In-Depth Myeloid Cell Characterization in the Murine Syngeneic CT.26 Colon Carcinoma Model by 10-Color Flow Cytometry
Matt Thayer, David Draper, Dan Sains, Maryland Rosenfeld-Franklin, Scott Wise. | MI Bioresearch 800 Technology Dr., Ann Arbor, MI 48108

Introduction
The efficacy of immune-modulating anti-cancer therapeutic antibodies that have been FDA-approved in recent years, such as anti-CTLA-4 and anti-PD-1, has driven growing interest in methods that provide a mechanistic understanding of drug function. Development of new monoclonal antibody combinations with immune-modulatory effects requires more powerful immunophenotyping techniques capable of in depth cell characterization. To this end, using the CT26 murine syngeneic colorectal cancer model we have developed a 10-color flow cytometry antibody panel that focuses on the identification of tumor-infiltrating immune cell subsets derived from myeloid lineage precursors utilizing the high-throughput-capable 4-laser, 14-color Attune NxT Flow Cytometer. The panel includes a combination of antibodies against CD45, CD3, CD49b, CD335, CD11b, CD11c, Ly-6G, Ly-6C, F4/80, and CD115. By excluding cells of lymphoid lineage, we show that this panel facilitates analysis of myeloid derived cells including natural killer (NK) cells, macrophages, neutrophils, dendritic cells (DCs), and monocytic or granulocytic myeloid-derived suppressor cells (mMDSCs and gMDSCs) subsets in tumor and peripheral blood. In addition, this antibody combination allows for a more complete analysis of MDSC cells which can differentially express several disease-relevant myeloid specific markers including Ly-6G, Ly-6C, F4/80, CD11c, and CD115. This panel was utilized to characterize changes in the myeloid subset between control and anti-PD-L1 treated mice.

Materials & Methods
Balb/c mice (Envigo) were subcutaneously implanted with CT.26 tumor cells and treated with either anti-mPD-L1 antibody (10F.9G2: BioXCell) or anti-rat isotype control antibody (LTF-2: BioXCell). On the last day of treatment, mice were terminated for immunophenotypic analysis of CD45+ cells and NK cells and a decrease in circulating DCs.

Results & Conclusions
• Treatment with anti-PD-L1 increased the abundance of CD45+ cells and NK cells in tumors.
• Treatment with anti-PD-L1 altered the composition of the MDSC milieu from mMDSC dominant to gMDSC dominant.
• Treatment with anti-PD-L1 resulted in a reduction of immature dendritic cells circulating in blood and increased the number of detected NK cells.

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