In-Depth Myeloid Cell Characterization in the Murine Syngeneic CT.26 Colon Carcinoma Model by 10-Color Flow Cytometry

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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 mono- and combination therapies with immune-modulatory effects requires more powerful immunophenotyping techniques capable of in depth cell characterization. To this end, using the CT.26 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, CD19, 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 and 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 tumor-derived cells and blood by flow cytometry. Tumors were processed into single-cell suspensions using gentleMACS™ Dissociators (Miltenyi Biotec). Samples were acquired on an Attune™ NxT Flow Cytometer (Life Technologies) and data were analyzed using Flowjo software (Treestar).

Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.

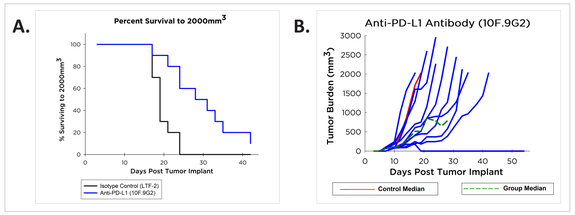


Figure 1. Treatment efficacy data. Survival (A) and tumor growth (B) curves from anti-PD-L1 treatment and isotype control groups demonstrate differential tumor growth rates resulting from treatment efficacy. Treatment with anti-PD-L1 resulted in ~40% response rate, with the majority being partial responders.

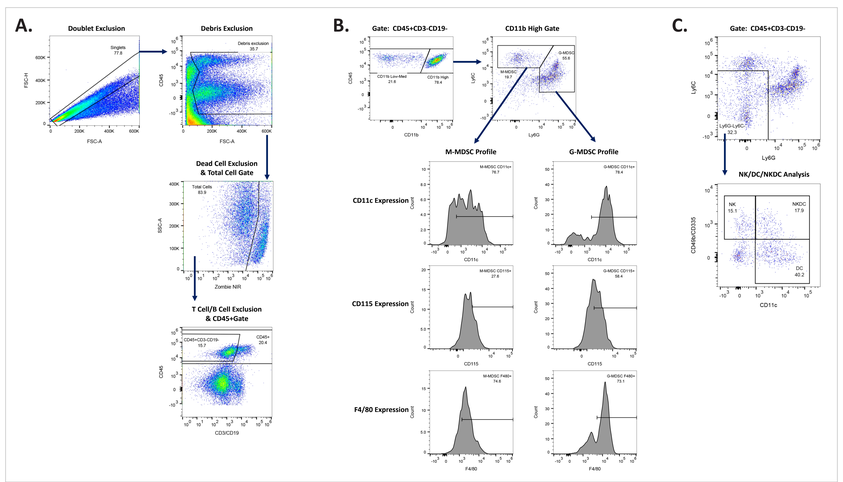


Figure 2. Gating strategy for identifying populations of interest in tumor samples. (A) Doublet discrimination, debris and T/B cell exclusion gates. (B) Myeloid lineage markers on gMDSCs and mMDSCs. (C) Myeloid cell exclusion and identification of DCs and NK cells.

	CD45+ (% Total Cells)	NK (% CD45+)	DC (% CD45+)	NKDC (% CD45+)	G-MDSC (% CD45+)	CD11c+ (% G-MDSC)	CD115+ (% G-MDSC)	F480+ (% G-MDSC)	M-MDSC (% CD45+)	CD11c+ (% M-MDSC)	CD115+ (% M-MDSC)	F480+ (% M-MDSC)
Isotype Control	24.4	4.2	9.5	4.8	33.3	64.6	61.7	56.9	17.5	74.9	29.3	72.8
Anti-PD-L1 Treatment	30.9	4.7	13.5	4.6	22.0	69.4	62.3	55.9	31.2	78.6	38.2	75.7

