P845 IDENTIFICATION OF MULTIPLE MYELOMA BIOMARKERS VIA LIQUID BIOPSY BASED ON CELL-FREE DNA USING A CAPTURE-HYBRIDIZATION PANEL

**Topic:** 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

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**Background:**

Multiple myeloma (MM) is a haematological malignancy characterized by clonal proliferation of pathogenic CD138+ plasma cells (PPCs) in bone marrow (BM). In recent years, the treatment of MM has shown great evolution. However, most patients who achieve a complete response eventually relapse. For that, an early detection of PPCs could be very beneficial for MM patients and therefore, a sensitive detection of this stage could allow early therapeutic interventions. Liquid biopsy using cell-free DNA (cfDNA) as a minimally invasive approach could be an alternative not only for the diagnosis but also for the detection of recurrences.

**Aims:**

The main objective of this study was the quantification of MM patient-specific biomarkers in cfDNA compared to PPCs and BM samples.

**Methods:**

Patients with active MM were treated with VRD, autologous stem cell transplantation and maintenance.

We studied gDNA from 23 PPCs from BM aspirates, 16 gDNA from whole BM and 12 cfDNA from peripheral blood samples obtained at diagnosis. Capture panel was aimed to detect most common and relevant aberrations known in MM such as coding regions of 37 genes involved in MM progression and drug resistance, canonical IGH and IGK to capture IGH rearrangements and regions to cover traditional translocations. Capture libraries were generated with SureSelect Reagent kits (Agilent Technologies). We used 50 ng of genomic DNA for both PCCs and BM and for cfDNA a median of 10-200 ng input. Final libraries were run on an Illumina NextSeq 500 platform. A specific bioinformatics pipeline was applied: alignment to the hg38 genome, SNVs and Indels identification by combination of variant callers, and IGH/K rearrangements with MiXCR.

**Results:**

A total of 36 different mutations were identified in the PPCs with frequencies ranging from 0.011-0.570. Comparison between PPCs and cfDNA showed that 20 out of 23 (87%) mutations identified in the PPCs were observed in the cfDNA, whereas 32 out of 35 (91%) mutations were observed in BM. Moreover, 6 out of 11 (55%) mutations identified in the PPCs were observed in cfDNA and BM (Figure 1A). In 5 out of 5 patients at least 1 mutation of those evaluated in PPCs was detected in cfDNA. Interestingly, for the classical oncogenes (NRAS, KRAS and BRAF), 5 of 8 (63%) hotspot mutations were observed in cfDNA and 7 of 8 (88%) in BM. Using the ratio between the observed frequency in cfDNA/BM and that observed in PPCs, we estimated an average tumour burden of 6% in cfDNA and a mean clonality of ~30% in BM. In addition, 5 out of 5 translocations identified in PPCs were observed in BM and unexpectedly 1 out of 2 was observed in cfDNA. Regarding IGH/K rearrangements, 50% of the patients presented at least one rearrangement in the cfDNA fraction that was identified in the PPCs, and 93% in the BM fraction. Furthermore, the average reduction in frequency observed in cfDNA and BM was ~12% compared to the frequency.
seen in PPCs. Finally, 2 out of 5 patients showed the same IG rearrangement in the three fractions studied (Figure 1B).

**Summary/Conclusion:**

Most of molecular alterations identified in PPCs seemed to be present in peripheral blood of patients with MM, surprisingly, even translocation breakpoints could be seen in cfDNA. However, liquid biopsy monitoring is limited by signal dilution, as an approximately 1-log reduction in cfDNA was observed compared to PPCs, which should be considered in bioinformatic analyses. On the other hand, *IGH*/*K* rearrangements could be identified in fewer patients, but the signal reduction was only ~12%, confirming IG rearrangements as strong biomarkers for MM in cfDNA.