Characterization of Waldenström macroglobulinemia/Lymphoplasmacytic Lymphoma using Single Cell Transcriptomic Analysis

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Background:

Single cell (sc) RNA and B cell receptor (BCR) sequencing can be used to characterize and enhance our understanding of intra-tumoral heterogeneity and gene expression profiles versus bulk approaches. We applied these techniques to lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinemia (WM) patient samples to better understand the cellular make-up and gene expression profiles of tumor cells.

Methods:

Paired scRNA-seq and scBCR-seq were performed on fresh bone marrow (BM) aspirate samples from patients with symptomatic (n=26) and smoldering (n=3) WM, as well as a peripheral blood (PBMC) sample from 1 patient with smoldering LPL. All samples were subjected to CD138+ and CD19+ selection. cDNA libraries were prepared from these enriched samples, followed by simultaneous 5' gene expression (scRNA-seq) and scBCR-seq. Single-cell data was processed as previously described (Dang et al., Cancer Cell, 2023). The mutation status of MYD88, CXCR4, TP53, DNMT3A, TET2, and ASXL1 for each patient was determined by clinical molecular diagnostics (Figure. 1A, 1B).

Results:

We profiled 247,916 high-quality cells including 111,848 B cells (21,382 progenitor/precursor/immature B cells and 90,466 mature B cells) and 136,068 plasma cells (PCs). We also obtained scBCR-seq data on 223,062 cells, of which 198,403 had paired scRNA-seq data. Clonotype was defined as monoclonal (frequency≥5), polyclonal (frequency<5), and undetected. Unsupervised clustering analysis revealed 10 clusters in B cells, including 4 precursor, 2 naïve, 3 memory and 1 atypical B cell clusters. Additionally, 3 clusters were identified in PCs (Figure. 1C).

More than half of the naïve/germinal center (GC) B, unswitched memory B, IgE memory B, and PCs were monoclonal, while immature B, naïve B, S100A10+ memory B, and atypical B cells were polyclonal. Monoclonal cells within a single patient displayed multiple phenotypes despite sharing the same clonotype (Figure.1D). MYD88-mutated patients had higher proportions of naïve and unswitched memory B but lower proportions of precursor B, switched memory B, and CXCR4+ PCs compared to MYD88 wild-type patients. $MYD88^{mut}CXCR4^{mut}$ patients (N=10) had

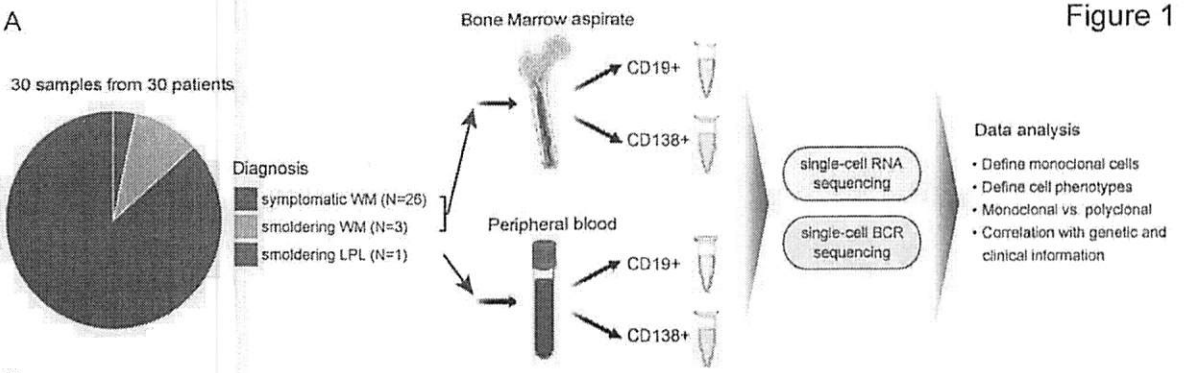
more PCs, but fewer naïve/GC B than MYD88^{mut}CXCR4^{wt} patients (N=15). Smoldering WM patients had higher proportions of proliferating pro/pre-B, naïve B, and unswitched memory B than symptomatic WM patients. Patients with prior covalent BTK inhibitor treatment had more plasmablasts but fewer naïve/GC B compared to those without prior treatment (Figure. 2A).

