Discovery and preclinical characterization of anti-Siglec-15 antibodies that rescue T cells from macrophage-mediated immune suppression

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Abstract # 1638

Background: Siglec-15 is an immunosuppressive sialic acid-binding Ig-like lectin expressed by myeloid cells, tumor associated macrophages (TAMs) and some human tumors. Siglec-15 expressed by TAMs inhibits anti-tumor immune responses by engaging unknown immune checkpoint(s) on T cells. Interactions between Siglec-15 on TAMs and sialy Tn (sTn) antigen found on tumor cells contributes to the immunosuppressive tumor microenvironment. Notably, the mutually exclusive expression of Siglec-15 and the checkpoint ligand PD-L1 by cancer cells emphasizes Siglec-15 as an attractive target for cancer immunotherapy

Methods: Anti-Siglec-15 antibodies were cloned from B cells derived from rabbits immunized with human Siglec-15. Cells were cultured at clonal density, and IgG antibodies in supernatants were evaluated for binding to human, murine and cynomolgus Siglec-15. Variable-regions from positive hits were sequenced, cloned, and expressed as recombinant rabbit human IgG1 Fc chimeras. Anti-Siglec-15 chimeric antibodies were evaluated in a panel of functional and phenotypic assays using primary human macrophages and T cells, and then prioritized for evaluation in murine tumor models.

Results: Thirty-one rabbit anti-Siglec-15 clones were expressed as rabbit-human IgG1 chimeras based on binding to recombinant human, cynomolgus and murine Siglec-15 proteins, binding to Siglec-15 expressing cell lines, and lack of binding to other Siglec family members. A subset of these clones inhibited the binding of Siglec-15 to sTn. The top 5 clones were identified in functional screens modeling Siglec-15 mediated immune suppression. The clones rescued the NFAT promotor activity of a T cell reporter cell line as well as the proliferative and IFN-γ response of anti-CD3 activated human T cells from the inhibitory activity of recombinant Siglec-15-Fc protein. Selected clones also relieved M2c-macrophage-mediated immune suppression in M2c/T cell coculture assays by restoring T-cell proliferation and IFN-γ secretion. The prioritized clones are currently under evaluation in in vivo tumor models for PK and efficacy.

Conclusions: We identified novel anti-Siglec-15 antibodies that restore T cell effector function following suppression by recombinant and cell-expressed Siglec-15. These data support further development of these anti-Siglec 15 antibodies as an anti-cancer immunotherapy.

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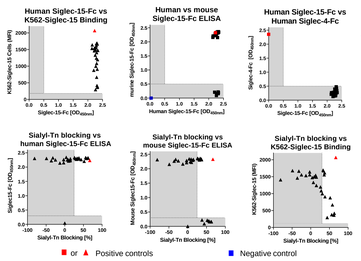
Selection of Top Anti-Siglec-15 Antibody Clones

Specificity of binding to Siglec-15, and blocking of sialyl-Tn binding to Siglec-15 led to the selection of top clones



Top Abs clones were selected based on:

- Binding to soluble Siglec-15-Fc ELISA (human, murine, cynomolgus)
- Binding to K562 cells expressing human Siglec-15
- Lack of binding to human Siglec-3, -4, -7, -8, -9 and -11
- Blocking of sTn binding to Siglec-15

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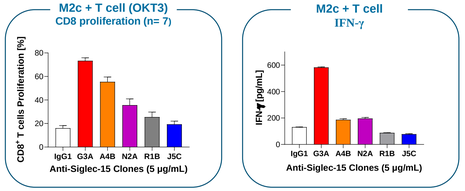
Anti-Siglec-15 Antibodies Relieve M2c Macrophage Mediated Immune Suppression in Coculture Assay with Human CD8+ T cells

