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 controls vs MM were confirmed 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 deregulated in MM 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 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 lactate dehydrogenase (rs=0.397 and 0.439, all p<0.05) and a negative correlation with levels of monoclonal immunoglobulin, β2-microglobulin and C-reactive protein (rs=-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 (rs=0.417, p<0.05); miR-222 and miR-34a were associated positively with percentage of plasma cell infiltration in the bone marrow (rs=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.

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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 Exvititech© 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©). 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 precursors 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 the 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 at 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 BM was collected from bone marrow (BM) 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%.

The clonogenic potential, tested by plating cells in conditioned media, resulted higher for CD19+ and/or bone marrow, except for a disappear-
ance of CD19 and CD20 in sPCL and decrease of CD117 in pPCL. Lower expression of CD56, CD27 and overexpression of CD44 with nestin was character-
istic for both PCs when compared to MM. Supported by IGA NT12425 grant.