Using Mutographs to Define the Molecular Landscape and Cell of Origin of WM

To address the molecular routes and differentiation pathways leading to the development of Waldenstrom's macroglobulinemia (WM) we carried out a single-cell (sc) multiomic analysis on a series of *MYD88* mutated cases and identified 2 subtypes of disease, MBC-like and PC-like, based on gene expression of key PC and MBC regulating genes including *XBP1, JCHAIN, BCL6*, and *PAX5*.

The subtypes are characterized by their capacity to differentiate towards a PC and exhibit unique transcriptomic, chromatin accessibility, and genomic profiles. The MBC-like subtype is unable to differentiate beyond the MBC stage, upregulates key MBC genes, and is characterized by BCR and PI3K signaling. In contrast, the PC-like subtype is able to differentiate towards a PC, upregulates key PC genes, and has enhanced NF-kB signaling and an unfolded protein response.

Pseudotime trajectory analysis of combined scRNA-sequencing and scATAC-sequencing confirms the variable differentiation capacity of each subtype and elucidates potential mechanisms for this. Transcription factors important in the control of the extent of differentiation include *SPI1*, *SPIB*, *BCL11A*, *XBP1, NFKB1, and RELB*. The existence of the disease subtypes was validated using hierarchical clustering analysis of bulk RNA-seq data from a secondary set of WM cases.

Furthermore, using whole genome sequencing combined with sc and bulk RNA-seq we show that *CXCR4, HIST1H1E, NIK,* and *ARID1A* mutations occur predominantly in the MBC-like subtype and 6q deletions in the PC-like subtype. The variable differentiation blockade seen in WM manifests itself clinically as 2 disease subtypes with distinct epigenetic, mutational, transcriptional, and clinical features with potential implications for WM treatment strategies.

Key takeaway messages are that Single-cell multiome analysis identifies 2 distinct subsets of Waldenstrom's Macroglobulinemia; Memory B-cell like and Plasma-cell like, which are distinguished by the extent to which they are able to complete differentiation towards a mature plasma cell. Transcription factor motif enrichment suggests that the transcription factors SPI1, SPIB, and BCL11A are related to blocked differentiation at the MBC-like stage and the transcription factor XBP1 is responsible for driving clonal PC-like cells towards plasma cells.

The MBC-like and PC-like groups have distinct transcriptomic and chromatin accessibility profiles, with the MBC-like showing aberrant BCR signaling and the PC-like showing increased NF-kB activity and an upregulated unfolded protein response.

While both subsets of WM are characterized by MYD88 mutations, CXCR4 mutations are predominantly seen in the MBC-like group and del 6q copy number abnormalities in the PC-like subgroup consistent with their distinct molecular pathogenesis.