Antibody Blockade of the Immunoinhibitory Receptor Siglec-10 Polarizes Tumor-associated Myeloid Cells and Promotes Anti-tumor Immunity

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BACKGROUND

- Myeloid cells are the most abundant immune cells within the tumor microenvironment (TME) where they play important roles regulating anti-tumor immunity
- Targeting myeloid-specific inhibitory receptors to modulate the TME is an attractive strategy to improve the therapeutic outcome of current cancer immune therapies
- Siglec-10 is an inhibitory receptor expressed on tumor-associated macrophages (TAMs) and dendritic cells that regulates immune cell activation via immunoreceptor tyrosine-based inhibitory motifs
- Recently, Siglec-10 was shown to induce immunosuppression and promote tumor immune escape through interaction with CD24
- In addition to CD24, CD52 and vascular adhesion protein-1 (VAP-1) have been shown to drive immunosuppression via Siglec-10, indicating that Siglec-10 functions as an inhibitory receptor through multiple ligands
- We report here that Siglec-10 expression is upregulated in human tumors and blockade of Siglec-10 with a monoclonal antibody (mAb) enhances proinflammatory responses and delays tumor growth in vivo by modulating myeloid cell function

METHODS

- Siglec-10 expression was evaluated in human tumors by flow cytometry and RNA-sequencing
- An anti-human Siglec-10 mAb that blocks ligand binding and induces receptor internalization was generated using hybridoma technology and recombinantly produced on mouse IgG1 backbone
- To assess the in vivo activity of a Siglec-10 mAb, transgenic mice expressing human Siglec-10 were generated
- Siglec-10 mAb activity was evaluated in vivo using a TLR-mediated lung inflammation model
- Anti-tumor activity of a Siglec-10 mAb was determined using an MC38 syngeneic colon adenocarcinoma mouse model

### 1. Siglec-10 is upregulated in multiple human cancers
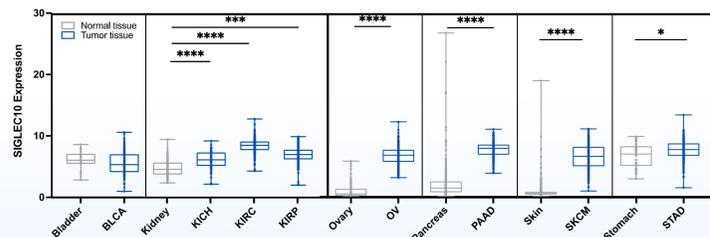
SIGLEC10 mRNA expression levels in diverse human cancers from the TCGA (The Cancer Genome Atlas) and normal matched-tissue from the GTEX (Genotype Tissue Expression Project). Boxes show the median and whiskers indicate min to max expression. * $P<0.01$, **** $P<0.0001$, unpaired, Mann-Whitney U test. BLCA, bladder urothelial carcinoma; KICH, kidney chromophobe; KIRC, kidney renal cell carcinoma; KIRP, kidney renal papillary carcinoma; OV, ovarian adenocarcinoma; PAAD, pancreatic adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma

2. Siglec-10 expression inversely correlates with patient survival and prognosis in colon adenocarcinoma
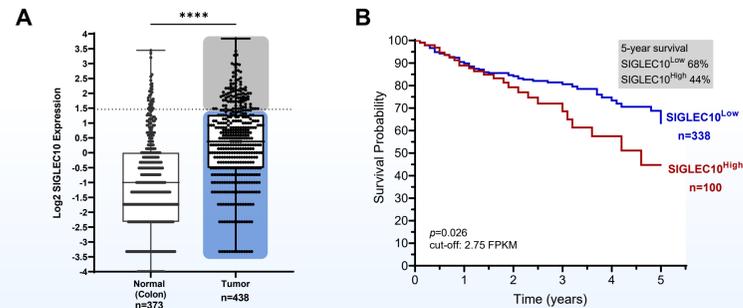
**A.** SIGLEC10 expression data from colon adenocarcinoma cohort (TCGA) was analyzed and compared to tissue-matched healthy individuals (TCGA and GTEX cohorts). Gray box: SIGLEC10 high, Blue box: SIGLEC10 low. , **** $P<0.0001$, unpaired, Mann-Whitney U test.
B. Tumor samples were separated in two cohorts based on level of SIGLEC10 with cut-off of 2.75 FPKM. Kaplan-Meier survival curves were plotted for both cohorts and 5-year survival were determined.

