AL009, a Fusion Protein and Multi-Siglec Inhibitor, Repolarizes Suppressive Myeloid Cells and Potentiates Anticancer Effects

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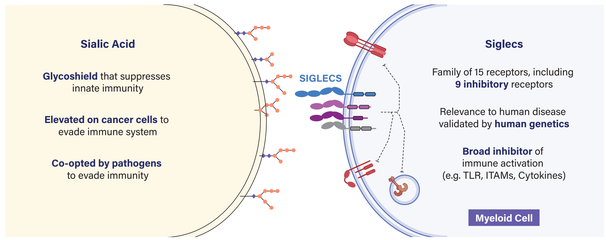
Background

- Siglecs are a family of cell surface receptors, expressed predominantly on myeloid cells, that often function to promote immune tolerance through interactions with sialic acid terminal residues in the glycocalyx (**Figure 1**)[1-3]
- Siglecs have an overlapping expression profile on myeloid cells, requiring targeting of multiple Siglecs for a robust effect[4]

Figure 1. Visualization of Sialic Acid-Siglec Synapse



- 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[5]
- AL009 is an engineered Siglec-9 extracellular domain Fc-fusion molecule that acts as a sialic acid trap and repolarizes suppressive myeloid cells to activate an anticancer immune response

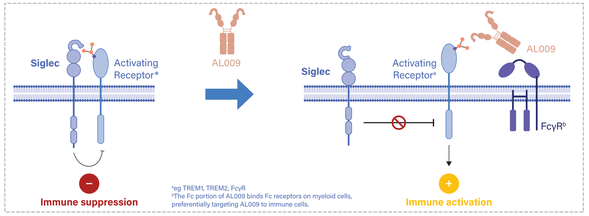
Methods

- MDSCs were generated from healthy human donors. CD14+ monocytes were isolated from human blood and differentiated for 7 days in RPMI media containing 10 ng/mL hGM-CSF and 10 ng/mL hIL-6
- Induction of CCL4 by Siglec-Fc fusion proteins was measured after 48 hours of treatment
- For measurement of CD86, CD163, and TNFα in the presence of AL009, MDSCs were treated with AL009 for 48 hours, followed by analysis of surface markers by flow cytometry and quantification of TNFα in the supernatant using the LEGENDplex™ immunoassay
- T-cell function in the presence of MDSCs with various treatments was assessed by treating MDSCs for 48 hours with the indicated treatments, followed by coculture with autologous CD8+ T cells in the presence of Dynabeads® Human T-Activator CD3/CD28. Cells were cultured for an additional 4 days, followed by quantification of IFNγ in the culture supernatant by LEGENDplex™
- E0771, a breast cancer syngeneic model enriched in monocytic MDSCs, and MC38, a colon cancer model, were used to measure AL009m (AL009 with a mouse IgG Fc) activity in solid tumor models. C57BL/6 mice expressing human Siglec-3, 7, and 9 (hSiglec Tg mice) were used in all tumor model experiments. All treatments were dosed twice weekly via intraperitoneal injection
- For the B16F10 melanoma metastasis model, hSiglec Tg mice were treated once with 27 µg anti-TRP1-mIgG2a and twice weekly with 10 mg/kg AL009m starting 1 day after intravenous B16F10 injection
- To measure pharmacodynamic markers in mice with AL009 treatment, huNOG-EXL mice (engineered to express hGM-CSF and hIL-3) were engrafted with human CD34+ hematopoietic stem cells and inoculated subcutaneously with A375 melanoma cells. When tumors reached an average volume of ~400 mm^3, mice received intraperitoneal injections of 10 mg/kg isotype control or AL009 on days 16 and 19, followed by sacrifice and analysis by flow cytometry on day 20. For the syngeneic tumor study, hSiglec Tg mice were implanted with E0771 cells, and treated with AL009m on days 11, 14, and 18, followed by sacrifice and analysis by flow cytometry on day 19

Results

- AL009 blocks Siglec-mediated cis inhibition of activating receptors on monocytes and macrophages (**Figure 2**)

