

Preclinical development of first-in-class antibodies targeting Siglec-9 immune checkpoint

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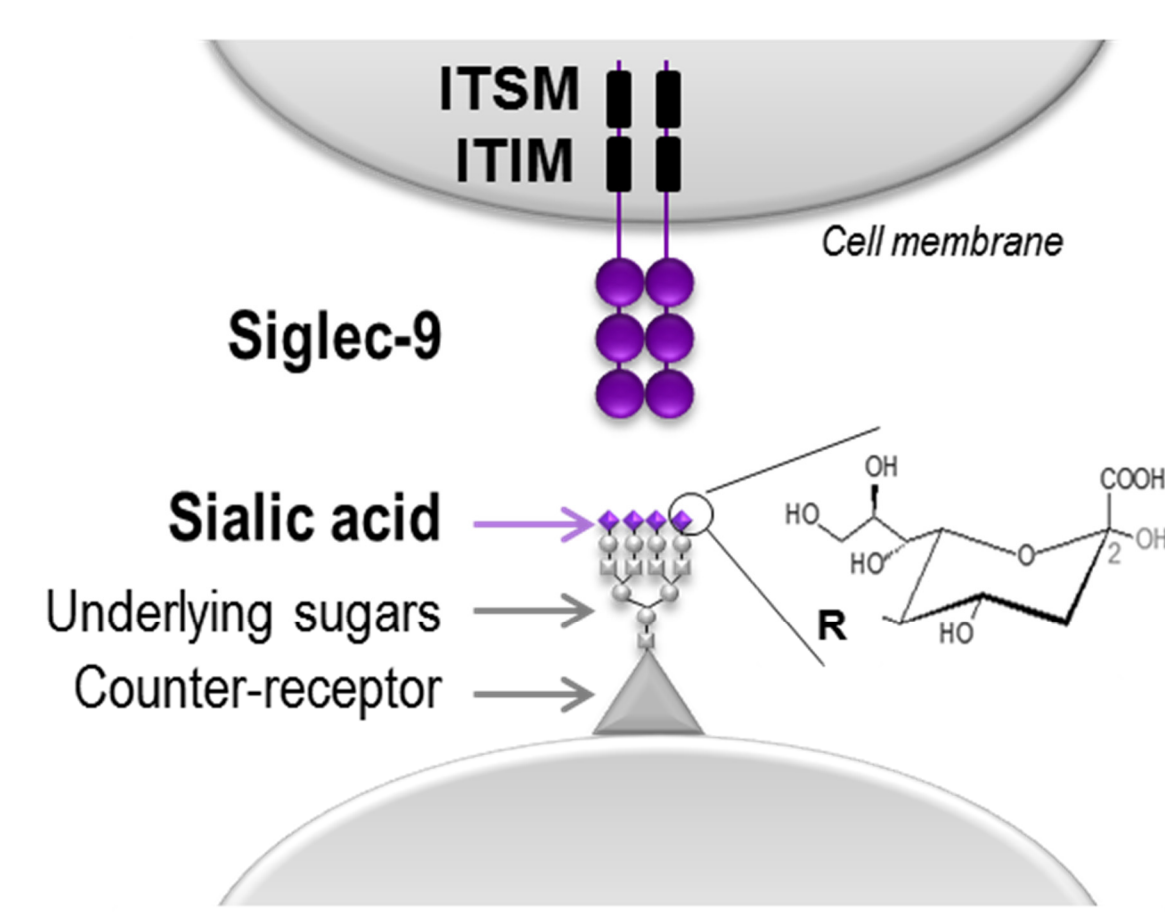
for cancer immunotherapy