3. Siglec-10 is selectively expressed on myeloid cells and upregulated in human tumor samples
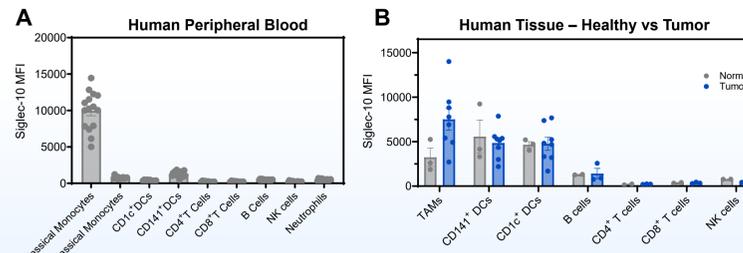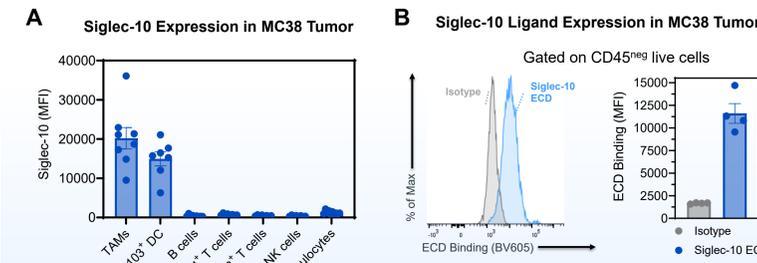

A. Immunophenotyping of Siglec-10 expression on human peripheral blood from healthy donors by flow cytometry.
B. Siglec-10 expression on immune cell subsets from dissociated human normal (gray) or tumor (blue) tissues.

4. Siglec-10 mAb enhances type-1 cytokine production in a model of TLR-mediated lung inflammation
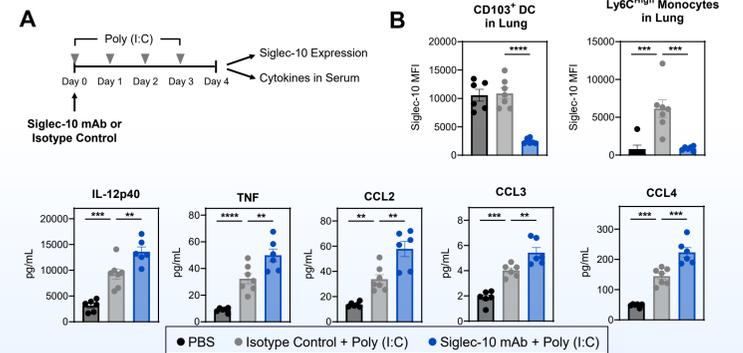

A. Schematic of experimental TLR-mediated lung inflammation model in Siglec-10 transgenic mice.
B. Siglec-10 expression levels on CD103+ DCs and Ly6C high MHC+ inflammatory monocytes in lung tissue from mice intranasally challenged with poly (I:C) and dosed with a Siglec-10 mAb (blue) or isotype control (gray) compared to PBS challenged mice (black). Cytokines and chemokines levels in serum of challenged mice (bottom). Data are plotted as means ± SEM. ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$ as determined by one-way ANOVA with Tukey's multiple comparisons.

5. Siglec-10 is selectively expressed on TAMs and DCs in MC38 colon adenocarcinoma model


A. Immune profiling of Siglec-10 expression in dissociated MC38 tumors from Siglec-10 transgenic mice by flow cytometry.
B. Representative histogram (left panel) and level (right panel) of Siglec-10 ECD binding to MC38 cells by flow cytometry in comparison to isotype control.

### 6. Siglec-10 mAb monotherapy reduces tumor progression
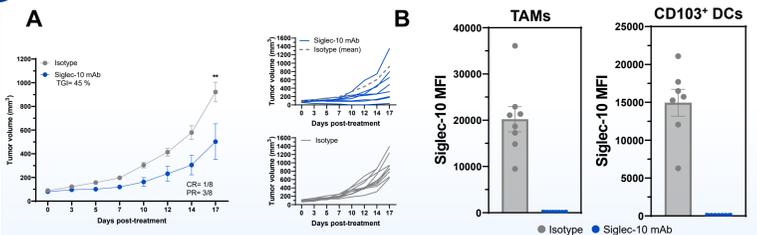
A. Siglec-10 transgenic mice were challenged with MC38 cells and tumor volume was monitored twice weekly. Once tumor was established, mice were treated with Siglec-10 mAb (blue), or isotype control (gray) every 2 days for a total of 8 doses.
B. Siglec-10 expression levels on TAMs and DCs in MC38 tumors after dosing with Siglec-10 mAb or isotype control. Data are plotted as mean +/- SD (n=8-10 mice/group). TAMs were gated on CD45+/CD11b+/F4/80+/MHC II+ and CD103+ DCs were gated on CD45+/CD11b+/CD11c+/CD103+ * $P<0.05$; *** $P<0.005$ as determined by two-way ANOVA with Sidak's multiple comparisons. CR, complete response; PR, partial response; TGI, tumor growth inhibition.

