Characterization of Suppressive Myeloid Cells in Solid Tumors to Refine Disease Selection in a Phase 1 Study of The Multi-Siglec Inhibitor AL009

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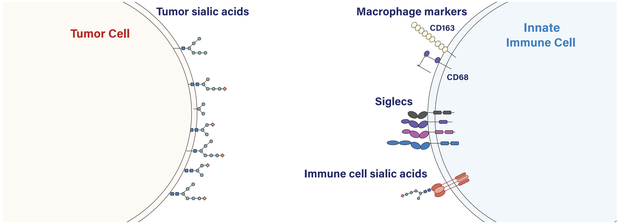
Background

- Inhibitory sialic acid–binding immunoglobulin-type lectins (Siglecs) are a family of cell surface receptors expressed predominantly on myeloid cells that recognize sialic acid glycans and potentiate immune suppression (**Figure 1**)[1,2]
- Tumors increase the expression of sialic acid glycans and co-opt the immunosuppressive effects of Siglecs, driving tumor resident immune cells toward a cancer-permissive phenotype (**Figure 1**)[1,3]
  - $CD68^+CD163^+$M2-like macrophages have also been implicated in cancer-induced immune suppression and CD68 and CD163 have been evaluated as biomarkers of therapeutic response[4]
- Disrupting Siglec-sialic acid signaling could confer a therapeutic benefit in cancer, particularly those cancers with high levels of myeloid-derived suppressor cells (MDSCs), which often display inhibitory Siglecs[5]
- AL009 acts as a sialic acid trap and a multi-Siglec inhibitor, repolarizing suppressive myeloid cells and activating an anti-cancer response
- Here we present data that help to refine disease selection for a phase 1 study of AL009 and identify potential predictive biomarkers for clinical use

Figure 1. Schematic Depicting The Synapse Between A Hypersialylated Tumor Cell And An Innate Immune Cell Displaying Immune Inhibitory Siglec Receptors



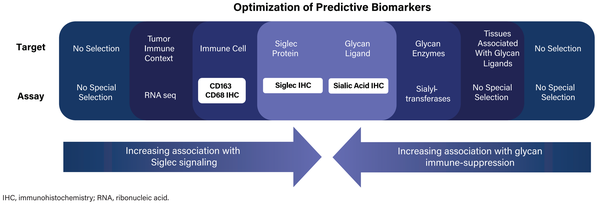
Methods

- Tissue microarrays (TriStar Technology Group, USA) with paired solid tumor samples from 432 patients with 33 tumor types were analyzed for CD163, CD68, and a representative Siglec for multi-Siglec inhibitors by immunohistochemistry (IHC)
  - IHC scoring methodology was prespecified, focusing on the proportion of cells expressing each of the above markers. Scoring was on a 4-point scale and based upon the number of cells stained with the marker of interest in reasonable proximity to the tumor (tissue without tumor was not scored)
    - IHC 0 was less than 10 stained cells per high power field, IHC 1 was 10-20 stained cells per high power field, IHC 2 was 20-100 stained cells per high power field, and IHC 3 was greater than 100 stained cells per high power field
  - Sequential thin sections of tissue microarrays were assessed for each of the antibody stains providing similar tumor specimen geography for comparative analyses
  - Immune cell sialic acid staining was estimated referencing parallel stained sections with CD163, CD68, or CD86 (CD86 staining data not shown)

Results

- Potential predictive biomarkers, such as IHC staining and analysis of CD163, CD68, Siglecs, and sialic acid residues, were selected based on their relevance to Siglec signaling and glycan immune suppression (**Figure 2**)

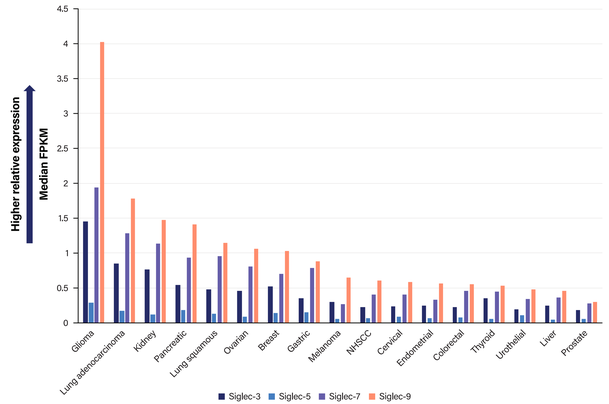
Figure 2. Diagram Of The Rationale For Identification of Potential Predictive Biomarkers



IHC, immunohistochemistry; RNA, ribonucleic acid.

- Siglec-9 has high relative expression and appears to consistently reflect proportionate expression for other inhibitory Siglecs (Siglecs -3, -5, and -7) across a variety of tumor types based on data from The Cancer Genome Atlas (TCGA) (**Figure 3**)

Figure 3. Relative Expression of Inhibitory Siglecs Across Tumor Types



FPKM, fragments per kilobase million.