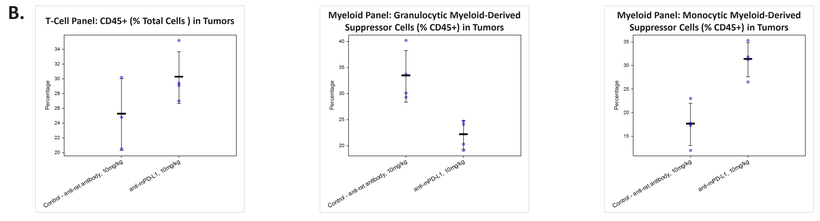
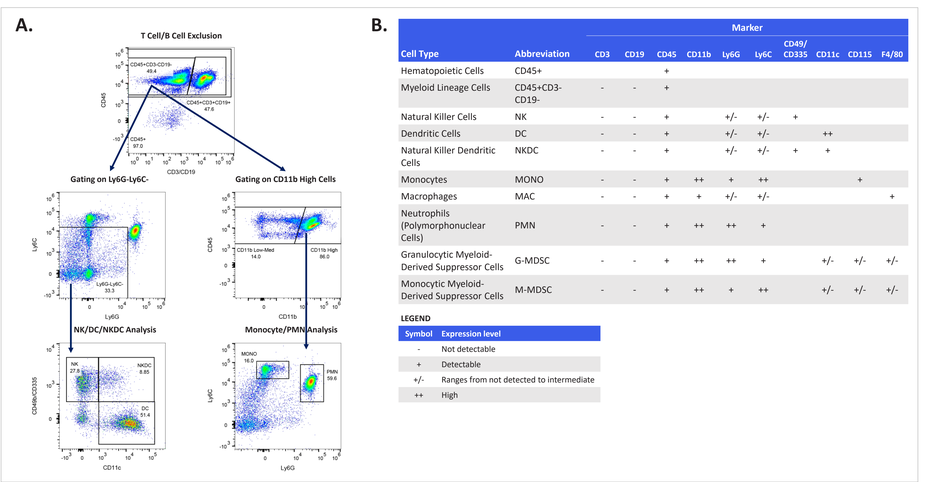


Figure 3. Immunophenotyping data from tumor. (A) Tabular data from tumor analysis. (B) Notable trends, including an increase in CD45+ cells and a shift in the balance of gMDSCs and mMDSCs.



B.

Cell Type	Abbreviation	Marker									
		CD3	CD19	CD45	CD11b	Ly6G	Ly6C	CD49/ CD335	CD11c	CD115	F4/80
Hematopoietic Cells	CD45+			+							
Myeloid Lineage Cells	CD45+CD3-CD19-	-	-	+							
Natural Killer Cells	NK	-	-	+		+/-	+/-	+			
Dendritic Cells	DC	-	-	+		+/-	+/-		++		
Natural Killer Dendritic Cells	NKDC	-	-	+		+/-	+/-	+	+		
Monocytes	MONO	-	-	+	++	+	++			+	
Macrophages	MAC	-	-	+	+	+/-	+/-				+
Neutrophils (Polymorphonuclear Cells)	PMN	-	-	+	++	++	+				
Granulocytic Myeloid-Derived Suppressor Cells	G-MDSC	-	-	+	++	++	+		+/-	+/-	+/-
Monocytic Myeloid-Derived Suppressor Cells	M-MDSC	-	-	+	++	+	++		+/-	+/-	+/-

LEGEND

Symbol	Expression level
-	Not detectable
+	Detectable
+/-	Ranges from not detected to intermediate
++	High

Figure 4. Gating strategies in blood and cell identification strategy. (A) Gating strategy for identifying populations of interest in blood. (B) Cell identification strategy.

	CD45+ (% Total Cells)	Monocytes (% CD45+)	PMN (% CD45+)	DC (% CD45+)	NK (% CD45+)	NKDC (% CD45+)
Isotype Control	95.6	8.1	28.3	8.3	4.6	1.5
Anti-PD-L1 Treatment	94.3	9.7	29.5	6.4	7.7	2.1

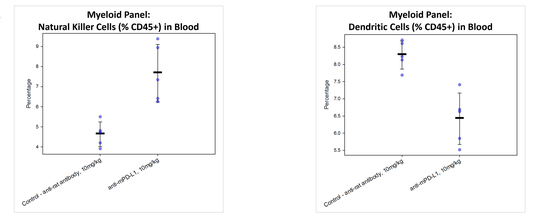


Figure 5. Immunophenotyping data from blood. (A) Tabular data from blood analysis. (B) Notable trends, including an increase in circulating NK cells and a decrease in circulating DCs.

Results and 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 gMDSC dominant to mMDSC 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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