Preclinical characterization of humanized anti-Siglec-15 antibodies that rescue T cells from macrophage-mediated immune suppression

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Abstract #262

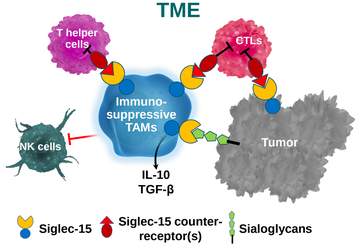
Background: Siglec-15 is an immunosuppressive sialic acid-binding Ig-like lectin expressed by myeloid cells, tumor associated macrophages (TAMs), and some human tumors. Interactions between Siglec-15 on TAMs and sialoglycans on cancer cells contribute to the immunosuppressive tumor microenvironment. Furthermore, Siglec-15 expressed by TAMs inhibits anti-tumor immune responses by engaging unknown immune checkpoint(s) on T cells. Notably, the mutually exclusive expression of Siglec-15 and the checkpoint ligand PD-L1 in the tumor tissue emphasizes Siglec-15 as an attractive target for combination immunotherapy. An anti-Siglec-15 antibody is currently being evaluated in clinical trials for the treatment of cancer.

Methods: Anti-Siglec-15 antibodies were cloned from B cells derived from rabbits immunized with human Siglec-15 protein. The antibodies were evaluated for binding to human and cynomolgus Siglec-15 by enzyme-linked immunosorbent assay (ELISA). Top clones were selected based on activity in functional and phenotypic assays using primary human macrophages and T cells and were subsequently fully humanized. The humanized anti-Siglec-15 IgG1 antibodies were screened for binding to human and cynomolgus Siglec-15 by ELISA, binding to cells expressing Siglec-15, and ability to rescue T cell functional activity (proliferation and IFN-γ) from M2c-mediated immune suppression in vitro. The pharmacokinetics of lead humanized Siglec-15 antibodies and their anti-tumor activity were evaluated in humanized mouse models.

Results: We have identified a panel of fully humanized anti-Siglec-15 antibodies that bind to recombinant human and cynomolgus Siglec-15 proteins, to Siglec-15-expressing cell lines and immunosuppressive M2c macrophages without appreciable binding to other Siglec family members. Lead antibodies were identified using functional screens modeling Siglec-15-mediated immune suppression by M2c macrophages. These antibodies restored T cell immune responses in two different M2c/CD8 T cell coculture assays. In the first model, lead antibodies rescued the proliferative and IFN-γ responses of anti-CD3-activated human T cells from the inhibitory activity of M2c macrophages. In the second model, these antibodies restored the ability of exhausted CD8 T cells to secrete IFN-γ in the presence of M2c macrophages. Lead antibodies demonstrated a half-life of 6-11 days in humanized FcRn mice, and tumor growth inhibition in humanized NSG-SGM3 mice.

Conclusions: We have identified novel humanized anti-Siglec-15 antibodies that restore effector function of activated and exhausted T cells from M2c-mediated immune suppression, with excellent half-life and anti-tumor activity in humanized mouse models. These data provide a strong rationale for further development of these antibodies for anti-cancer immunotherapy.

1 Siglec-15 is a target for cancer Immunotherapy in the TME



- TAMs contribute to tumor immune evasion by suppressing anti-tumor immune responses and by promoting a pro-tumorigenic milieu.
- Siglec-15 expressed by TAMs inhibits anti-tumor immune responses by engaging unknown immune checkpoints on T cells.
- Tumor-expressed sialoglycans may bind to Siglec-15 on TAMs and induce the secretion of immunosuppressive cytokines.

RESULTS

2 Rabbit/human chimeric anti-Siglec-15 antibodies relieve Siglec-15-mediated immune suppression of human T cells

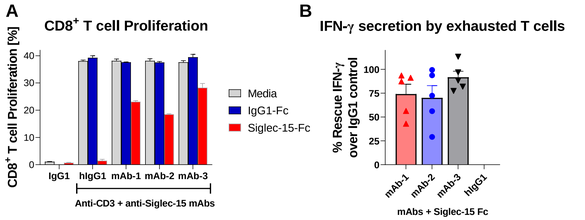


Figure 2. Relief of Siglec-15-Fc-mediated suppression. (A) PBMC-derived CD8+ T cell proliferation, (B) Rescue of exhausted T cell IFN-γ response.

3 Humanized variants of mAb-1 and mAb-2 bind to human and cynomolgus Siglec-15 by ELISA

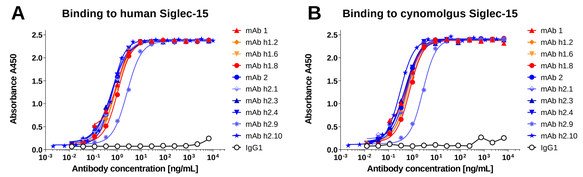


Figure 3. Top antibody clones were selected based on binding to (A) human and (B) cynomolgus Siglec-15-His.

