C084

PROSPECTIVE EVALUATION OF PERIPHERAL BLOOD CD26+ LEUKEMIA STEM CELLS IN CHRONIC MYELOID LEUKEMIA PATIENTS DURING TKI DISCONTINUATION (FLOWER-TFR STUDY)

A. Sicuranza[1], D. Raspadori[1], P. Pacelli[1,2], P. Pregno[3], E. Abruzzese[4], M. Annunziata[5], S. Galimberti[6], F. Sorà[7], M. Crugnola[8], L. Luciano[9], A. Iurlo[10], D. Cattaneo[10], M. Liberati[11], I. Ferrigno[1,2], C. Marzano[1], S. Ciofini[1], L. Puccetti[1], M. Bocchia[1]

[1]University of Siena, Hematology Unit, Siena; [2]University of Siena, Department of Medical Biotechnologies, Siena; [3]Hematology Division, Azienda Ospedaliera Città della Salute e della Scienza, Torino; [4]. S.Eugenio University Hospital, Hematology Unit, Roma; [5]Hematology, Cardarelli Hospital, Napoli; [6]Department of Hematology, Clinical and Experimental Medicine, University of Pisa, Pisa; [7]University Cattolica del Sacro Cuore, Roma; [8]Hematology Unit, Maggiore Hospital University of Parma; [9]Hematology, Federico II University, Napoli; [10]Hematology Division, Foundation IRCCS Ca'Granda Ospedale Maggiore Policlinico, Milano; [11]Azienda Ospedaliera S. Maria, Division of Onco-Hematology, Terni, Italy

Background and rationale: In order to better identify CML patients (pts) suitable for an efficacious treatment free remission (TFR) are warranted additional biological criteria to molecular response. Leukemia stem cells (LSCs) are supposed to be the reservoir of disease. We first showed in a cross-sectional study that residual circulating CD34+/CD38-/CD26+ CML-specific LSCs are still detectable in the peripheral blood (PB) of the majority CML pts in sustained TFR (66%) despite stable and deep molecular response. Aims In prospective FLOWER-TFR multicenter study we monitored by flow-cytometry the number of circulating CD26+LSCs in CML pts from the time of TKI discontinuation until molecular relapse, if any.

Methods: CML pts meeting the current molecular criteria for TKI withdrawal entered this study. At time of stopping TKI treatment (baseline) and at +1, +2, +3, +6, +12 months (mos) after discontinuation and at any time of molecular relapse, CML pts were evaluated for number of PB CD34+/CD38-/CD26+LSCs by centralized flow-cytometry analysis and for BCR-ABL transcript levels by QRT-PCR assay.

Table 1. Patient's characteristics and Results:

TOTAL PATIENTS		72
Median age at diagnosis		68 (19-71)
Sex	Male	39 (54%)
	Female	33 (46%)
Sokal skore	High	10/72 (14%)
	Intermediate	22/72 (30,5%)
	Low	36/72 (50%)
	n.a.	4/72 (5,5%)
	IMATINIB	44
	NILOTINIB	20
	DASATINIB	8
Median TKI treatment duration before discontinuation (months, range)		103 (38-232)
Median duration of treatment according to TKI (months, range)	IMATINIB	124 (38-232)
	NILOTINIB	92.5 (50-151)
	DASATINIB	65.5 (59-170)
Measurable circulating CD26+ LSCs at time of discontinuation	YES	37/72 (51%)
	NO	35/72 (49%)

	TOTAL (72)	TFR SUSTAINED (52)	TFR LOSS (20)
CD26LSC+ detectable BCR-ABL/ABL ratio detectable	10/72(14%)	6/10 (60%)	4/10 (40%)
CD26LSC+ detectable BCR-ABL/ABL ratio undetectable	27/72 (37,5%)	19/27 (70%)	8/27 (30%)
CD26LSC+ undetectable BCR-ABL/ABL ratio detectable	16/72 (22%)	12/16 (75%)	4/16 (25%)
CD26LSC+ undetectable BCR-ABL/ABL ratio undetectable	19/72 (26,5%)	15/19 (79%)	4/19 (21%)