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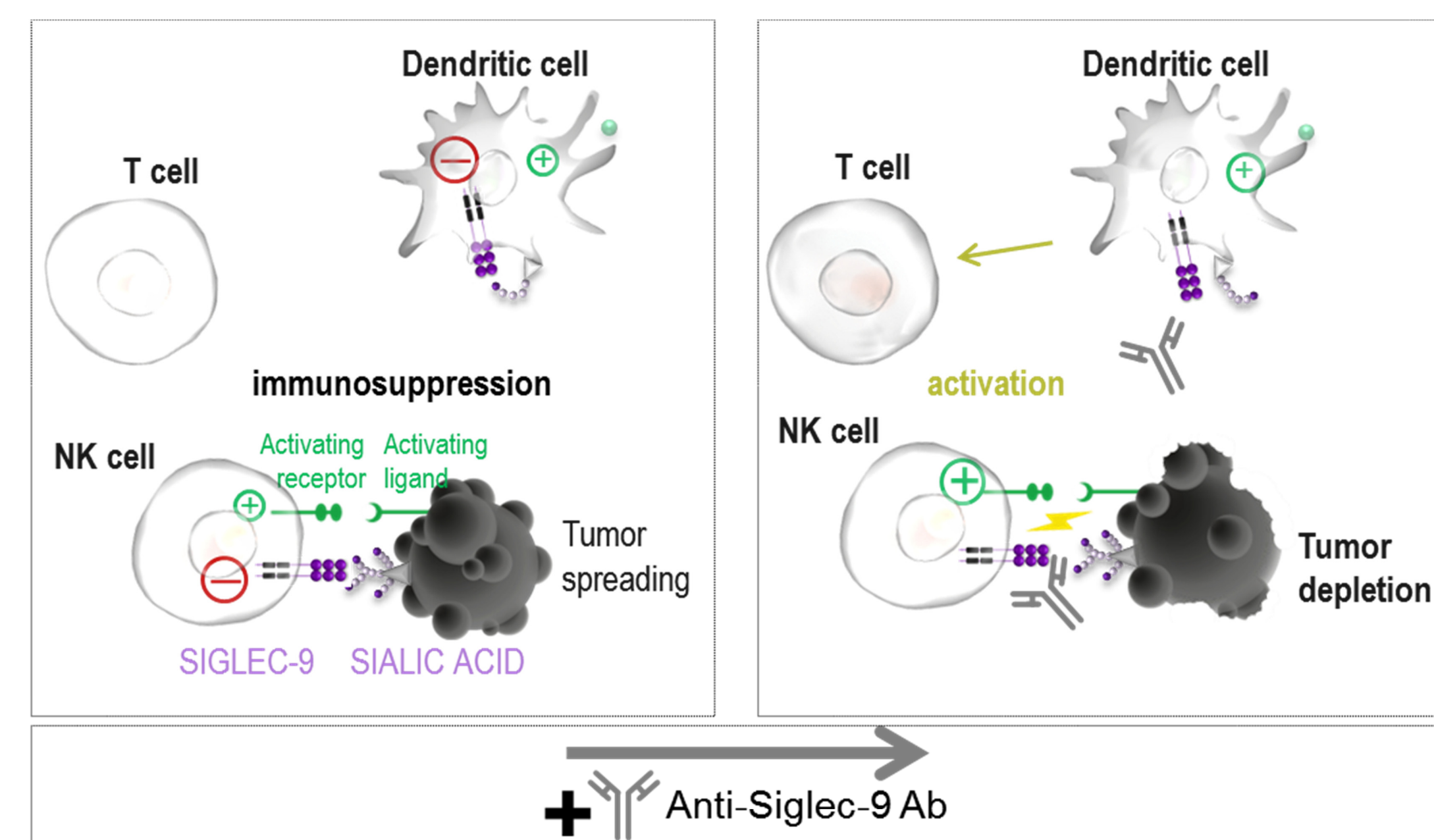
Abstract

Siglecs comprise a family of 15 members of sialic acid-binding receptors that vary in their expression on immune cells and their specificity for sialic acid-containing ligands. Siglec-9 is an inhibitory receptor expressed on NK and myeloid cells (including dendritic cells, monocytes and neutrophils) and binds preferentially to α 2-3-linked sialic acids. Sialic acids are nine-carbon sugars, usually in the terminal positions of sugar chains. They are involved in tumor cell malignancy and are reported as a mechanism of escape from immune surveillance. Siglec-9 can interact with tumor- and self-sialic acids and dampen immune cell functions. Thus, Siglec-9-sialic acid interaction disruption may promote anti-tumor immunity.