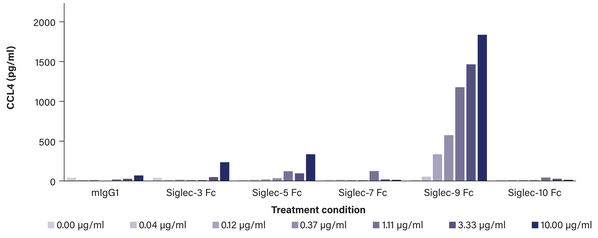
Figure 2. Schematic of AL009 Mechanism of Action



Monocyte/macrophage

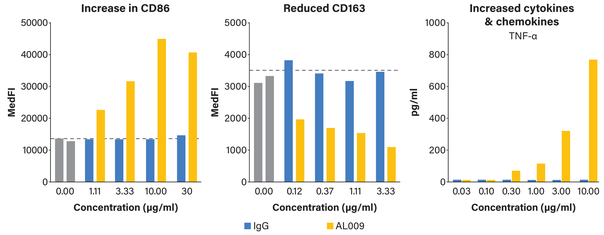
- Of the multiple Siglec-Fc protein fusions tested, Siglec-9 Fc was the most potent immune activator in an assay measuring induction of the proinflammatory chemokine CCL4 (**Figure 3**)
- In a glycan array, AL009 had broader sialic acid binding than other Siglec-Fc fusion proteins tested (data not shown)
  - Truncation of Siglec-9 reduced binding
  - Based on the array tested, Siglec-9 Fc has the potential to block all Siglec-9, Siglec-15, and Siglec-10 ligands, as well as the majority of Siglec-3 and Siglec-7 ligands, and many Siglec-5 ligands
- AL009 blocks multiple Siglec receptors, including 3, 5, 7, 9, and 10, on myeloid cells (data not shown)

Figure 3. Induction of CCL4 by a Panel of Siglec Receptor–Fc Fusion Proteins



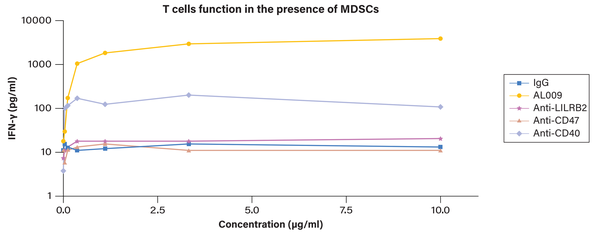
- Treatment with AL009 led to a dose-dependent increase in CD86, a decrease in CD163, and an increase in TNFα, consistent with a repolarization of the MDSCs toward a proinflammatory phenotype (**Figure 4**)
  - A similar upregulation in IL-6, IL-10, CCL3, CCL4, RANTES, CXCL1, and CXCL9 was observed (data not shown)

Figure 4. Effect of AL009 on Repolarizing Suppressive M2 Macrophages to Activating M1



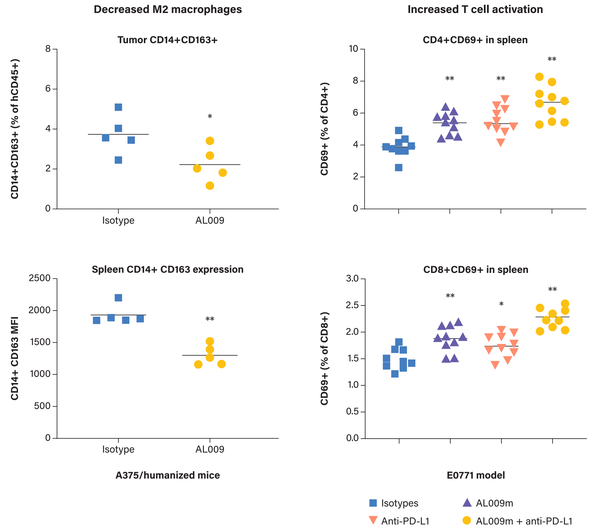
- When compared with other anticancer immunotherapies, AL009 showed greater potency in blocking MDSC suppression of T cells across a range of concentrations (**Figure 5**)

Figure 5. AL009 Prevents MDSC Suppression of T Cells



- Pharmacodynamic markers show enhanced immune activation with AL009m treatment in mouse models (**Figure 6**)
  - Suppressive M2 macrophages in tumor cells and the spleen were decreased
  - T-cell activation was increased, particularly with AL009m/anti-PD-L1 combination treatment