Differential gene expression analysis between monoclonal and polyclonal B cells showed high expression of *JCHAIN*, *BCL2*, *XBP1*, *ZNF804A*, *CDKN1A*, and *HSPA5*, among others in monoclonal B cells. Pathway analysis revealed upregulated apoptotic signaling, B cell activation, response to endoplasmic reticulum stress, and glucose starvation, with downregulated B cell mediated immunity and actin filament-based processes in monoclonal B cells. Comparing monoclonal and polyclonal PCs, we observed elevated expression of *JCHAIN*, *IGHM*, *CD79A*, *CXCR4*, *ZNF804A*, and a variety of ribosome genes, et al., with downregulated activity in B cell activation, phagocytosis, humoral immune response and complement activation in monoclonal PCs (Figure. 2B).

Conclusions:

Noted intra-tumoral heterogeneity and differences in gene expression between monoclonal and polyclonal cells may help inform new therapeutic sequences for pre-clinical and clinical investigation.

Figure 1



B

Diagnosis	Symptomatic WM		Smoldering WM		Smoldering LPL	
n total	26		3		1	
Age, median (range), years	64	(46-92)	64	(60-73)	83	(83)
Gender, n (%)						
Female	11	42.3	2	66.7	1	100.0
Male	15	57.7	1	33.3	0	0.0
Race (ethnicity), n (%)*						
White (Non-hispanic or Latino)	25	96.2	2	66.7	1	100.0
White (Hispanic or Latino)	1	3.8	0	0.0	0	0.0
Other Race (Hispanic or Latino)	0	0.0	1	33.3	0	0.0
Mutations, n (%)						
MYD88	23	88.5	3	100.0	0	0.0
CXCR4	9	34.6	1	33.3	0	0.0
TP53	4	15.4	0	0.0	0	0.0
DNMT3A	4	15.4	1	33.3	0	0.0
TET2	0	0.0	1	33.3	0	0.0
ASXL1	1	3.8	0	0.0	0	0.0
Treatment status						
Treated	21	80.8	0	0.0	0	0.0
Untreated	5	19.2	3	100.0	1	100.0
Prior covalent BTK inhibitor (Ibrutinib, Acalabrutinib,						
Yes	12	46.2	0	0.0	0	0.0
No	14	53.8	3	100.0	1	100.0

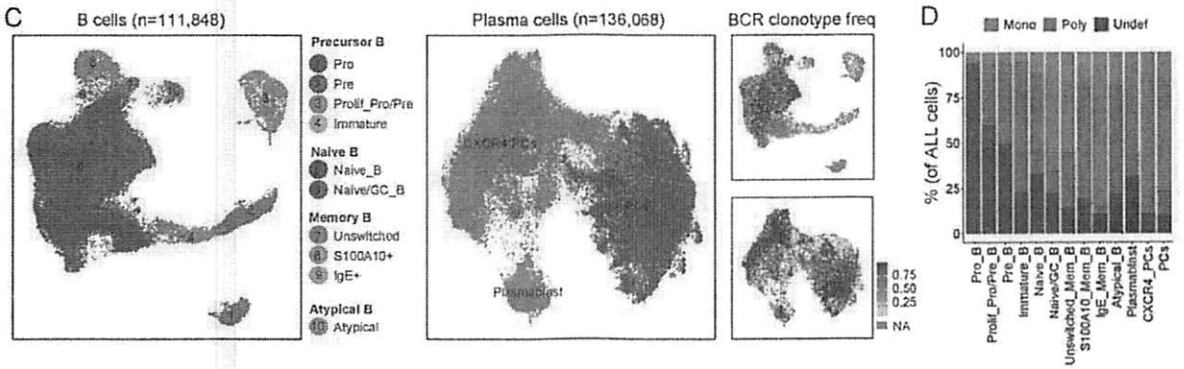


Figure 1. Workflow, sample information and single cell transcriptome map of this study. (A) A schema depicting the workflow of this study. **(B)** Patient characteristics. **(C)** Uniform manifold approximation and projection (UMAP) visualization of unsupervised clustering analysis of B cells and plasma cells that passed quality filtering. Cells were color coded for their phenotypes (left and middle) and BCR clonotype proportion (right). **(D)** Stacked bar plot showing the composition of each cell population regarding the clonotype groups.