Anti-Siglec-15 antibodies restore CD8+ T cell proliferation and IFN-γ secretion



Representative of n=3 experiments

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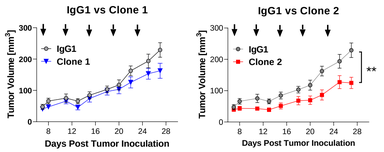
Anti-Siglec-15 Clones Inhibit Tumor Growth In Humanized Mice

Clone 2 inhibits human SK-MEL-5 melanoma tumor growth in humanized NSG-SGM3 mice



Dosing with 10 mg/kg i.p. at 7, 11, 15, 19, 23

- Anti-Siglec-15 clone 2 achieved 53% TGI
- Clone 1 showed 33% TGI

% Tumor Growth Inhibition

mAb	Day 20	Day 22	Day 25	Day 27
Clone 1	10%	26%	23%	33%
Clone 2	57%	59%	40%	53%

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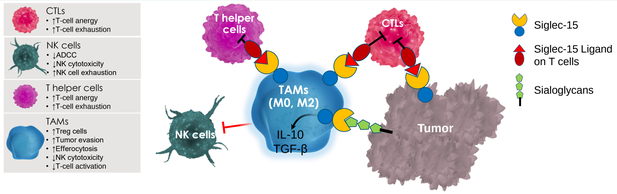
Siglec-15 is a Target for Cancer Immunotherapy in the TME



- M2 TAMs contribute to tumor immune evasion by suppressing anti-tumor immune responses and by promoting a tumorigenic milieu. Targeting immunosuppressive myeloid cells and their inhibitory modalities is an attractive strategy to enhance clinical responses to CPI therapy.
- Siglec-15 expressed by TAMs inhibits anti-tumor immune responses by engaging unknown immune checkpoint(s) on T cells.
- Tumor expressed sialoglycans may bind to Siglec-15 on TAMs and induce the secretion of immunosuppressive cytokines.
- We identified novel anti-Siglec-15 antibodies that restore T cell effector function following suppression by recombinant and cell-expressed Siglec-15, supporting further development of these anti-Siglec 15 antibodies as an anti-cancer immunotherapy

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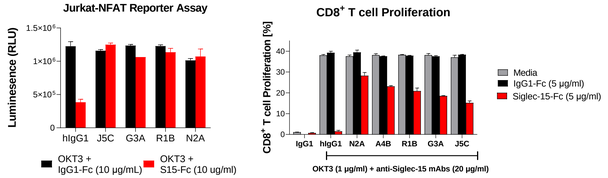
Anti Siglec-15 Clones Rescue T Cell Proliferation from Siglec-15 Mediated Immune Suppression

Anti-Siglec-15 mAbs relieve Siglec-15-Fc mediated suppression of Jurkat and human CD8+ T cell proliferation



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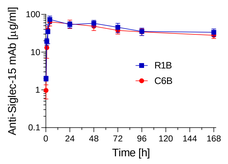
Anti-Siglec-15 Clones Pharmacokinetic (PK) Profiles in BALB/c Mice



Mice were dosed with 10 mg/kg (i.p)
Blood draws at 0.25, 1, 2, 4, 24, 48, 72, 96 and 168h.

Parameter	R1B	C6B
Dose	10 mg/kg	10 mg/kg
$t_{1/2}$ (h)	148 h	225 h
$t_{1/2}$ (Days)	6.18	9.4
T_{max}	4 h	4 h
C_{max}	74 mg/ml	63 mg/ml
AUC $_{(0-t)}$	7456 mg/ml x h	6739 mg/ml x h
AUC $_{(0-inf)}$	14655 mg/ml x h	15831 mg/ml x h

Exposure and half-life of OncoResponse-clones are in the typical range for human IgG1