4 Humanized Siglec-15 antibodies bind to cell-expressed Siglec-15

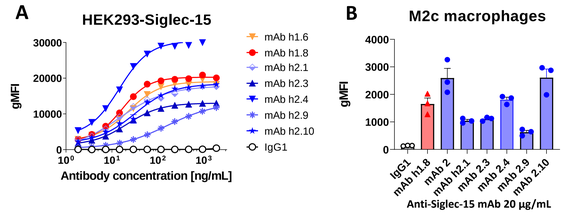


Figure 4. Humanized antibodies bind to Siglec-15 expressed by (A) HEK-293 cells and (B) human M2c macrophages.

5 Humanized anti-Siglec-15 mAbs rescue CD8+ T cells from M2c macrophage mediated immune suppression

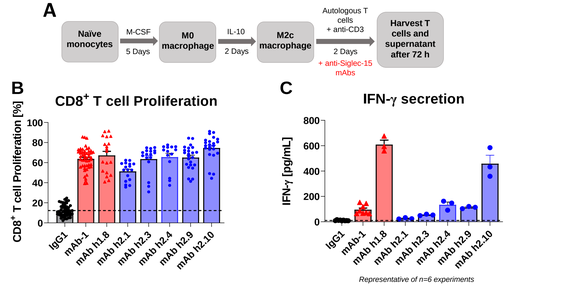


Figure 5 A) Schematic of M2c/T cell coculture assay. B) Treatment with anti-Siglec-15 antibodies restores human CD8+ T cell proliferation and C) enhances IFN-γ secretion.

6 Anti-Siglec-15 mAbs restore the IFN-γ response of exhausted human T cells from in the presence of M2c macrophages

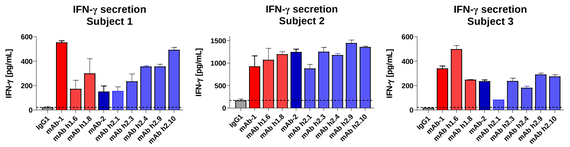
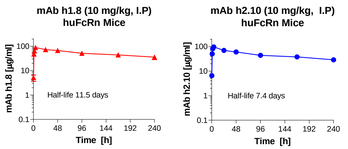


Figure 6. Anti-Siglec-15 mAbs (5 µg/mL) relieve M2c macrophage-mediated immune suppression and enhance IFN-γ secretion by exhausted human T cells.

7 Anti-Siglec-15 mAbs have a typical IgG1 PK profile in humanized FcRn mice



Parameter	Unit	mAb h1.8	mAb h2.10
Dose	mg/kg	10	10
$t_{1/2}$	days	11.5	7.4
T_{max}	h	4	4
C_{max}	µg/mL	89	97
AUC (0-t)	µg/mL*h	12692	11348
AUC (0-inf)	µg/mL*h	26942	18757

Mice were dosed with 10 mg/kg (i.p.), N = 4 per group, Blood draws at 0.25, 1, 2, 4, 24, 48, 96, 168 and 240 hr.

Figure 7. PK profile of humanized anti-Siglec-15 antibodies in humanized FcRn mice.

8 SK-MEL-5 tumor model in humanized NSG-SGM-3 mice

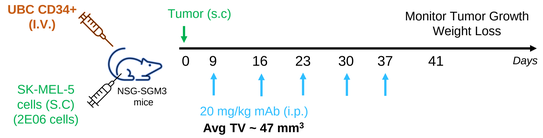
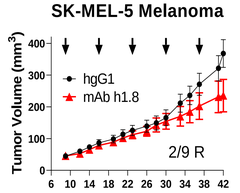


Figure 7. Humanized NSG-SGM3 mice were injected with 2 x 10^6 SK-MEL-5 cells. Tumor growth proceeded for 9 days prior to initiating antibody treatment.

9 Clone mAb h1.8 inhibits tumor growth in the humanized NSG-SGM3 mouse model



Group	Tumor Growth Inhibition (%) d37	d42	Regression (%) d42
IgG1 control	NA	NA	0
mAb h1.8	37	41	22

N= 9 per group; Tumor subcutaneous in humanized mice.
Dosing: 20 mg/kg i.p.
Dosing Days: 9, 16, 23, 30, 37, Average tumor volumes ~ 47 mm3 at first dose

Figure 8. Anti-Siglec-15 antibodies demonstrate anti-tumor activity in SK-MEL-5 tumor model in humanized NSG-SGM3 mice.

Summary

- The anti-Siglec-15 antibodies block the interaction of Siglec-15-Fc with an immune suppressive checkpoint on T cells and restore T cell effector function.
- The humanized anti-Siglec-15 mAbs:
  - Relieve CD8+ T cells from M2c macrophage-mediated immune suppression
  - Restore the IFN-γ response of exhausted T cells
  - Have a typical human IgG1 PK profile in humanized FcRn mice
- Select anti-Siglec-15 mAbs inhibit tumor growth in the SK-MEL-5 tumor model in humanized NSG-SGM3 mice.
- These results support further development of lead anti-Siglec-15 antibodies for cancer immunotherapy

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