**Preclinical development of first-in-class antibodies targeting Siglec-9 immune checkpoint for cancer immunotherapy**

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**Abstract**

1. Discovery of first-in-class anti-Siglec-9 blocking antibodies

We discovered high affinity anti-Siglec-9 antibodies that block the interaction between Siglec-9 and its ligands. These antibodies potently enhance NK cell cytotoxicity by blocking interactions with sialic acid expressed on tumor target cells. We also show that anti-Siglec-9 antibodies improve anti-tumor response induced by the blockade of the immune checkpoint NKG2A. Using flow cytometry analyses, we show that Siglec-9 is expressed on several immune cell types including lymphocytes and myeloid cells pointing to potential multiple modes of action. Removal of sialic acid on monocyte-derived dendritic cells unblocks Siglec-9 suggesting interactions with self-sialic acids. Finally, we show that Siglec-9 expression is maintained on tumor-infiltrating immune cells using immunohistochemistry (IHC) and that Siglec-9 is upregulated on circulating T cells in cancer patients suggesting a potential role on adaptive immunity.

**Proposed Mode of Action**

1. **Siglec-9 is highly expressed on myeloid cells and upregulated on circulating T cells in cancer patients**

A. Siglec-9 is highly expressed on circulating myeloid cells

B. Tumor-infiltrating immune cells express Siglec-9

C. Siglec-9 interacts with self-sialic acids on moDCs

D. Siglec-9 is upregulated on circulating T cells in cancer patients

**Conclusion**

- New first-in-class anti-Siglec-9 antibodies block the interaction of Siglec-9 with its sialic acid ligands.
- Siglec-9 is an inhibitory receptor and its blockade enhances NK cell cytotoxicity.
- Siglec-9 blockade synergizes with other immune checkpoints blockade (e.g. NKG2A blockade)
- Large expression on multiple immune cell types including myeloid, NK and T cells in cancer patients points to potential multiple modes of actions.
- Taken together, these data support the development of anti-Siglec-9 blocking antibodies for cancer immunotherapy.