Background: Suppressive myeloid cell populations in the tumor microenvironment (TME) are associated with worse survival of cancer patients and low effectiveness of T cell checkpoint inhibitors. Recently, several early clinical trials have produced positive data for therapies aimed at repolarizing immunosuppressive myeloid populations in the TME. One new macrophage repolarizing target, PSGL-1 (P-selectin glycoprotein ligand-1), is expressed at high levels on suppressive tumor-associated macrophages (TAMs) and in vitro differentiated M2 macrophages. PSGL-1 has been shown to have an immune-modulatory activity, which includes its role in maintaining an immuno-suppressive macrophage state.

Methods: To assess the ability of PSGL-1 antibodies to convert macrophages and the tumor microenvironment from an immuno-suppressive toward a pro-inflammatory state, we employed in vitro primary macrophage and multi-cellular assays, ex vivo patient-derived tumor cultures, and a humanized mouse PDX model.

Results: We have determined that our lead anti-PSGL-1 antibody repolarized M2-like macrophages to a more M1-like state both phenotypically and functionally as assessed in primary in vitro macrophage assays. Transcriptomics profiling of M2c macrophages showed that the anti-PSGL-1 antibody upregulated TNF-alpha/NF-kB and chemokine-mediated signaling, while downregulating oxidative phosphorylation, fatty acid metabolism and Myc signaling pathways, consistent with a broad M2-to-M1 shift of the macrophage state. Furthermore, these repolarized M1-like macrophages enhanced the inflammatory response in complex multi-cellular assays. Pre-clinical efficacy of the anti-PSGL-1 antibody was demonstrated using ex vivo cultures of fresh patient-derived tumors that preserve the cellular heterogeneity of the TME. Anti-PSGL-1 increased production of inflammatory cytokines and chemokines involved in immune activation of the TME and T cell recruitment. Lastly, our lead anti-PSGL-1 antibody also showed in vivo anti-tumor effect in a humanized mouse PDX model of melanoma. The antibody suppressed tumor growth to a significantly greater degree compared to anti-PD-1. At the cellular and molecular levels, the anti-PSGL-1 treatment led to a more enhanced inflammatory microenvironment, including a reduced M2:M1 macrophage ratio, and an increase in systemic pro-inflammatory mediators. Compared to anti-PD-1 monotherapy, anti-PSGL-1 alone and in combination with anti-PD-1 increased the fraction of effector CD8+ T cells among the infiltrating T cells. Significant combination effects of anti-PSGL-1 plus anti-PD-1 were seen at the cellular and molecular levels within the tumor tissue, the spleen, and peripheral blood.

Conclusions: The data presented here provide biological and mechanistic support for clinical testing of antibodies targeting PSGL-1 for the treatment of cancer.

Ethics Approval: All legal and ethical requirements were met with regards to the humane treatment of animals described in the study. The animal study was conducted in compliance with IACUC PROTO202000042 and the institutional assurance certification of the University of Massachusetts Medical School. The University of Massachusetts Medical School is fully accredited by AAALAC and has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW).

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