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Summary

- We have identified anti-Siglec-15 antibodies that:
  - rescued T cells from Siglec-15 mediated immune suppression
  - rescued the NFAT promotor activity of a T cell reporter cell line
  - rescued the proliferative and IFN-γ response of anti-CD3 activated human T cells from Siglec-15-Fc mediated immune suppression
- The top clones relieved M2c-macrophage-mediated suppression of T cell activation and proliferation in M2c/T cell coculture assays
- Selected clones showed a typical PK profile in BALB/c mice
- A mouse reactive anti-Siglec-15 clone showed 41% tumor growth inhibition in a syngeneic murine CT26 colon carcinoma tumor model
- A representative anti-Siglec-15 clone inhibited human SK-MEL-5 melanoma tumor growth by 53% in humanized NSG-SGM3 mice

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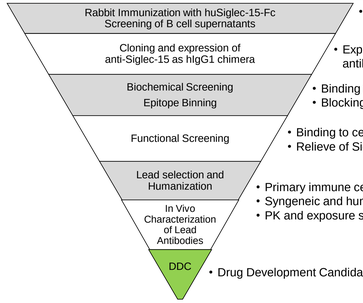
Screening Cascade for the Discovery of Anti-Siglec-15 Antibodies



- Binding to human, cynomolgus and murine Siglec-15
- Expression/purification of anti-Siglec-15 antibodies
- Binding to human, cynomolgus and murine Siglec-15
- Blocking of sTn and counter-screens
- Binding to cells expressing Siglec-15
- Relieve of Siglec-15 mediated immune suppression
- Primary immune cell based functional assays
- Syngeneic and humanized mouse tumor models
- PK and exposure studies
- Drug Development Candidate

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Profile of Representative Anti-Siglec-15 Clones

	Binding K562-huS15 cells	ELISA Binding to Siglec-15 Fc ($OD_{450\,nm}$)		Sialyl-Tn % blocking of Siglec-15 Binding (n=3)		% Rescue of Proliferation in Jurkat-NFAT (n-3)	PBMC & Siglec-15 Fc assay (n=6)			
Clone ID	EC_{50} [nM]	Human S15 [0.1 µg/ml]	Cyno S15 [0.1 µg/ml]	Human Siglec-15 [2.5 µg/ml]	Cyno Siglec-15 [2.5 µg/ml]	Jurkat-NFAT [10 ug/ml]	Jurkat-NFAT [2.5 ug/ml]	$CD8^+$ T cell Proliferation	$CD4^+$ T cell Proliferation	IFN-γ (% Rescue over IgG1)
R1B	0.53	1.55	1.43	87%	82%	90%	44%	59%	46%	64%
N2A	0.14	1.99	2.0	70%	26%	98%	35%	42%	31%	92%
G3A	0.14	1.64	1.79	81%	74%	76%	58%	42%	34%	75%
A4B	0.12	2.28	2.26	82%	79%	95%	66%	41%	33%	42%
J5C	0.13	1.21	1.1	89%	86%	104%	46%	30%	23%	47%

- Demonstrated binding to K562-human-Siglec-15 cells
- Performed well in cell-based functional assays
- Demonstrated binding to cynomolgus Siglec-15 protein
- Distinct binding epitopes between all clones

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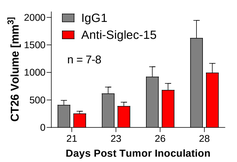
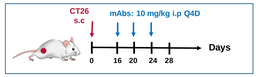
Representative Anti-Siglec-15 Clone Shows Tumor Growth Inhibition in a Murine Carcinoma Tumor Model





% Tumor Growth Inhibition (CT26)

mAb	Day 21	Day 23	Day 26	Day 28
Anti-Siglec-15	47%	42%	28%	41%

- Anti-Siglec-15 clone is cross-reactive to murine Siglec-15
- Dosing of antibodies initiated at an average tumor volume of 95 mm^3
- Treatment with representative clone achieved a 41% TGI by Day 28

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Acknowledgements

Scientific Advisors
Anil Singhal
Mike Gallatin
Albert Yu

Bob Lechleider
Cliff Stocks
Meagan Welsh
Meghan Zuck

Phil Hammond
Randi Simmons
Ray Fox

Michael A. Curran
James Welsh
David Hong

Patients who provided precious tissue samples for this study

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