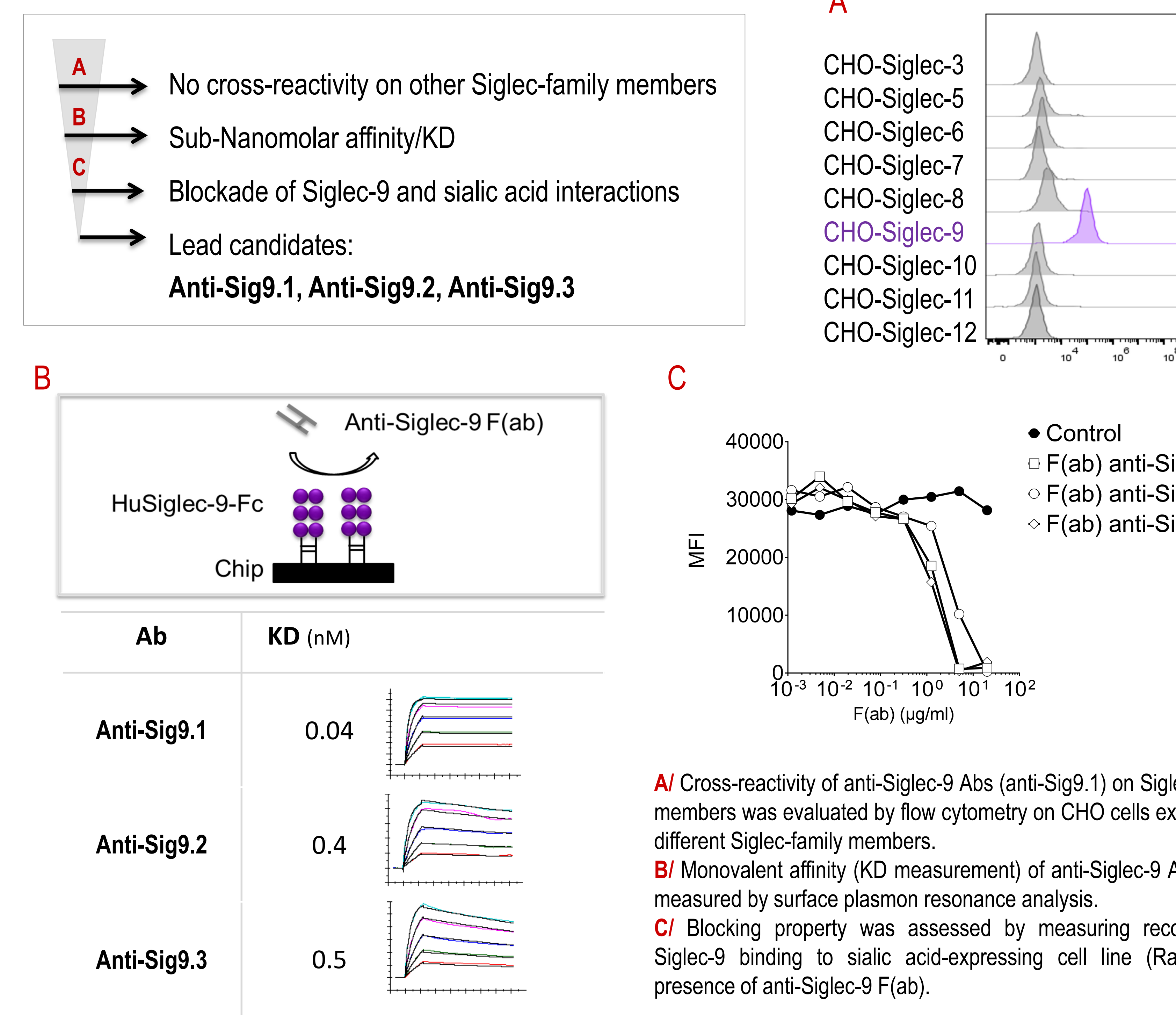
We discovered high affinity anti-Siglec-9 antibodies that block the interaction between Siglec-9 and its ligands. These antibodies potentially enhance NK cell cytotoxicity in vitro by blocking interactions with sialic acid expressed on tumor target cells. We also show that anti-Siglec-9 antibodies improve 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 uncovers Siglec-9 suggesting interactions with self-sialic acids. Finally, we show that Siglec-9 expression is maintained on tumor-infiltrated immune cells using immunohistochemistry (IHC) and that Siglec-9 is upregulated on circulating T cells in cancer patients suggesting a putative role on adaptive immunity.

