haematologica

the hematology journal

19th Congress of
the European Hematology Association
Milan, Italy, June 12 - 15, 2014

ABSTRACT BOOK

2014 | s1

ISSN 0390-6078
Journal of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 99, supplement no. 1, June 2014
www.haematologica.org

(NK:MM) compared to autonomous growth of human MM cells (RPMI-8226, NCI-H929 and OPM-2 cell lines). Patients NK cells (n=8) reduced 57,9±12,1% (32:1) colonies generation of MM cells without evidence of NK cell dose relationship. In contrast, patient NKAEs (n=6) reduced 93,6±2,7% (32:1) colonies growth, showing a strong dose-dependent relationship as expected (Figure 1A). At 8:1 ratio MM cell destruction by NK cell was higher on clonogenic MM cells (48,2±6,9% in MM patients and 58,3±4% in HS) than corresponding bulk MM cells (28,8±2,8% in MM patients and 21,1±4,5% in HS). Then NK cell (n=18) and NKAEs (n=5) phenotype were compared. NKAEs showed overexpression of NKG2D and NKp30 receptors. Blocking NKAEs NKp30 receptor (n=2) reduced 38% MM cells lysis, while NKG2D blockade decreased 12% lysis. NKAEs methylcellulose cultures (n=3) showed 15% and 20% increased colonies growth when NKp30 or NKG2D receptors were previously blocked respectively. Flow cytometry analysis of MM cells exhibited that side population have same expression profile of NKG2D ligands (ULBP-1, ULBP-2, ULBP-3, MICA, MICB) when compare to no side population cells in MM cell lines (U-266, RPMI-8226, NCI-H929, OPM-2, L-363, JJN-3, MM.1S), however they have down regulated apoptosis receptors (FAS, TRAIL-DR4, TRAIL-DR5) and DNAM-1 ligands (CD112 and CD155) expression (Figure 1B).

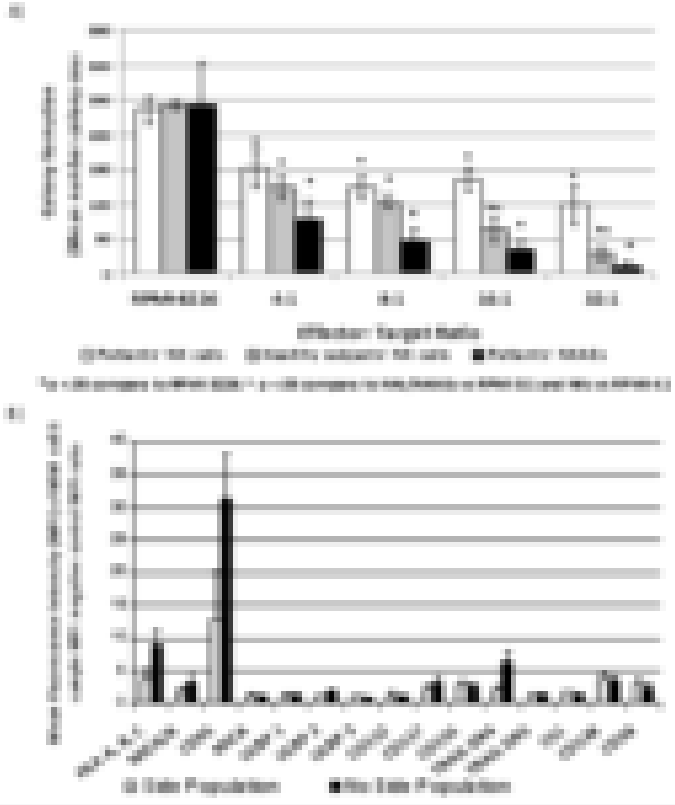

Figure 1. A) Clonogenic assay of MM cell line R PMI-8226 after exposure to MM patients' NK cells, healthy subjects' NK cells and MM patients' N KAEs. B) Membrane receptor expression profile analysis by flow cytometry of side population and no side population from MM cell lines (n=7).

Summary and Conclusions: NK cells are effective against MM clonogenic tumor cell. Patient NK cell stimulation produces NKAEs that have enhanced cytotoxic activity against MM clonogenic tumor cells. Integrity of NKp30 and NKG2D receptors is critical in NK cell activity against MM clonogenic cells. NKG2D ligands of MM side population could be a therapeutic target for MM treatment.

