Single cell multiomics for the phenotypic characterization of clonal B cells in patients with IgM MGUS and Waldenström's Macroglobulinemia

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Background: The importance of flow cytometry monitoring of circulating tumor cells and measurable residual disease in multiple myeloma has grown significantly. By contrast, its role in Waldenström's Macroglobulinemia (WM) remains ill defined. This is partly explained because of the higher intratumoral heterogeneity that spans from B lymphocytes to plasma cells (PC), and because of the limited knowledge on the phenotypic aberrancies of clonal cells.

Aim: Use an unbiased and holistic approach to identify new phenotypic markers helpful in the discrimination between normal vs clonal B lymphocytes and PC, towards the development of spectral flow cytometry panels.

Methods: Single-cell RNA and B-cell receptor sequencing (scRNA/BCR-seq) was performed using 10X Genomics in B-cell precursors, mature B lymphocytes and PC isolated by FACS from 5 healthy adults, 6 IgM MGUS and 17 WM patients. Predominant BCR gene rearrangements were identified in each patient with IgM MGUS and WM, and differentially expressed genes were considered if an average log2FC > |0.25| and an adjusted *P*-value < 0.05 were observed.

Results: After stringent quality control filtering, scRNA/BCR-seq yielded paired immunoglobulin gene rearrangements and transcriptomes in 118,532 cells and uncovered 48 clusters, which were classified into B-cell precursors (n=7,251), mature B

lymphocytes (n=94,519) and PC (n=16,762) based on differential expression of genes such as CD10 (*MME*), CD19, CD22, CD20 (*MS4A1*), CD27, CD38, CD79a, CD138 (*SDC1*), CD179a (*VPREB1*), CD269 (*BCMA*) and *XBP1*.

Patient-specific predominant clones were detected in 1.4%, 61% and 86.5% of total B-cell precursors, mature B lymphocytes and PC from IgM MGUS patients. The respective frequencies in WM were 3.9%, 70% and 40%. The number of differentially expressed genes between normal vs clonal B-cell precursors, mature B lymphocytes and PC in patients with IgM MGUS were 1,027, 1,162 and 772. The respective numbers of differentially expressed genes in WM were 601, 816 and 1,129.

In total, 61 antigens were differentially expressed in one or more cell types from WM patients vs healthy adults. The most promising new markers for the phenotypic identification of clonal cells were CD9, CD44, CD72, CD210 and CD267. In total, 52 antigens were differentially expressed in one or more cell types from IgM MGUS patients vs healthy adults. There was similarity in the new aberrant markers identified in IgM MGUS and WM. However, differential expression between normal vs clonal cells from IgM MGUS patients was less evident when compared to differences between healthy adults and WM.

Among all new aberrant markers, CD29, CD44, CD83 and CD210 were differentially expressed in the three cell types from IgM MGUS patients vs healthy adults, and CD44, CD79b, CD82, CD229 and CD268 were differentially expressed in the three cell types from WM patients vs healthy adults.

Conclusions: We provided a transcriptional atlas of the entire clonal B lineage from patients with IgM MGUS and WM, which surprisingly includes B-cell precursors. This unique dataset is being leveraged for the identification of new markers towards improved detection of clonality based on more comprehensive panels using spectral flow cytometry, which will be presented at the meeting.

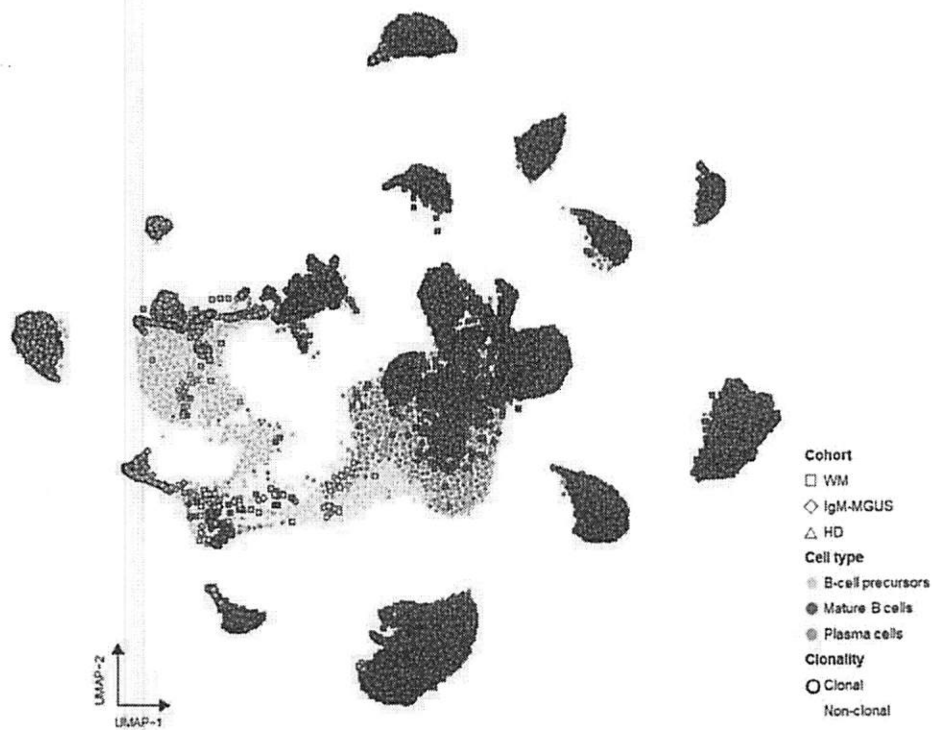

Figure 1. UMAP representation of the 118,532 cells analyzed, comprising 7,251 B-cell precursors, 94,519 mature B cells and 16,762 plasma cells, each represented with a different color. Healthy adults (HD), IgM MGUS and WM patients are represented with different geometric shapes. Patient-specific predominant clones are delineated by a black outline.