting inhibitors of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) for the treatment of cancer

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Introduction. TDO/IDO catabolise tryptophan (Trp) leading to the formation of immunosuppressive kynurenine (Kyn) pathway metabolites that dampen the tumour immune response. Given that both IDO and TDO have been shown to be up-regulated in cancers, selective IDO inhibitors may have a limited effect in cancers harbouring both TDO and IDO. We have discovered multiple novel chemical series of both highly selective and dual-acting inhibitors of IDO/TDO. Herein we describe their in vitro and in vivo characterisation.

Material and method. In vitro assays measured the effects of TDO/IDO inhibitors on Kyn production in cancer cells, hPBMCs and effects on T cell proliferation in co-culture systems. In vitro ADME properties and in vivo PK/PD profiles were measured by standard methods.

Result and discussion. Selective IDO inhibitors ablate Kyn production in IDO-expressing cells (Interferon-γ (IFNγ)-treated SKOV3 cancer cells and human peripheral blood mononuclear cells (hPBMCs)) with potencies in the low nM-μM range. Selective TDO inhibitors are unable to modulate Kyn levels in (hPBMCs) suggesting that IDO is the primary Trp-catabolising enzyme in these immune cells. As expected, TDO inhibitors ablate Kyn production in TDO-expressing A172 glioma cells with potencies in the low nM-μM range. Dual-acting TDO/IDO inhibitors, exemplars of which exhibit nM potency, are required to fully inhibit Kyn levels in IFNγ-stimulated A172 cells expressing both enzymes. These novel compounds also relieve inhibition of T-cell proliferation in cancer cell/T cell co-culture assays. Compounds bearing nM potency also display highly favourable physico-chemical properties with stability in human/rodent plasma and hepatocytes. Importantly, in vivoPK/PD studies show prolonged reduction of the Kyn/Trp ratio in plasma. Furthermore these compounds are active against murine IDO/TDO facilitating their use in in vivo syngeneic mouse IDO/TDO tumour models.

Conclusion. We describe the characterisation of multiple novel chemical series of potent, selective and dual-acting TDO/IDO inhibitors. These data demonstrate the utility of applying highly selective and dual-acting inhibitors of TDO/IDO as probes for defining the Kyn-producing component from tumour and immune cells. The robust in vitro and in vivo properties of these molecules shows a highly favourable profile compared to compounds related to those in clinical trials, thus supporting the preclinical/clinical development of these inhibitors as immunotherapeutic agents.

Conflict of interest: Ownership: IOmet Pharma Limited

Poster Session: Tumour Immunology II

731 Novel immunotherapeutic regimen consisting of adoptive T-cell transfer and cytokine-coding adenoviruses for the treatment of melanoma

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Introduction. Adoptive cell transfer (ACT) has shown promising results in clinical trials for melanoma, with response rates ranging from 40% to 72%. We aimed to enhance the efficacy of ACT by coupling it with adenoviruses coding for immunostimulatory cytokines.

Materials and Methods. The non-replicating adenoviruses that were constructed (Ad5-CMV-mIFNg, Ad5-CMV-mIL2, Ad5-CMV-mIFNb and Ad5-CMV-mTNFa) were confirmed to produce biologically active murine cytokines in vitro. These viruses were then used in combination with adoptive transfer of OT-1 TCR transgenic T-cells to treat C57BL/6 mice bearing B16-OVA melanoma tumors. The animals were administered intraperitoneally with 1.5 x 106 CD8+-enriched OT-1 cells with or without intratumoral injections of cytokine-coding adenoviruses (1 x 109 viral particles/tumor).

Results. Combination treatment with Ad5-CMV-mIL2 and OT-1 resulted in statistically significant antitumor efficacy when compared with either monotherapy and untreated control (combination vs. virus, p=0.026; combination vs. OT1, p=0.003; combination vs. mock, p<0.001). Similar results were obtained with other cytokine-coding virus and OT-1 combinations, except for mIFNb. In further experiments a triple combination of Ad5-CMV-mIL2 + Ad5-CMV-mTNFa (1:1 ratio) and OT-1 T-cells were used to treat B16-OVA; improved antitumor efficacy was observed with the triple combination over dual agent therapies (triple combination vs. Ad5-CMV-mIL2 + OT-1, p=0.001; triple combination vs. Ad5-CMV-mTNFa + OT-1, p=0.049).

