

Atypical CD4+/CD33+ Inflammatory B-cells are Enriched in MGUS/Smoldering and Early Disease Progression in Post Therapy Patients with Waldenstrom Macroglobulinemia

Nicholas Tsakmaklis¹, Filip Garbicz², Maria Luisa Guerrero¹, Hao Sun¹, Shirong Liu¹, Amanda Kofides¹, Sofia Quezada¹, Nour Mashmouhi¹, John M. Hatcher¹, Alberto Guijosa¹, Christopher J. Patterson¹, Andrew R. Branagan³, Shayna R. Sarosiek¹, Jorge J. Castillo¹, Ruben Carasco², Steven P. Treon¹, Zachary R. Hunter¹

¹ Bing Center for Waldenstrom Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA.

² Department of Pathology, Dana-Farber Cancer Institute, Boston, MA, USA.

³ Division of Hematology and Oncology Massachusetts General Hospital Cancer Center, Boston, MA.

Background: *MYD88* mutated Waldenstrom's Macroglobulinemia (WM) is comprised of two molecular subtypes, B-cell like (BCL) and plasma cell like (PCL), with unique transcription and mutation profiles, and distinct cell of origin based on methylation analysis (Hunter et al Blood 2023; 142(S1): 756; Gagler et al, Blood 2025; 146(10):1225-1238). During MGUS and smoldering disease, these subtypes share a similar transcriptional profile known as Early WM. The Early WM profile consists of the co-expression of BCL and PCL signature genes that are not expressed in other non-malignant bone marrow (BM) cells but are expressed in BCL (*KLF14* and *GRIK3*) and PCL (*WNK2* and *PRDM5*) subtypes of WM. We utilized the WM evolutionary score (EScore), an independent gene signature that correlates with disease progression, with low score indicating early WM, and higher score depicting late stage WM. (Hunter et al Blood 2023; 142 (Supp. 1): 756). Analysis of EScore identified a population of cells with aberrant expression of T-cell, myeloid, pre/pro B-cell and stem cell markers that were prevalent at the early disease stages. These cells also appeared to promote inflammation by elevated expression of myeloid chemokines and S100 alarmins but become less predominant with WM progression. These results were validated in external datasets of MGUS and WM, single cell sequencing and multi-parameter flow cytometry. Herein, we aimed to show existence of these atypical WM B-cells by double immunohistochemistry (IHC) and evaluate the impact of the WM subtype and EScore gene signatures in previously treated WM.

Methods: Nuclear PAX5 and CD4, CD33 or S100A8/9 heterodimer dual IHC assays were optimized and tested on blocks from our multi-omic cohort of 249 untreated WM patients used in the development of the WM subtypes and EScore. RNA and whole exome sequencing were performed on an independent cohort of CD19+ BM samples from 137 previously treated patients derived from their first biopsy at our Institution including 45 on active therapy.

Results: The double IHC studies successfully identified PAX5+CD4+, PAX5+CD33+ and PAX5+S100A8/9+ WM B-cells. While rare, the S100A8/9+ WM cells demonstrated robust expression in line with the myeloid cells. CD33+ WM cells were the most common and the CD33 appeared to be restricted to the Golgi apparatus in WM rather than the cell surface as was observed in the myeloid compartment. CD4 was observed on the cell surface as expected. EScore, BCL and PCL subtype scores were imputed in the cohort of 137 WM patients. K-means clustering was used on these scores to identify 41 BCL, 52 PLC and 44 Early WM samples. As observed in the untreated population, Early WM showed low EScore levels versus the BCL or PCL subtypes ($p < 0.005$ vs both PCL and BCL, respectively) while the subtypes grew increasingly distinct at higher EScore levels. As observed in our untreated cohort, Early WM was characterized by expression of both BCL and PCL signature genes including *WNK2*, *KLF14*, *GRIK3* and *PRDM5*. Early WM was also associated with a longer time to next therapy from study biopsy compared with the mature BCL/PCL subtypes for all samples ($p=0.034$) as well as when excluding samples on active therapy ($p=0.026$). Low EScore values were again associated with the high

expression of T-cell genes (*CD4*, *CD8A*, *CD3E*; $p < 0.0001$), myeloid genes (*CD33*, *CD14*, *FCGR3A*; $p < 0.0001$), stem cell genes (*KIT*, *PROM1*; $p < 0.001$), and inflammatory genes (*S100A8*, *S100A9*, *CXCL1*, *CXCL8*; $p < 0.0001$) indicating reemergence of these atypical B-cells in Early WM subtyped previously treated patients. These genes regressed to near normal levels at the highest EScore levels. In contrast to our previous findings, the pre/pro B-cell signature genes including *MYB*, *RAG1*, and *IGLL1* as well as the stem cell marker *CD34* were largely invariant with EScore, indicating some differences in the biology of these cells in the previously treated population.

Conclusions: Atypical WM B-cells expressing T-cell, myeloid, and inflammatory markers are enriched in MGUS/smoldering WM and at early stages of disease progression in previously treated patients. The re-emergence of these atypical cells with an Early WM signature indicates that the biology of WM progression is similar between these two populations and that these cells may be resistant to many of the current WM therapies. Our studies provide a framework to investigate the role of atypical WM B-cells in disease progression and opportunities for therapeutic exploitation.