Targeting Siglec-15 with NC318, a Novel Therapeutic Antibody to Enhance Anti-Tumor Immunity

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BACKGROUND

Siglec-15 (S15), a member of sialic acid-binding immunoglobulin-type lectins, is a highly conserved Type I cell surface protein, which was previously reported to play a role in osteoclast differentiation and bone remodeling[1,2]. Here we describe S15 as a novel co-inhibitory ligand expressed on tumors and myeloid cells that suppresses T cell function and promotes cancer growth. Blocking S15 by antibody enhances anti-tumor immunity in preclinical models.

[1] Macauley MS et al. Nat Rev Immunol. 2014 Oct;14(10):653-66.
[2] Hiruma Y et al. Bone. 2013 Mar;53(1):87-93.

SIGLEC-15 KNOCK OUT MICE

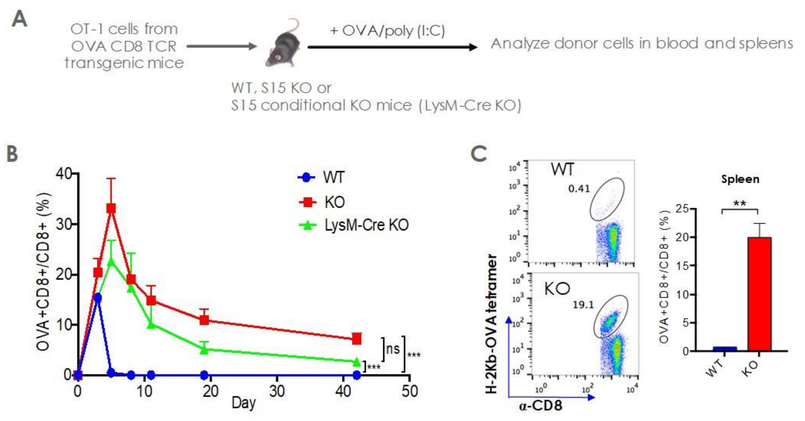


Figure 1: Myeloid-Lineage Cell Expression of S15 Inhibits Antigen-Specific CD8⁺ OT-I T Cell Responses *in Vivo*

A: Brief outline of the study. B: The kinetics of OT-I T cell expansion and contraction in the blood of WT, S15 KO and LysM-Cre S15 KO mice at indicated time points after OVA/poly (I:C) immunization (n=4/group). Analysis shown is OT-I T cells as a percentage of total CD8⁺ T cells. C: OT-I T cell proliferation in mouse spleens 5 days after transplantation. Analysis shown is percentage of OT-I of total CD8⁺ T cells.

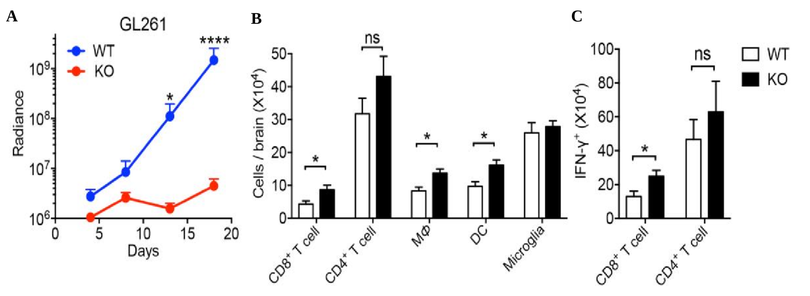


Figure 2: S15 Suppresses Anti-Tumor Immunity and Promotes GL261 Tumor Growth

A) GL261luc tumor growth in wild type or S15 KO mice post intracranial injection. B/C) Flow cytometry analysis of tumor-infiltrating immune cells on Day 14 after GL261 tumor inoculation. $CD8^+$ T cells, $CD4^+$ T cells, $CD11b^+$ $CD45^{high}$ macrophages (MØ), $CD11b^+$ $CD45^{low}$ microglia, and $CD11c^+$ dendritic cells (DC) in total brain mononuclear cells were quantified. B) Brain mononuclear cells were further re-stimulated with irradiated GL261-luc cells for 5 days. C) Total number of IFN-γ-producing $CD8^+$ T cells and $CD4^+$ T cells was determined based on live cell counting and intracellular cytokine staining. *P < 0.05.