Conclusions. In summary, these results support the further development of a novel immunotherapeutic approach that comprises adoptive T-cell transfer and cytokine-coding adenoviruses for the treatment of melanoma.

Conflict of interest: Other substantive relationships: Employee of TILT Biotherapeutics Ltd.

732 How adenosinergic pathways in the myeloma niche are instrumental in reorganizing normal bone to favor tolerogenesis and local growth

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Introduction. Different factors contribute to the growth of multiple myeloma (MM) in the bone marrow (BM) niche. Among these, hypoxia and acidic pH trigger a shift to anaerobic glycolysis, reducing ATP and increasing NAD+. These conditions induce a subtle bias of adenosinergic pathways working in the niche. ATP leads the canonical CD39/CD73 pathway of adenosine (Ado) production. NAD+ activates an alternative pathway mediated by the actions of the ectoenzymatic CD38/CD203a/CD73 pathway.

Our working hypothesis is that Ado plays a key role in MM tolerogenesis and growth in the BM myeloma niche.

Materials and Methods. BM aspirates from MGUS (6), SMM (6) and MM (30) patients were processed avoiding fast cellular uptake of Ado and degradation to inosine. Ado was assayed by HPLC.

Results and discussion. Ado levels ranged from 10-200 μM in BM plasma, where products (nucleotides and nucleosides) and cells [myeloma (MM), osteoblasts (OB), osteoclasts (OC) and stromal cells (SC)] converge. Ado variability (Table) likely reflects heterotypic interactions taking place among myeloma cells on the one side, and osteogenic cells (OB, OC and SC) on the other. Co-cultures of MM with OC displayed an increase in Ado levels (88%±10), while co-cultures with OB and SC lines induced decreased levels (41%±12 and 29%±14, respectively). Fluctuating Ado levels suggest that adenosinergic pathways operates in a trans-associated configuration. Preliminary experiments indicate that Ado is operative on the AMPK-mTOR checkpoint, leading to local immunosuppression (i.e., reduced proliferation of T cells). Ado evaluation under acidic and hypoxic conditions are now underway to mimic bone resorptive environment typical of the MM niche.

Conclusions. Ado concentrations in BM aspirates from MGUS, SMM and MM disorders support the view that bone and myeloma are equipped with the ability to maintain Ado at micromolar levels. Ado profiles may appear peculiar of different pathological conditions and, consequently, become an early indicator of aggressiveness of the disease or a marker of disease progression. An hypothesis is that myeloma cells usurp the normal organization of bone, replacing it with a niche that maximizes local growth and tolerogenesis.

Adenosine (μM)	MGUS (n = 6)	SMM (n = 6)	MM (n = 33)
Mean value	25,56	29,99	55,98
Limit values	3,42 - 60,30	7,58 - 69,70	7,88 – 165,1
±SD	27,58	29,99	55,98

No conflict of interest.