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CIRCULATING MIR-34A AND MIR-130A AS BIOMARKERS OF EXTRAMEDULLARY DISEASE

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Background: MicroRNAs (miRNA), short non-coding regulatory RNAs, are implicated in deregulation of critical pathways involved in multiple myeloma (MM) and extramedullary form of MM (EM). Circulating miRNAs are highly stable and are both detectable and quantifiable in a range of accessible body fluids; thus, they have the potential to be useful diagnostic biomarkers, as was shown in our previous study on MM. Here, we have identified a specific serum miRNA profile in patients with extramedullary disease and correlated it with clinically important parameters.
Aims: The goal of this study was to identify circulating miRNA signature using Taqman Low Density Arrays and assay specific quantitative PCR (qPCR) on a cohort of patients with extramedullary disease, MM patients and healthy controls, and to compare miRNA levels with clinical parameters.
Methods: One hundred serum samples obtained from relapsed EM patients, newly diagnosed MM patients and healthy donors (HD) were evaluated for this study. Screening analysis of 667 miRNAs was performed on 5 EM samples, 5 MM samples and 6 HD samples using TaqMan Low Density Arrays (TLDA). Levels of 4 differentially expressed miRNAs from TLDA (p<0.05) between EM *vs* MM, and EM *vs* HD were confirmed by qPCR using absolute quantification approach on 35 EM, 35 MM and 30 HD serum samples. Receiver Operating Characteristic (ROC) analysis was used to calculate specificity and sensitivity of each miRNA and their combination. Biochemical characteristics were also available for EM and MM patients. P values <0.05 were considered as significant.
Results: MiRNA TLDA profiling revealed 14 deregulated miRNAs (all p<0.05, adjusted p<0.41) between MM patients and EM. Further, 20 miRNAs were on the top of the list of deregulated miRNAs between EM and HD serum samples (all p<0.05, adjusted p<0.40). MiR-222, miR-130a, miR-34a and miR-195 were further verified on a bigger cohort of EM, MM and HD samples. MiR-130a was significantly down-regulated, miR-222 and miR-34a were up-regulated in EM samples when compared with HD (all p<0.005); moreover, miR-130a was down-regulated and miR-34a up-regulated also in EM when compared with MM sera (p<0.06). To discriminate EM from other groups, ROC curve was calculated. To distinguish EM from HD, it revealed highest sensitivity of 74.3%, specificity of 90.0% and area under the curve (AUC)=0.879 for the combination of miR-130a and miR-34a. Further, when EM *vs* MM were compared, this combination of miRNA revealed sensitivity of 54.3% and specificity of 80% with AUC=0.675. In the cohort of EM patients, miR-130a significantly correlated with most of clinically relevant parameters; there was a positive correlation with level of hemoglobin and thrombocytes count (r_s=0.397 and 0.439, all p<0.05) and a negative correlation with levels of monoclonal immunoglobulin, β_2-microglobulin and C-reactive protein (r_s=-0.398, -0.427 and -0.488, all p<0.05). This miRNA was also associated with higher ISS stage (p=0.017). Further, miR-222 correlated positively with lactate dehydrogenase (r_s=0.417, p<0.05); miR-222 and miR-34a were associated positively with percentage of plasma cell infiltration in the bone marrow (r_s=0.435 and 0.562, p<0.05).
Summary and Conclusions: Altogether, our first observations demonstrate that circulating miR-130a and miR-34a may be promising biomarkers for patients with extramedullary disease and prompt further studies in this field.
Grants support: NT14575, NT12130, MUNI/11/InGA17/2012, CZ.1.07/2.3.00/20.0046 and CZ.1.07/2.3.00/20.0019 of the Ministry of Education of the Czech Republic

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THE EFFECT OF BORTEZOMIB ON DIFFERENT CELL SUBSETS: AN APPROACH FOR AN INDIVIDUAL PERSONALIZED MEDICINE

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Background: Our automated flow cytometry-based Exvitech© platform allows the measurement of bortezomib induced depletion of different bone marrow cell populations. We can evaluate in the same myeloma bone marrow sample, the efficacy of bortezomib in depleting myeloma plasma cells as well as the toxic-

