Early Subclones Showing Aberrant Co-expression of Stem Cell, T cell and Myeloid Genes Can Be Detected by Flow Cytometry in MYD88 Mutated Waldenström's Macroglobulinemia patients

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BACKGROUND

Previous studies have described the occurrence of MYD88^{L265P} mutation (MYD88^{MUT}) in CD34+ hematopoietic stem/progenitor cells and B cell precursors, as well as both CD19+ and CD138+ cells co-expressing the T cell marker CD3 in Waldenström's Macroglobulinemia (WM) patients.[1-4] Our RNA-Seq analysis of 249 untreated MYD88^{MUT} WM patients showed aberrant expression of stem cell, pre-B-cell, T-cell and myeloid genes in early WM B clones. This expression pattern was associated with inferior progression free survival with BTK inhibitor therapy and diminished with advancing disease.[5] We therefore sought to validate this early transcriptome signature at the protein level using flow cytometry and evaluate its potential as a clinical assay to monitor WM disease evolution.

METHODS

RNA-Seq predictions were validated on an independent cohort of 4 untreated MYD88 mutated WM patients and 3 healthy (HD) samples. Patients' characteristics are shown in **Table 1**. We designed a 13-color panel for flow cytometry detection of surface markers CD19, CD138, kappa and lambda immunoglobulin (Ig) light chains, CD34, CD117 (KIT), CD4, CD8A, CD33, FCGR3A (CD16), CEACAM8 (CD66b), pre-BCR (using an antibody that recognizes a unique conformational epitope of the BCR complex formed by the association of IGLL1 and VPREB1); Zombie Yellow was used to label dead cells. Peripheral B (PBMC) and/or bone marrow mononuclear cells (BMMC) were isolated from WM and HD samples by density gradient centrifugation using Ficoll-Paque medium. Data acquisition was performed for at least 10^6 nucleated cells per tube in BDLSR Fortessa flow cytometer using the FACSDiva software. At least 145,000 events were recorded per sample with a median of 176,945 events for WM and 1,941,174 events for HD (range 144,711-1,973,780).

RESULTS

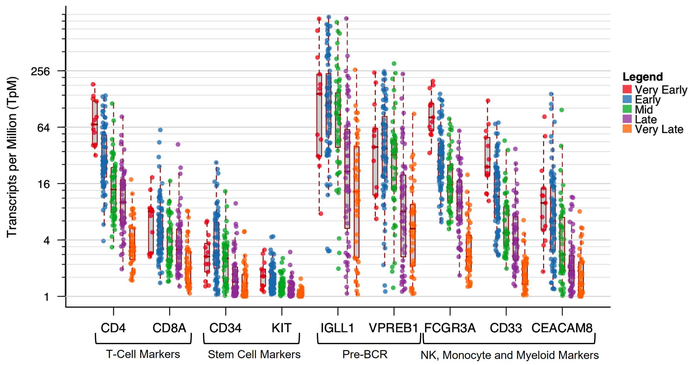

Figure 1. Transcriptome analysis of 249 untreated WM samples based on a novel subtype and early/late evolutionary staging classification for WM[6] revealed that CD19+ bone marrow WM cells aberrantly express stem cells, T cells, precursor B cells and NK, Monocyte and Myeloid markers at an early stage of the disease. The expression of all these markers is progressively downregulated as the disease progresses overtime.

Table 1	WM1	WM2	WM3	WM4
Age, yo	68	50	88	53
MYD88, mutational status	MUT	MUT	MUT	MUT
CXCR4, mutational status	MUT	MUT	NA	WT
BM involvement, %	15-20	95	>10	30
IgM, mg/dL	536	1151	686	4236
Hb, g/dL	13.2	8.1	13.4	11.3
PLT, K/uL	330	52	272	316
WBC, K/uL	9.42	3.53	10.17	5.64
Lymphocytes, %	13.9	25	42.6	19
Free light chain kappa, mg/l	115.8	34.3	57.7	10.4
Free light chain lambda, mg/l	8.1	6.6	14.6	109.1
kappa/lambda	14.3	5.2	3.95	0.1
Flow, source	BMMC	PBMC, BMMC	PBMC	PBMC

