

Serum levels of the S100A8/S100A9 heterodimer are elevated in Waldenstrom's Macroglobulinemia patients, and are not impacted by ibrutinib monotherapy.

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Introduction: The mechanisms orchestrating immune dysregulation in Waldenstrom's Macroglobulinemia (WM) remain poorly understood. Such insights are important given the investigation of cellular therapies for WM. Bone marrow (BM) myeloid-derived suppressor cells (MDSC) are expanded in WM patients and can suppress CD8+ T cell function, a critical component for cellular therapy success. S100A8 and S100A9 alarmins are known regulators of MDSC formation and function. S100A8 and S100A9 are expressed by WM cells in untreated early/smoldering WM but downregulated in late/symptomatic cases (Hunter et al, ResearchSquare Preprint 2025). S100A8 and S100A9 promote T cell exhaustion and mediate resistance to BCMA-directed CAR T cell and bispecific antibody therapies in multiple myeloma (Aleman et al, ASH meeting 2025). Here we sought to investigate the role of S100A8 and S100A9 in WM and gain mechanistic insights into pathways regulating their release.

Methods: S100A8 and S100A9 expression was profiled across BM populations in 17 WM and 6 healthy donors (HD) by scRNA-Seq. We performed spectral flow cytometry on BM (BMMC) and/or peripheral blood mononuclear cells (PBMC) from untreated and relapsed/refractory WM patients to BTKi-containing therapeutic regimens and HD controls to phenotypically profile monocytic (M-MDSC) and polymorphonuclear MDSC (PMN-MDSC) and the expression of the S100A8 and S100A9 receptors CD33, CD69, RAGE and TLR4. Intracellular staining for p-BTK and p-HCK was performed in 6 samples. IHC was performed to assess S100A8 and S100A9 in untreated WM samples. Circulating plasma levels of 11 MDSC-related inflammatory mediators, including the S100A8/S100A9 heterodimer, were assessed in 35 untreated WM and 5 HD by Luminex multiplex and ELISA immunoassays. Changes in circulating levels of the S100A8/S100A9 heterodimer were assessed by ELISA in 61 longitudinal samples from 24 WM patients treated with Ibrutinib monotherapy in a prospective phase 2 trial in treatment-naïve patients (NCT02604511). The analyzed samples were collected at screening (C0) and cycle 6 (C6) for 11 WM; C0, C6 and long-term VGPR (samples collected at ≥24 months) for 7 WM; and C0, C6 and progression for 6 WM patients. Correlation was tested using Spearman's rho in GraphPad Prism.

Results: By scRNA-Seq, CD14⁺ classical monocytes emerged as the predominant expressors of *S100A8* and *S100A9*. Spectral flow cytometry analysis showed greater enrichment of granulocytic vs monocytic populations in untreated WM BM samples. In BM samples from untreated and BTKi-relapsed/refractory WM, LOX1⁺ PMN-MDSCs were significantly more abundant than LOX1⁻ granulocytes (MFI 5,988 vs. 1,110; $p = 0.021$). LOX1⁺ PMN-MDSC showed constitutive p-BTK activation across HD and WM samples. Within the monocyte gate, CD14⁺CD11b⁺CD15⁻HLA-DR⁺ monocytes were enriched vs CD14⁺CD11b⁺CD15⁻HLA-DR^{low/-} (M-MDSC) in patient BM and PB. BM M-MDSC upregulated CD69 vs HLA-DR⁺ monocytes. By IHC, we confirmed *S100A8* and *S100A9* expression from both tumor and myeloid cells in untreated WM. A trend towards higher plasma levels of the *S100A8/S100A9* heterodimer was observed in WM (median 1,023 ng/ml, range 249-3,285) vs HD (median 703 ng/ml, range 325-1,940, $p=0.09$). No difference in baseline levels was observed based on patient subtype and evolutionary score. Higher *S100A8/S100A9* heterodimer levels positively correlated with absolute neutrophil count and CXCL8 plasma levels, a potent neutrophil and MDSC-attracting chemokine. Following Ibrutinib treatment, no significant changes in plasma levels of the *S100A8/S100A9* heterodimer were observed at C6, regardless of CXCR4 status and attainment of a major response, long-term VGPR or progression.

Conclusions: Our study identified the BM expansion of LOX1⁺ PMN-MDSC which showed constitutive BTK activation in untreated and BTKi relapsed/refractory WM patients, the enrichment of HLA-DR⁺ monocytes and the persistence of circulating plasma levels of the *S100A8/S100A9* heterodimer following Ibrutinib treatment. Our study provides a framework for further investigation of the immunosuppressive role of the *S100A8* and *S100A9* alarmins and MDSC interplay in WM progression and resistance, including in the setting of WM-directed cellular therapies.

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Abstract Title: Serum levels of the S100A8/S100A9 heterodimer are elevated in Waldenstrom's Macroglobulinemia patients, and are not impacted by ibrutinib monotherapy.

Conference: IWWM13