Proposed Mode of Action

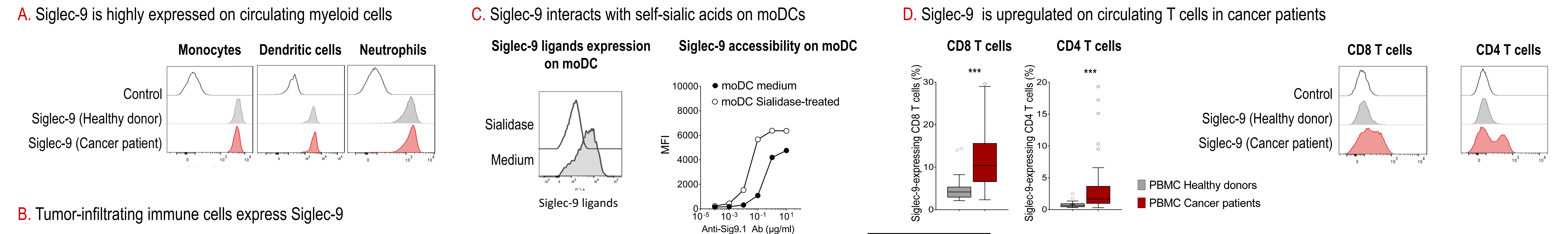


Taken together, these data support the development of anti-Siglec-9 blocking antibodies for cancer immunotherapy, potentially in combination with other immune checkpoint inhibitors. The antibodies displaying the most interesting features were successfully humanized and are currently in preclinical development.

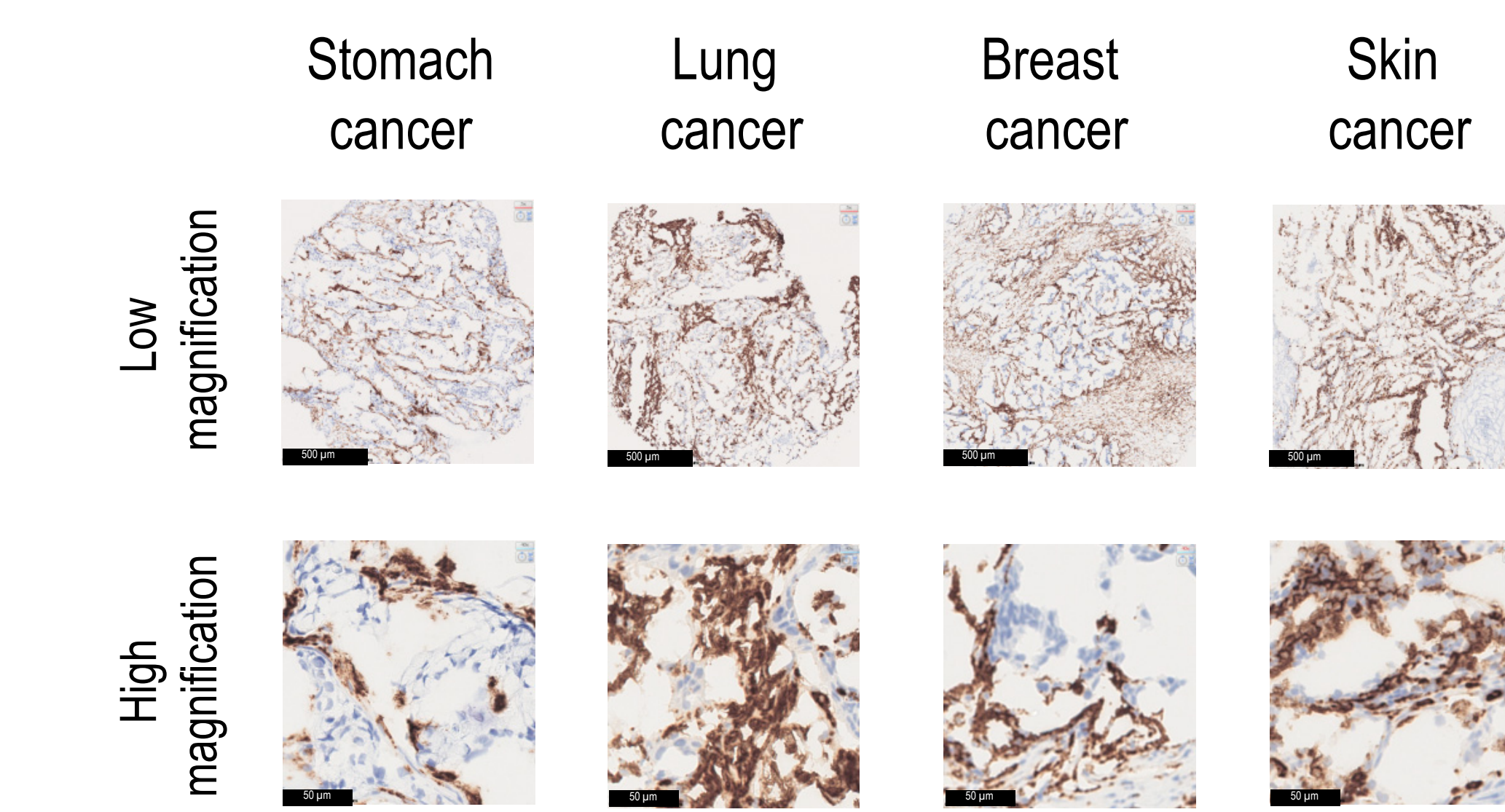
1. Discovery of first-in-class anti-Siglec-9 blocking antibodies