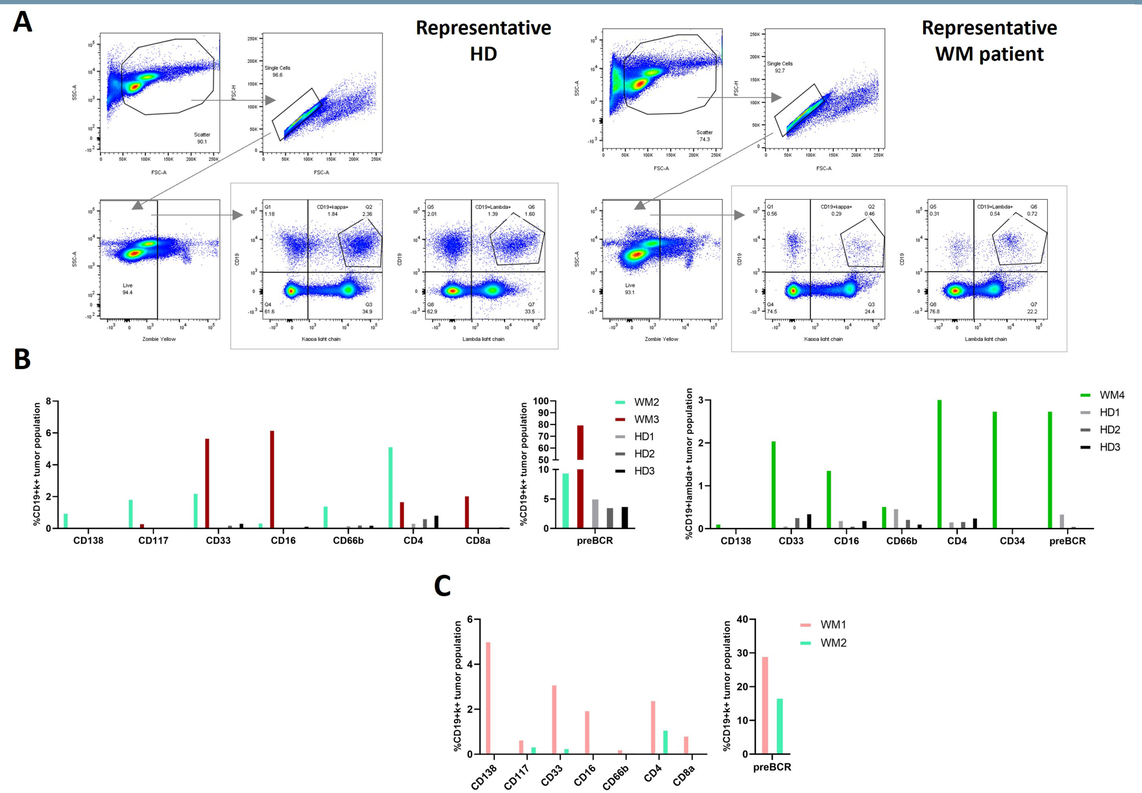

Figure 2. **Flow cytometry**. **A)** The adopted gating strategy is shown in a representative HD and WM patient. **B)** Detection of the CD19+kappa+ (left panel) and CD19+lambda+ (right panel) clone demonstrating aberrant co-expression of the studied genes is shown in PBMC from both HD and WM patients. In CD19+kappa+ cells, CD34 expression was detected in <0.1% cells. No expression of KIT and CD8a was observed in CD19+lambda+ cells. **C)** Detection of the CD19+kappa+ clone demonstrating aberrant co-expression of the studied genes is shown in BMMC from WM patients. CD34 expression was detected in <0.1% CD19+kappa+ cells in WM2 (not shown).

CONCLUSIONS

In WM primary cells, we observed the existence of a clonal B cell population that appears to re-activate stem cell programming early on and it is progressively replaced by clones bearing more typical B cell markers as the disease progresses. This study lays the foundation to develop a widely accessible methodology to accurately diagnose IgM MGUS/early-stage WM and monitor disease evolution. Flow-based sorting of these subclones will allow targeted multi-omic studies that will provide insight into disease pathogenesis and targeted therapeutic applications.

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