733 Anti-cancer drug Combretastatin (CA)-4 and its novel analogue CA-432 display anti-inflammatory properties in T-cells

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The purpose of this study was to explore a potential role for anti-cancer agent CA-4 and its novel analogue CA-432 as anti-inflammatory agents. Firstly, the capacity of T-cell lymphocytes to migrate and localise in tissues is important in their protective function against infectious agents, however, the ability of these cells to migrate and infiltrate the tumour microenvironment is also a major contributing factor in the development of cancer and cancer metastasis. T-cell migration requires ligand (ICAM-1)/integrin(LFA-1) interaction, activating intracellular signalling pathways which result in a distinct polarised morphology, with an actin-rich lamellipodium and microtubule-rich uropod. Secondly, the pro-tumorigenic cytokine TNF-α has been shown to exacerbate inflammation through modulating the NF-κB pathway in cancer cells. Key to this function are the inhibitor of apoptosis proteins (IAPs) which serve as endogenous inhibitors of programmed cell death, such as XIAP and survivin, and initiators of the TNFα receptor complex such as cIAP-1 and cIAP-2. Crucially, the IAPs are involved in inflammation, cell cycle, cell death and cell migration and as such they are often overexpressed in cancer, therefore making them an attractive chemotherapeutic target. Microtubule-targeting agents (MTAs) are amongst some of the most widely used anti-cancer drugs and several, including colchicine, have been shown to exhibit anti-inflammatory activity. Combretastatin (CA) A-4 is a MT-destabilising agent that possesses potent anti-tumour and anti-vascular properties both in vitro and in vivo. CA-432, a novel cis-restriciting analogue containing a β-lactam ring, has recently been synthesised to circumvent the problem of CA-4 isomerisation from a biologically active cis-conformation to a more thermodynamically stable but inactive trans-isomer.

Our lab have previously demonstrated the anti-cancer and anti-vascular properties of CA-4 and CA-432 in various cancer cell lines, CML patient samples and in vivo mouse models. Effects of CA-4 and its novel cis-restricting analogue CA-432 on human T-cell morphology and migration were assessed using high content analysis (HCA). Active migration of primary blood T-cells was explored using a transwell assay. The mechanisms and signalling pathways involved in mediating these effects were then examined using confocal microscopy and western blotting. Cytotoxicity of the compounds in various cell lines including Jurkat and HuT-78 cells was assessed using a viability assay and flow cytometry. Effects of CA-4 and CA-432 on TNF-α-induced NFκB activity in Jurkat and HEK 293T cells was determined using a luciferase assay and EMSA. The signalling pathways involved were then explored using western blotting for protein expression. CA-4 and CA-432 treated cells displayed altered T-cell migratory polarity in HuT-78 and peripheral blood T-lymphocyte (PBTL) cells and inhibited active migration of PBTLs. Both compounds induced activation of the RhoA / RhoA associated kinase (ROCK) signalling pathway, leading to the phosphorylation of myosin light chain (MLC), acto-myosin contractility and impaired migration. GEF-H1 is a MT-associated nucleotide exchange factor which activates RhoA upon release from MTs, The siRNA-mediated depletion of GEF-H1 in Hut-78 T cells prevented CA-induced phosphorylation of MLC and attenuated the formation of actin-rich membrane protrusions and cell contractility leading to inhibition of cell migration. CA-4 and CA-432 also showed anti-inflammatory potential through dampening pro-inflammatory TNF-α-induced NFκB activity in Jurkat T-cells. Inhibition of IκBα degradation and p65 nuclear translocation was followed by decreased NFκB reporter gene expression. CA-4 and CA-432 also displayed potent pro-apoptotic activity, demonstrated by suppressed cell proliferation and inhibition of anti-apoptotic IAP proteins XIAP, cIAP-1, cIAP-2 and survivin, followed by induction of PARP cleavage and enhanced apoptosis in Jurkats.

These results suggest an important role for combretastatins as anti-inflammatory agents. Therapeutic agents that target cytoskeletal proteins and are effective in inhibiting cell migration and dampening the pro-inflammatory response leading to increased apoptosis may open new avenues in the treatment of cancer and metastasis.

No conflict of interest.

736 C3a complement activation and blood coagulation drive a protumorigenic phenotype in neutrophils and promote intestinal tumorigenesis

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Introduction. Excessive activation of blood coagulation and neutrophil accumulation have been described in several human cancers. Hypercoagulation and thrombotic events are the second most common cause of death in cancer patients and therefore need to be carefully managed in the clinics. Further, neutrophilia has been extensively described as a poor prognostic factor in different cancers. To date, it is unknown whether the hypercoagulation and neutrophilia are linked and cooperate to favor tumor development and progression.