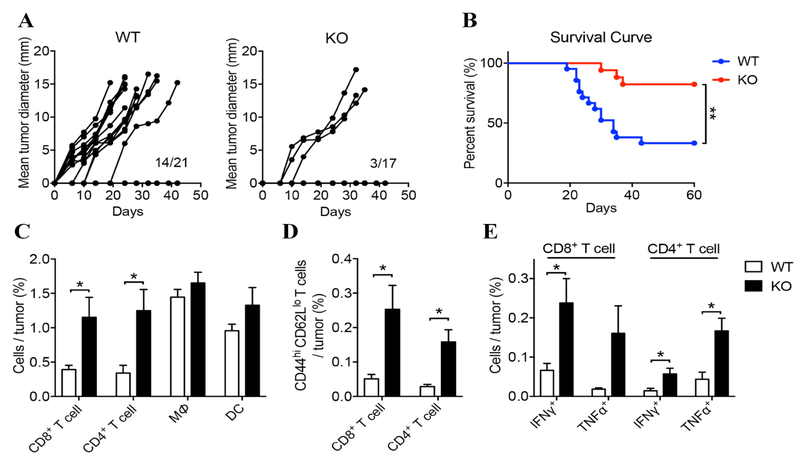


Figure 3: S15 Suppresses Anti-Tumor Immunity and Promote B16.GMCSF Tumor Growth

A & B) Subcutaneous B16.GMCSF tumor growth in S15 KO or C57Bl/6 wild type mice. Tumor incidence and growth of individual mice (A) as well as percent survival (B). Percentage of immune subsets (C) and T cells with effector phenotype (CD44hiCD62Llo) (D) among total cells in the tumors from wild type or S15 KO mice. E) The % of IFN-γ and TNF-α producing T-cells were shown based on intracellular staining of cells after ex vivo re-stimulation with PMA and ionomycin.

SIGLEC-15 SUPRESSES T CELL *IN VITRO*

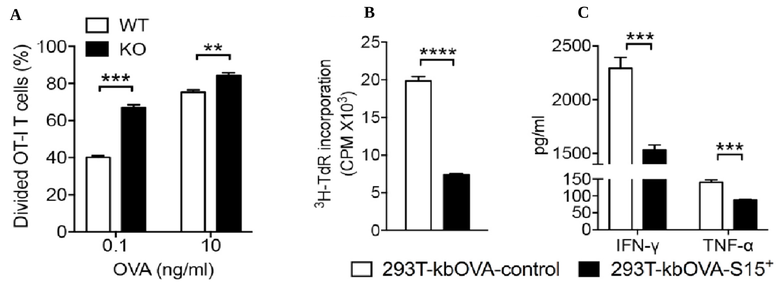
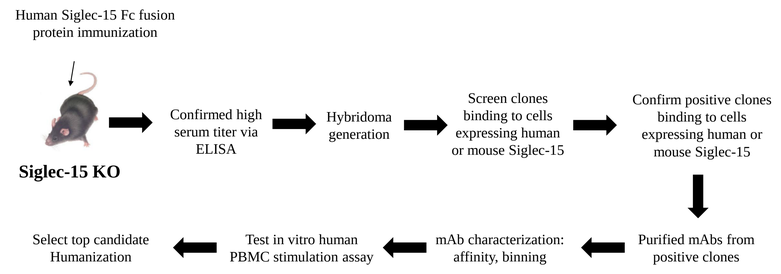


Figure 4: Siglec-15 Inhibits Antigen-Specific T Cell Responses *In Vitro*

A: Peritoneal macrophages from WT or S15 KO mice were harvested from C57Bl/6 wild type or S15 KO mice peritoneal cavities. B: 293TKbOVA cells were transfected with S15 or control transfection with empty vector. Irradiated 293TKbOVA-S15+ or 293T-KbOVA-control cells were co-cultured with purified OT-I T cells at 1:10 ratio. 3Hthymidine was added to the co-culture two days later. The following day OT-I T cell proliferation was measured by incorporation of 3H-thymidine. C: Supernatants from the cultured cells was harvested prior to addition of 3H-thymidine and assessed for levels of IFN-γ and TNF-α by ELISA.