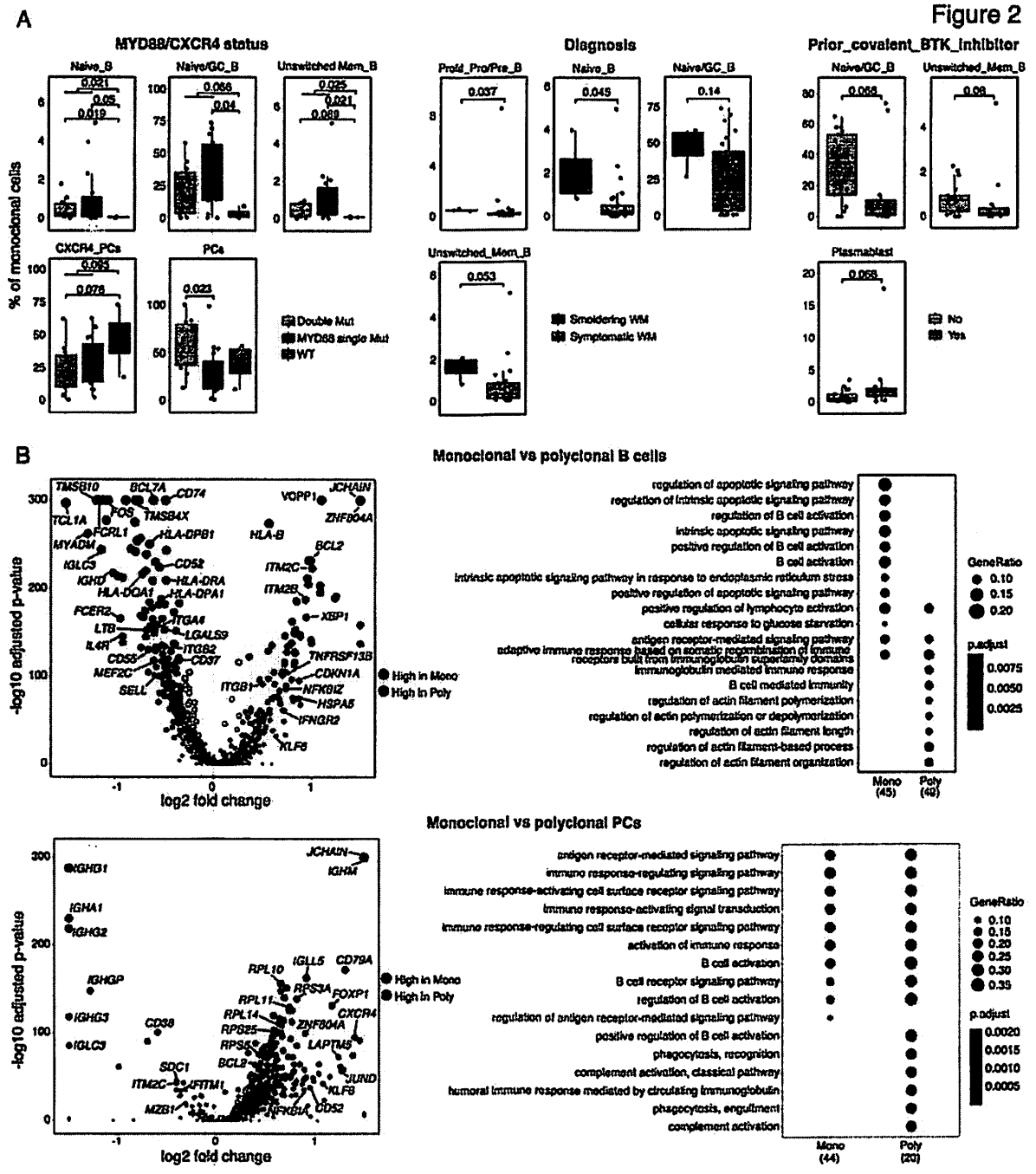


Figure 2. Correlation of cell phenotypes with genetic and clinical information, and differences in gene expression between monoclonal and polyclonal cells. (A) Boxplots showing the comparisons of cell population proportions among monoclonal cells across different groups and settings. **(B)** Volcano plots (left) and bubble plots (right) showing differentially expressed genes and enriched pathways between monoclonal and polyclonal B cells (top) and plasma cells (bottom).