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

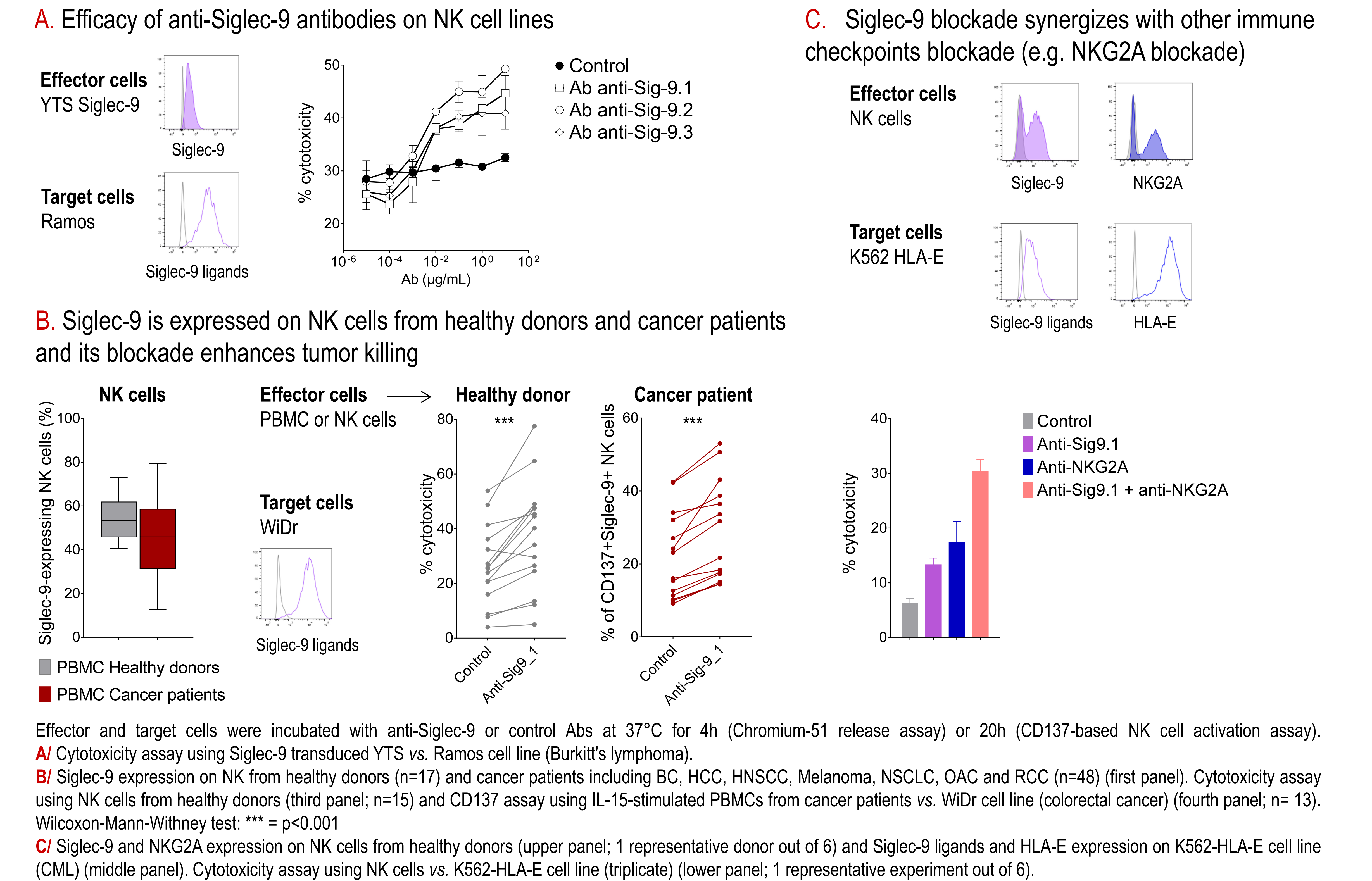


B. Tumor-infiltrating immune cells express Siglec-9



A/ Representative example of Siglec-9 expression on monocytes, myeloid dendritic cells and neutrophils from healthy donors and cancer patients (e.g. Breast cancer). B/ Representative example of cancer infiltrated by immune cells and having high Siglec-9 expression. Black scale bars correspond to 500 μ m (upper panel) or 50 μ m (lower panel). C/ Monocyte-derived dendritic cells (moDCs) were treated or not with Sialidase (from Vibrio Cholerae). Siglec-9-ligands and Siglec-9 expression was evaluated with recombinant Siglec-9-Fc protein and anti-Siglec 9 Ab, respectively. D/ Siglec-9 expression on CD8 and CD4 T cells from healthy donors (n=17) and cancer patients including BC, HCC, HNSCC, Melanoma, NSCLC, OAC and RCC (n=48). Box and Whiskers (Tukey). Wilcoxon-Mann-Whitney test: *** = p<0.001 (Left panel). Representative example of Siglec-9 expression on CD8 and CD4 T cells from healthy donors and cancer patients (e.g. Breast cancer) (right panel).

2. New immune checkpoint inhibitor: proof of concept on NK cells



Effector and target cells were incubated with anti-Siglec-9 or control Abs at 37°C for 4h (Chromium-51 release assay) or 20h (CD137-based NK cell activation assay). A/ Cytotoxicity assay using Siglec-9 transduced YTS vs. Ramos cell line (Burkitt's lymphoma). B/ Siglec-9 expression on NK from healthy donors (n=17) and cancer patients including BC, HCC, HNSCC, Melanoma, NSCLC, OAC and RCC (n=48) (first panel). Cytotoxicity assay using NK cells from healthy donors (third panel; n=15) and CD137 assay using IL-15-stimulated PBMCs from cancer patients vs. WiDr cell line (colorectal cancer) (fourth panel; n=13). Wilcoxon-Mann-Whitney test: *** = p<0.001 C/ Siglec-9 and NKG2A expression on NK cells from healthy donors (upper panel; 1 representative donor out of 6) and Siglec-9 ligands and HLA-E expression on K562-HLA-E cell line (CML) (middle panel). Cytotoxicity assay using NK cells vs. K562-HLA-E cell line (triplicate) (lower panel; 1 representative experiment out of 6).

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.