ANTIBODY DEVELOPMENT



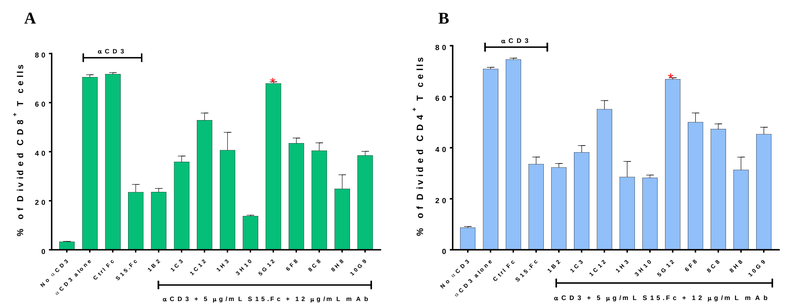


Figure 5: Anti Siglec-15 (S15) Clone 5G12 Reverses S15 Fc Fusion Protein-mediated Suppression on Human T cells.

Human PBMCs from healthy donor were labeled with CFSE and added to anti CD3 coated 96-well plate plus S15 Fc fusion protein and indicated S15 mAbs. Three days later, the cells were stained with anti CD4 and anti CD8 followed by FACS analysis of $CFSE^{low}$ cell population. A) Human CD8 T cell proliferation; B) Human CD4 T cell proliferation

Table 1: Average kinetics values

Clone	K_D (nM) (From 3 runs)
1H3	0.11 ± 0.02
5G12	0.30 ± 0.04
6F8	0.44 ± 0.08
10G9	0.55 ± 0.08
1C3	0.58 ± 0.13
3H10	0.60 ± 0.14
8C8	0.89 ± 0.25
8H8	1.30 ± 0.33
1C12	4.01 ± 1.05
1B2	4.33 ± 0.75

Table 2 : Summary of Parent 5G12 and NC318 Affinity Binding to Human Siglec-15

	K_D (nM)	R^2	K_{on} (1E+5/Ms)	K_{off} (1E-4/s)
Parent 5G12	0.37	0.994	3.18	1.19
NC318	0.35	0.999	3.22	1.12

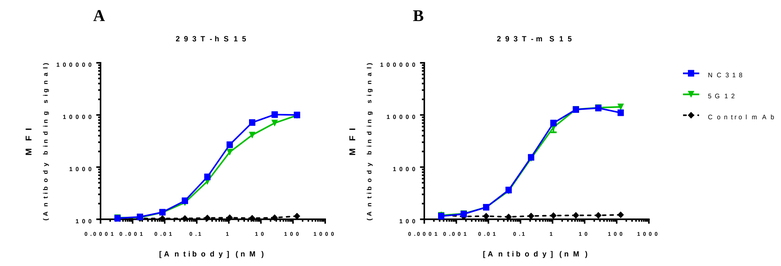


Figure 6: Binding of Anti-S15 Antibodies to Cells Expressing Mouse or Human S15

(A) 293T.hS15 cells (NC318 EC_{50} = 2.42 nM) and (B) 293T.mS15 cells (5G12 parent EC_{50} = 1.26 nM).

EFFECTS of 5G12 *IN VIVO*

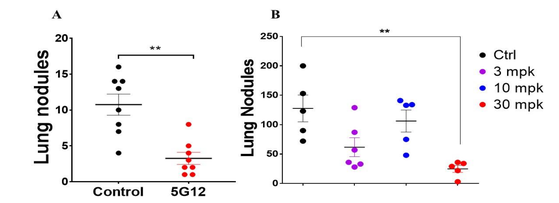


Figure 7: 5G12 Reduces MC38.mS15 Tumor Lung Metastatic Nodules.