Material and methods. In this study, we measured the coagulation parameters and characterized neutrophil effector functions and phenotype in the APCMin/+ mouse

model of intestinal tumorigenesis. Moreover, we evaluated tumor development and neutrophil function in bone marrow chimeras, in APCMin/+ mice treated with anticoagulants and in APCMin/+ mice lacking complement C3a receptor (APCMin/+/C3aRko mice). Finally, we evaluated coagulation parameters and neutrophil counts in a cohort of patients diagnosed with colorectal cancer or with small intestinal tumorigenesis.

Results and discussion. We showed that APCMin/+ mice undergo progressive and chronic hypercoagulation. Moreover, we found that tumor development was accompanied by a dramatic reduction in the lymphocytic populations and a consistent increase in neutrophil numbers. These events were driven by non-hematopoietic factors. Interestingly, tumorigenesis correlated with the appearance of low-density neutrophils (LDN) with a pro-tumorigenic N2 phenotype. Blood clots were shown to imprint an N2 phenotype. The development of N2 neutrophils, hypercoagulation and the increase in tumor burden were dependent on complement C3a receptor (C3aR) engagement. Notably, APCMin/+ mice lacking C3aR signaling showed a strong reduction in tumor number and did not experience neutrophilia and hypercoagulation. Finally, in a cohort of patients with small but not large intestinal cancer, we found a correlation between neutrophilia and hypercoagulation, suggesting that this is a feature of tumorigenesis in the upper intestinal tract.

Conclusions. Our findings highlight a novel link between coagulation, neutrophilia and complement activation. Moreover this study provides for the first time a mechanistic explanation for the tumor-promoting effects of hypercoagulation and opens possible new therapeutic targets.

No conflict of interest.

737 PD-1+ T cells mediate early phases of anti-tumor immunity in NSCLC

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Introduction. Over the last years, clinical trials have shown that non-small cell lung cancer (NSCLC) is responsive to immunotherapy targeting PD-1 pathway, which leads to inhibition of chronically stimulated PD-1+ T lymphocytes. Recently, it has been shown that PD-1 expression identifies tumor reactive melanoma-associated CD8+ T cells, suggesting a more complex role for this molecule in cancer. In NSCLC the characteristics of anti-tumor reactivity associated with PD-1 expression by tumor-associated lymphocytes (TALs) still need to be clarified, and the identification of immunological features which are relevant for response to PD-1/PD-L1 blocking therapy is required to better define the patients who will best benefit from such treatments.

Matherials and methods. We analyzed by flow cytometry and immunohistochemistry T cells infiltrating tumor specimens and corresponding non neoplastic lung tissues. To evaluate TALs functionality, we assessed by flow cytometry CD107a upregulation and IFN-γ and IL-2 production after either co-culture with the autologous tumor or stimulation with PMA/Ionomycin.

Results and discussion. We found that activated T cells are enriched in NSCLC, and that these cells are functional and tumor-reactive. An high percentage of TALs expressed PD-1, which identified more activated (CD38+HLA-DR+), proliferating (Ki67+) and cytotoxic (perforin+) cells compared to PD-1- TALs; furthermore, PD-1+ T cells reacted against the autologous tumor, supporting the hypothesis of functionality and anti-tumor activity associated with PD-1 expression in NSCLC. Importantly, we found enrichment at tumor site also of CD8+FOXP3+ 'early effector' T cells (EECs), a subset of lymphocytes recently described by our group in melanoma as functional tumor-reactive cells at the earliest stage of effector differentiation. EECs expressed PD-1, comprised more recently activated (CD27+) and functional (CD38+HLA-DR+, Ki67+, perforin+, T-bet+) cells compared to FOXP3-CD8+ T lymphocytes, and reacted against the autologous tumor.

Conclusions. These findings clarify the role of T lymphocytes in anti-tumor immune response in NSCLC, and suggest a continuous development of early phases of anti-tumor immunity. These results might contribute to explain the clinical activity of PD-1 pathway blockade in NSCLC not only on the basis of re-activation of chronically stimulated lymphocytes, but also through promotion of anti-tumor functions of PD-1+ 'early effector' T lymphocytes.

No conflict of interest.

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