ity on myeloid precursors, B-precursors, and mature lymphocytes. Sensitivity is measured by standard pharmacology with 8 concentration dose responses in each of these cell subpopulations.
Aims: To compare bortezomib drug efficacy in the target myeloma population with its expected hematotoxicity from depleting myeloid precursors. This data enables an *ex vivo* therapeutic index for each patient.
Methods: 10 bone marrow (BM) samples from patients diagnosed with MM were sent to Vivia from different hospitals across Spain within 24h. The whole sample in heparin tubes was diluted with 20% SBF retaining the erythrocyte population and serum proteins, and was plated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 12-hours, and then prepared for analysis by our flow cytometry-based Exvitech© platform. A multiple staining (CD45v450/Anexin-FITC/CD117-PE/CD34PerCP/CD38-APC/CD19APC.Cy7) was performed to identify and distinguish the following BM populations: plasma cells ($CD45^{-/+}/CD38^{+}/CD19^{-}$), myeloid precursors ($CD34^{+}/CD45^{-/+}CD117^{+}/CD19^{-}$), B-lymphocyte precursors ($CD34^{+}/CD45^{-/+}CD117^{-}/CD19^{+}$), and normal lymphocytes ($CD45^{+}/SSC^{lo}$). Drug response was evaluated as depletion of each cell population relative to the average of 6 control wells without drug in each plate. All processes have been automated increasing the accuracy of the analysis.
Results: Overall, the effect of bortezomib was clearly higher in the tumor population, since the toxicity to residual BM normal populations was low, demonstrating its selectivity for the pathologic plasma cells. However, there is a high inter-patient variability in the bortezomib's effect inside the plasma cell population and in the healthy cell populations that could correspond to the range of responses seen in the clinical outcome of the patients. Interestingly, we observed the opposite effect as expected in one patient sample, shown in the Figure 1; bortezomib has a non-selective action, with a similar effect in the plasma cells than in all the precursor populations with the exception of the normal residual lymphocytes. We would interpret this data as suggestive that the probability of hematological toxicity in this case could be especially high, and hence the patient may not be a good candidate for bortezomib based therapies.

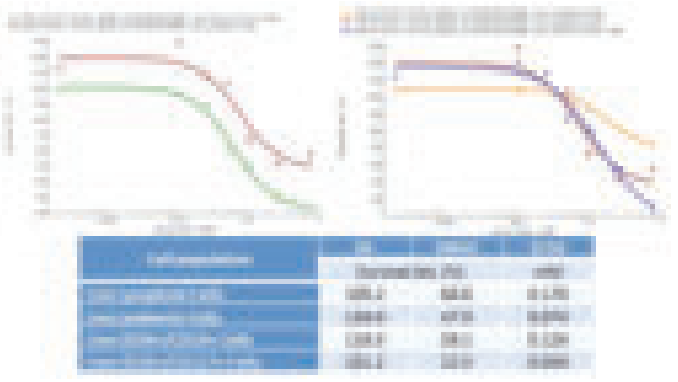

Figure 1.

Summary and Conclusions: These preliminary results show that Vivia Exvitech© platform is able to measure within the same sample the efficacy and toxicity of bortezomib on different BM populations. This platform enables measuring the effect simultaneously in the clonal plasma cells and in a putative stem cell precursor, myeloid precursors or mature lymphocytes. The example shown here for bortezomib is being extended to evaluate full drug combination treatments. This simultaneous analysis for bortezomib based treatments at the different cell levels might be able to predict the clinical response, the clinical follow up and possible hematological toxicities associated with these treatments. We pretend to establish for each individual patient an *ex vivo* hematologic therapeutic index based on the responses of each cell population to a given treatment.

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DOES CHARACTERISTIC PHENOTYPE FOR PLASMA CELL LEUKAEMIA EXIST?