A: Mice were treated with 5G12 on day 2 at 20 mg/kg, Q4D; B: Mice were treated with 5G12 on Day 3 at 3, 10 or 30 mg/kg, Q7D. ***p<0.01*

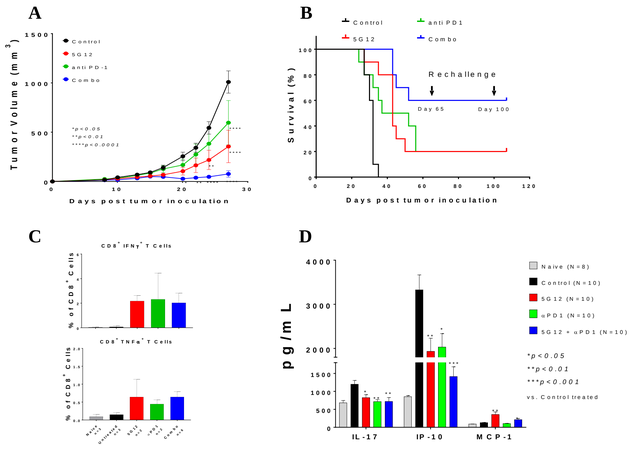


Figure 8: 5G12 Monotherapy or in Combination with Anti-PD1 In CT26/S15⁺ BMDM Tumor Model.

A: Tumor volume; B: Kaplan-Meier survival plot; C: CT26-specific $CD8^+IFN\text{-}\gamma^+$ and $CD8^+TNF\text{-}\alpha^+$ T cells in mouse spleen collected on Day 107; D: Serum collected on Day 30 and analyzed for 20 mouse cytokines (mouse 20-plex Luminex Kit from ThermoFisher).

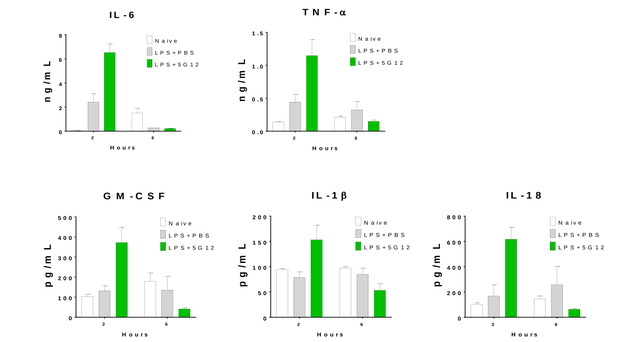


Figure 9: 5G12 Significantly Increases LPS-Mediated Immune Activation in Mice.

Mice were first injected with PBS or 5G12 (10 mg/kg) and challenged with LPS 1h later. Serum was collected 2h, 6h later and analyzed for pro-inflammatory cytokines.

EFFECTS of NC318 *IN VITRO*

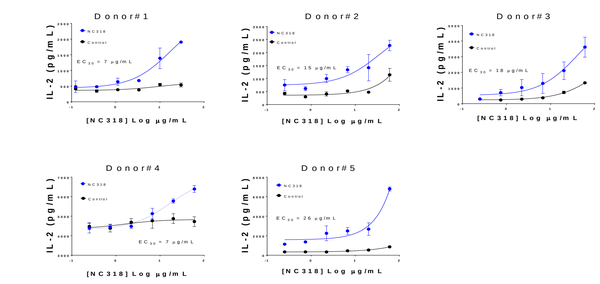


Figure 10: NC318 Dose Dependently Stimulates Production of IL2 in Co-Stimulated PBMC Cultures

Human PBMCs from healthy donors were added to anti-CD3 coated 96-well plate plus SEB together with serially diluted NC318 or isotype control mAb. Supernatant was collected three days later for IL-2 analysis.

CONCLUSION

- S15 immunosuppressive properties in the TME make it a rational target for immunotherapy.
- NC318 is a high affinity humanized IgG_1 mAb specific for S15 developed to reverse tumor immune suppression and promote an effective anti-tumor immune response.
- NextCure has completed IND-enabling studies and initiated evaluations of NC318 in patients with advanced malignancies: "**A Safety and Tolerability Study of NC318 in Subjects With Advanced or Metastatic Solid Tumor"** https://clinicaltrials.gov/ct2/show/NCT03665285