Results: 72 consecutive CML pts were enrolled. After a median observation time of 11 mos since TKI withdrawal (1-37 mos), 20/72 (28%) pts lost their molecular response and restarted TKI treatment while 52/72 (72%) are still in TFR; of note 12/72 (17%) pts have so far discontinued the treatment for ≤ 6 months. The median time to relapse after discontinuation was 4 mos (range 2-7 mos) (Table 1). At the time of discontinuation, residual CD26+LSCs, were detectable in 37/72 (51%) pts: of those 25/37 (67%) sustained TFR and 12/37 (33%) lost response. The median number of detected CD26+LSCs was 0.0237μ/L (range 0.0077-0.1197) with minimal fluctuation at different time points. On the other hand, 35/72, 49% pts showed no detectable CD26+LSCs at time of discontinuation: 27/35 (77%) pts maintained TFR and 8/35 (23%) pts lost response. No statistical correlation between BCR-ABL/ABLIS ratio and number of residual CD26+LSCs was found. However, we observed that pts in which both LSCs and BCR-ABL copies were detectable had the highest percentage of TFR loss while pts with both undetectable LSCs and BCR-ABL copies had the lowest probability to TFR loss (Table 1).

Conclusions: Our results confirm that CD26+LSCs are detectable at time of TKI discontinuation and during TFR. Moreover, the persistence of "fluctuating" values of CD26+LSCs do not hamper the possibility to maintain a stable TFR. Pts discontinuing TKIs with no detectable CD26+ and no detectable BCR-ABL copies appear to have less probability to undergo TFR loss (21%) compared to pts with both detectable CD26+LSCs and BCR-ABL (TFR loss 40%). However, no correlation between BCR-ABL/ABLIS ratio and number of residual CD26+LSCs was found. Additional studies evaluating CD26+LSCs ability to modulate the immune system through a variable expression of immune response inhibitory molecules are ongoing.

C085

MEK-INHIBITORS/ARSENIC TRIOXIDE COMBINATION THERAPY AS A NOVEL TREATMENT OPTION FOR TYROSINE KINASE INHIBITORS-RESISTANT PH+-LEUKEMIA

L. Mazzera,[1,2] M. Abeltino,[2] G. Lombardi,[1] A.M. Cantoni,[3] S. Jottini,[3] A. Corradi,[3] M. Ricca,[1] D. Arienti,[1] A. Peli,[4] R. Perris,[5,6] A. Bonati,[2] P. Lunghi[5,6]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini," Brescia, Italy; [2]Department of Medicine and Surgery, University of Parma, Parma, Italy; [3]Department of Veterinary Sciences, University of Parma, Parma, Italy; [4]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy; [5]Department of Chemistry, Life Sciences and Environmental Sustainability. University of Parma, Parma, Italy; [6]Centre for Molecular and Translational Oncology-COMT, University of Parma, Parma, Italy

Despite the unprecedented efficacy observed with Bcr-Abl tyrosine kinase inhibitors (TKIs) in the management of chronic myeloid leukemia (CML), resistance and intolerance towards first-, second- and third-generation Bcr-Abl TKIs are frequently reported. This calls upon the need to identify new therapeutic strategies that may improve the therapeutic outcome of TKI-resistant Ph+-leukemia patients. Based upon previous findings, we have hypothesized that combined treatment with MEK inhibitors and arsenic trioxide (ATO) may provide the wanted cytotoxic effects against TKIs-resistant Bcr-Abl leukemic cells. We therefore first analyzed the pharmacologic interactions between the MEK inhibitor PD0325901 (PD) and ATO on Bcr-Abl-positive leukemia cell lines displaying different levels of resistance to TKIs, using a fixed-ratio treatment paradigm. We found that combination treatment with PD and ATO resulted in a synergistic induction of apoptosis in AR230-R, LAMA-R, Ba/F3 p210Y253F, Ba/F3 p210T315I and K562-R TKI-resistant cell lines. We next determined whether PD and ATO also induced apoptosis of primary cells from patients with BCR-ABL-driven CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) who had manifested resistance or intolerance to the TKIs Imatinib, Nilotinib and/or Dasatinib, or carried the T315I BCR-ABL mutation. Mononuclear cells derived from blood or bone marrow of patients with CML or Ph+ ALL harboring native BCR-ABL or BCR-ABLT315I were then treated with PD, ATO or both compounds and cell death was monitored by either sub-G1 or Annexin V/PI analysis. Similarly to cell lines, we found that PD significantly ($P<0.02$; $n=5$) increased ATO-induced cell death also in primary TKI-resistant CML or Ph+ ALL cells. We further observed that the combination PD/ATO promoted the accumulation/activation of the proapoptotic and antiproliferative transcriptionally active (TA)-p73 isoforms and transcription of their proapoptotic target