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Background: Plasma cell leukaemia (PCL) is characterized by a presence of circulating plasma cells (PCs) in peripheral blood. Primary PCL (pPCL) occurs in patients with no evidence of multiple myeloma (MM) while secondary PCL (sPCL) is end-stage of relapsed and/or refractory MM. Detection of circulating PCs by flow cytometry is important for diagnosis determination and for discrimination of PCL from reactive plasmacytosis as well. Identification of phenotype profile characteristic for PCL could help in early treatment intervention.
Aims: Analyses of pPCL and sPCL to identify phenotype profile in comparison with MM.
Methods: Total of 86 patients was analysed: 12 patients with pPCL, 10 patients with sPCL and 64 newly diagnosed MM patients. Whole peripheral blood (PB) and/or bone marrow (BM) $CD38^{+}CD138^{+}$ PCs were analysed. Expression of surface antigens (CD19, CD20, CD27, CD28, CD44, CD56, and CD117) together with intracellular nestin was studied by flow cytometry. PCs were considered positive for given marker when its expression exceeds 20%.
Results: There were found similar relative number of PCs in peripheral blood of pPCL (26.7%) and sPCL (26.8%), on the other hand, infiltration of bone marrow was the highest in sPCL (55.6%) when compared to pPCL (39.2%) and MM (5.8%). No presence of $CD19^{+}$ and/or $CD20^{+}$ PCs was found in sPCL, but slightly increased number of positive cases was found in pPCL (16.7% for CD19 and/or CD20 in PB; 18.2% for CD19 and 27.3% for CD20 in BM) when compared to MM (4.7% for CD19 and 11.7% for CD20 in BM). Number of $CD56^{+}$ positive cases was higher in BM of MM (87.5%) then in pPCL (54.5%) and sPCL (66.7%), similar expression was found in PB (58.3% for pPCL and 50.0% for sPCL). Number of CD27 positive cases was the highest in BM of MM (MM 51.6%; pPCL 9.1%; sPCL 28.6%); the same positivity of CD27 was found in PB of both PCLs (25.0%). No big differences were detected in expression of CD28 in BM (30.0% in pPCL, 42.9% in sPCL and 22.6% in MM) and /or PB (16.7% in pPCL *vs.* 28.6% in sPCL). Surprisingly both PCLs expressed CD44 in 100% of BM samples, while MM in 70.7%; PB expression was lower in pPCL (85.7%) then in sPCL (100.0%). CD117 was mostly expressed in BM of sPCL (50.0%) and MM (43.5%) when compared to pPCL (10.0%), similar expression were found in PB of both PCLs (8.3% in pPCL *vs.* 14.3% in sPCL). Nestin, a marker of stem cell, was highly expressed in sPCL (80.0%) and pPCL (75.0%), but decreased in MM (36.1%); PB expression in PCLs was not so different (57.1% in pPCL *vs.* 66.7% in sPCL).
Summary and Conclusions: Phenotype profile of pPCL and sPCL did not differ so much in peripheral blood and/or bone marrow, except for a disappearance of CD19 and CD20 in sPCL and decrease of CD117 in pPCL. Lower expression of CD56, CD27 and overexpression of CD44 with nestin was characteristic for both PCLs when compared to MM.
Supported by IGA NT12425 grant.

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GENE EXPRESSION PROFILING AND COPY NUMBER ALTERATIONS OF CIRCULATING CLONOTYPIC B CELLS OF MULTIPLE MYELOMA NEWLY DIAGNOSED PATIENTS REVEALS PATHWAYS POTENTIALLY INVOLVED IN THE DISEASE PERSISTENCE

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Background: In recent years, increasing efforts have been devoted to adjust the Multiple Myeloma (MM) therapeutic approach, in order to get patients' "real cure", instead of "functional cure". Indeed, even if a significant fraction of MM patients are able to achieve complete responses, nevertheless they ultimately relapse. The persistence of Myeloma Propagating Cells (MPCs) has been supposed to be one of the major causes of MM drug-resistance. However, very little is known about the molecular background of MPCs, even if several studies suggested that they display phenotypic characteristics resembling the memory B cells that reside in the CD138- compartment.
Aims: To evaluate the genomic and genetic background MM CD138-B+ cells, located both in bone marrow (BM) and in peripheral blood (PBL) as compared to the CD138+ neoplastic clone.
Methods: We collected the CD138+ and CD138- cell fractions from 50 newly diagnosed MM patients. We isolated the B cell population and, whenever possible, the memory B cell clone. Clonogenic assays were performed using cell fractions obtained from RPMI-8226 and NCI-H929. For each cell fraction, we performed a sequencing of the IgH VDJ rearrangement. The complete set of genomic aberrations and the gene expression profile were performed according to standardized procedures (Affymetrix), using the SNPs array 6.0 and the HG-U133 Plus 2.0 array.
Results: The clonogenic potential, tested by plating cells in conditioned media, resulted higher for CD138- as compared to CD138+ cells. By VDJ rearrangement analyses, a clonal relationship between the CD138+ clone and the memory B cells clone was confirmed. SNPs arrays showed that both BM and PBL CD138+ cell fractions displayed exactly the same genomic macro-alterations. On the contrary, in the BM and PBL CD138-19+27+ memory B cell fractions any macro-alteration was detected, whereas several micro-alterations were highlighted. These micro-alterations were located out of any genomic variant regions and were presumably associated to MM pathogenesis, since, among others, *KRAS*, *WWOX* and *XIAP* genes are located in the amplified regions. The memory B cells were also characterized by the presence of several LOH