- Siglec-9, sialic acid, CD163, and CD68 IHC staining was high in gastric cancer, lung squamous cell carcinoma, and ovarian cancer samples (**Figures 4-6**)

Figure 4. Representative IHC Staining for Sialic Acid, CD163, CD68, And Siglec-9 In The Same Gastric Cancer Sample

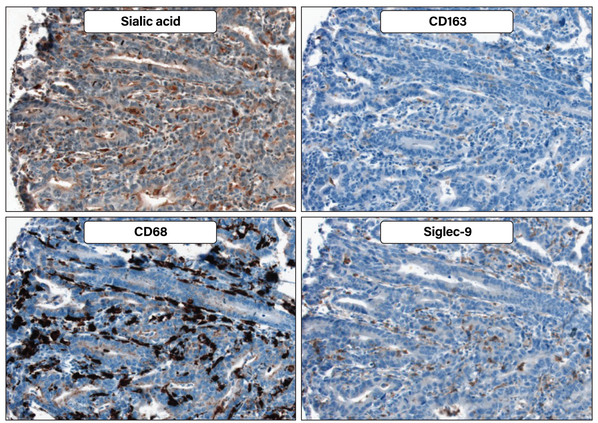


Figure 5. Representative IHC Staining for Sialic Acid, CD163, CD68, And Siglec-9 In The Same Lung Squamous Cell Carcinoma Sample

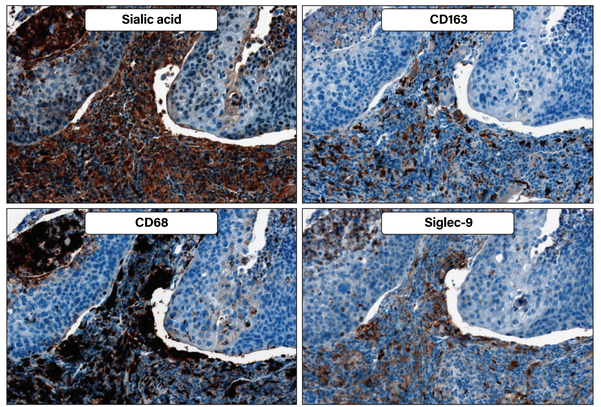
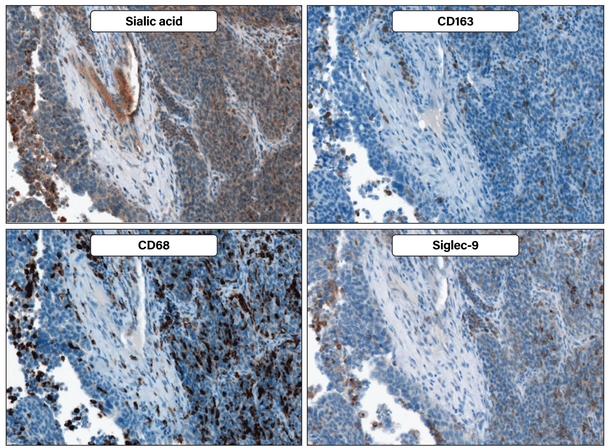
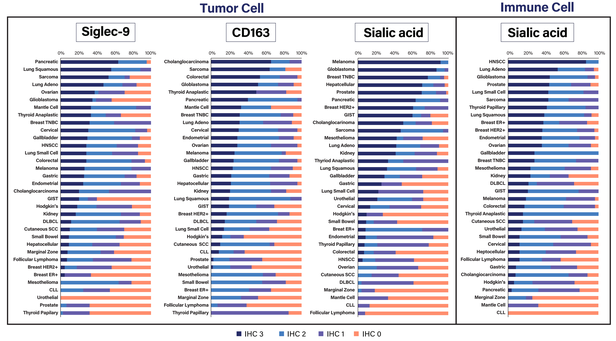


Figure 6. Representative IHC Staining for Sialic Acid, CD163, CD68, And Siglec-9 In The Same Ovarian Cancer Sample



- Tumor profiling by IHC identified gastric cancer, squamous cell lung cancer, and ovarian cancer as indications rich in MDSCs marked by high levels of CD163 and Siglec expression (**Figure 7**)

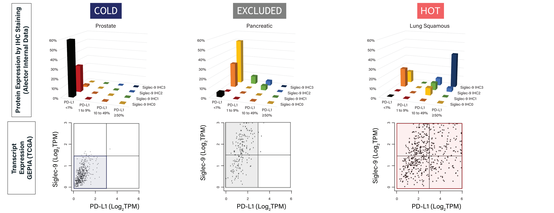
Figure 7. IHC Profiling In Tumor And Immune Cells For A Variety Of Tumor Types



CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; ER+, estrogen receptor positive; GIST, gastrointestinal stromal tumor; HER2+, human epidermal growth factor receptor 2 positive; HNSCC, head and neck squamous cell carcinoma; SCC, squamous cell carcinoma; TNBC, triple negative breast cancer.

- IHC staining and transcript expression from gene expression profiling interactive analysis (GEPIA) from TCGA both suggest that PD-L1 and inhibitory Siglecs each have unique expression patterns in some tumor microenvironments (cold and excluded) while they are both implicated in others (hot) (**Figure 8**)

Figure 8. PD-L1 And Siglec IHC Staining and TCGA Transcripts Across Different Tumor Immune States



GEPIA, gene expression profiling interactive analysis; IHC, immunohistochemistry; PD-L1 is programmed cell death ligand 1; TCGA, The Cancer Genome Atlas; TPM, transcripts per million.

Conclusions

- Inhibitory Siglecs generally share relative expression across the same tumor types, and Siglec-9 showed higher relative expression than most other inhibitory Siglecs, making it an ideal candidate to broadly represent inhibitory Siglec expression across a variety tumor types
- An IHC panel that marks CD163, CD68, and Siglecs was used to identify cancer indications rich in MDSCs
- As AL009's mechanism of action is to disrupt the Siglec-sialic acid signaling of MDSCs, we believe that patients with cancers high in CD163, CD68, and inhibitory Siglecs will be most likely to respond to AL009 treatment
  - This IHC panel will be utilized to retrospectively explore CD163, CD68, and Siglecs as predictive biomarkers in an upcoming phase 1 study
- The use of PD-L1 expression data from The Cancer Genome Atlas for various cancer indications provides further guidance on potentially effective combination therapies that include AL009

References

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