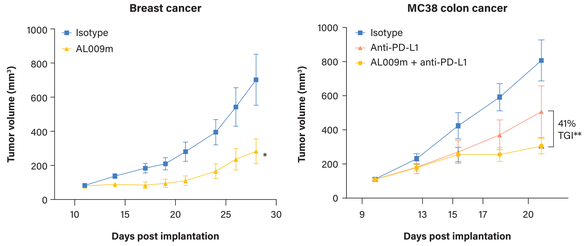
Figure 6. Pharmacodynamic Markers Show Enhanced Immune Activation With AL009, Anti–PD-L1, and Combination Treatment



*Indicates p-value<0.05
**Indicates p-value<0.01

- In vivo mouse models showed AL009 efficacy in reducing tumor growth (**Figure 7**)
  - Breast cancer tumor volume in AL009-treated mice was reduced compared with controls
  - Combination therapy of anti-PD-L1 and AL009 showed significantly greater inhibition of tumor growth than anti-PD-L1 alone in colon cancer and breast cancer tumors (**Figure 7**)

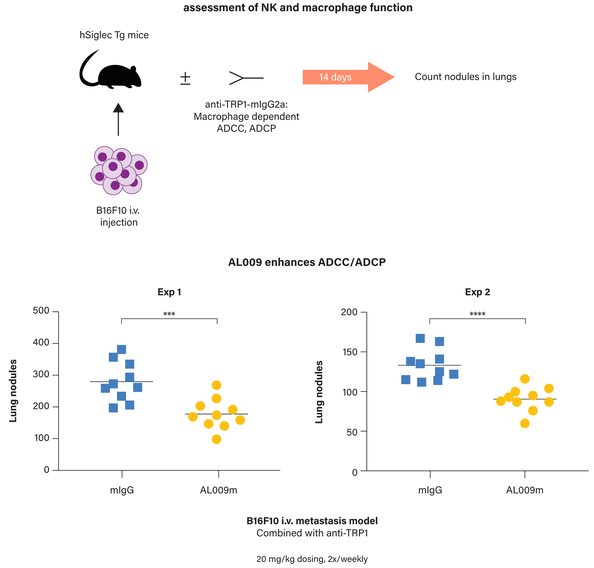
Figure 7. AL009 as Monotherapy and in Combination With Anti–PD-L1 in Breast Cancer and Colon Cancer Mouse Models



*Indicates p-value<0.05
**Indicates p-value<0.01
TGI, tumor growth inhibition.

- Combination therapy with anti-TRP1 led to a reduced number of lung nodules in a mouse metastasis model (**Figure 8**)

Figure 8. AL009 Synergizes With Targeted Anti-TRP1 Therapy to Reduce Lung Metastasis



***Indicates p-value<0.001
****Indicates p-value<0.0001

Conclusions

- AL009 targets and repolarizes myeloid cells without cell depletion
- Potent single-agent anti-tumor effects are observed in multiple models
- AL009 has potential efficacy in tumors that are unresponsive or refractory to standard immunotherapies
- Combination experiments with AL009 and anti-PD-L1 showed a greater effect than anti-PD-L1 alone
- Results from AL009 in vitro and in vivo animal data support further exploration of this novel therapeutic

Abbreviations

ADCC, antibody-dependent cellular cytotoxicity; **ADCP,** antibody-dependent cellular phagocytosis; **CCL,** chemokine C-C motif ligand; **CD,** cluster of differentiation; **CXCL,** chemokine C-X-C motif ligand; **Fc,** crystallizable fragment; **GM-CSF,** granulocyte macrophage colony-stimulating factor; **h,** human; **IFN,** interferon; **IgG,** immunoglobulin G; **IL,** interleukin; **MDSC,** myeloid-derived suppressor cell; **PD-L1,** programmed death-ligand 1; **RANTES,** regulated upon activation, normal T cell expressed and presumably secreted; **RPMI,** Roswell Park Memorial Institute; **Siglec,** sialic acid-binding immunoglobulin-type lectin; **Tg,** transgenic; **TNF,** tumor necrosis factor; **TRP1,** tyrosinase-related protein 1.

References

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Acknowledgments

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