7. Siglec-10 mAb monotherapy activates innate and adaptive immune response in tumor
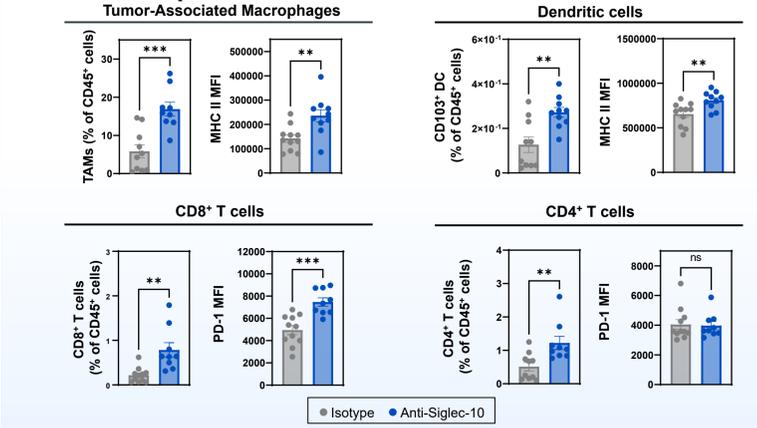

MC38 tumors were dissociated into single cells on Day 27 and flow cytometry was used to identify innate and adaptive immune cells. Mice treated with a Siglec-10 mAb (blue) displayed significantly increased percentages of TAMs, DCs, CD8+ and CD4+ T cells compared to mice dosed with an isotype control (gray). In addition, Siglec-10 mAb treatment induced activation of innate immune cells determined by MHCII expression and CD8+ T cells by PD-1 expression. ** $P<0.01$; *** $P<0.0004$ as determined by unpaired, two-tailed, t-test with Welch's test correction.

8. Siglec-10 mAb induces a global pro-inflammatory response in MC38 tumors
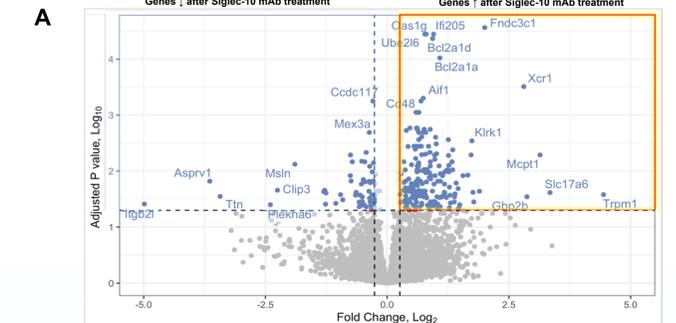
A. RNA-seq analysis of MC38 tumors from mice treated with a Siglec-10 mAb or isotype control. Volcano plot showing differentially expressed genes between anti-Siglec-10 and control group.
B. Correlative network showing interactions and clustering of cell types and inflammatory markers identified in the Siglec-10 mAb-treated group. Circle size indicates the fold change in gene expression and blue gradient indicates the p-value.

CONCLUSIONS/DISCUSSION
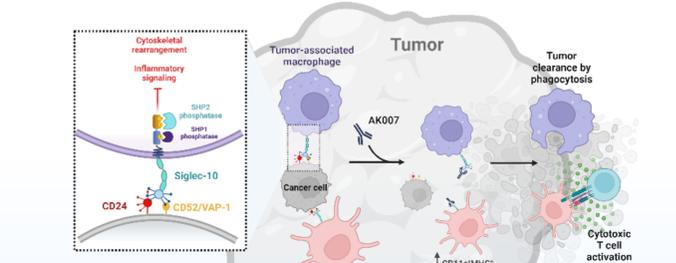
- Siglec-10 is highly expressed on tumor-associated myeloid cells and antibody blockade promotes anti-tumor immunity through activation of TAMs and dendritic cells
- Our findings highlight Siglec-10 as a promising myeloid cell target for enhancing anti-tumor immunity in solid tumors

References: Barkal et al., 2019. Nature. doi: 10.1038/s41586-019-1456-0; Yin SS, Gao FH. 2020. Front Immunol. doi: 10.3389/fimmu.2020.01324; Xiao et al., 2021. . Exp Hematol Oncol. doi: 10.1186/s40164-021-00230-5.