Targeting IL-15 delivery to PD-L1 Expressing Tumors using an Anti-PDL1 x IL-15 Cytokine Fusion IgM to Enhance T Cell and NK Cell Mediated Tumor Cytotoxicity

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Background

- Therapeutic antibodies inhibiting PD-1/PD-L1 have demonstrated clinical efficacy, although only a fraction of patients respond.
- Combinations are being explored to enhance responses including anti-PD-1/PD-L1 IgG antibodies with IL-15 pathway stimulating agents to remove PD-1 immunosuppressive signaling and enhance anti-tumor NK and memory CD8 T cell expansion and survival.
- We have engineered an anti-PD-L1 high affinity, high avidity pentameric IgM, to target PD-L1 expressing tumors and APCs, with an IL-15 and IL-15Rα Sushi domain fused to a joining (J) chain to generate **PDL1-ISA**.

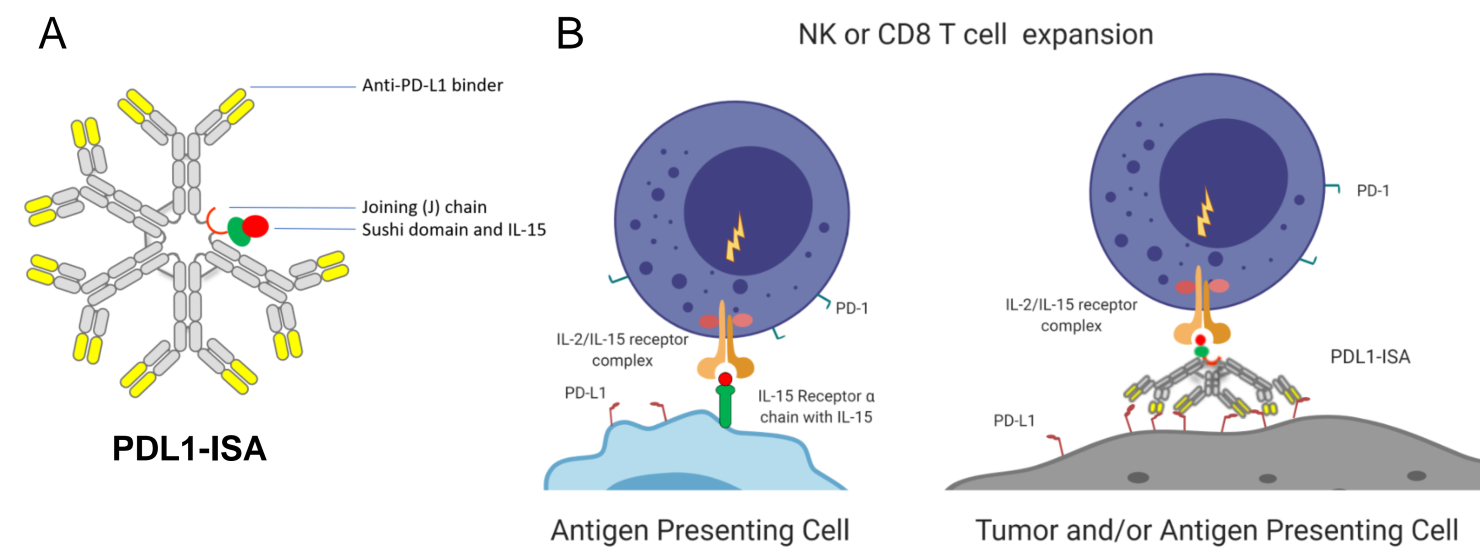


Figure 1. A) Structure of PDL1-ISA; **B)** Natural presentation of IL-15 versus presentation of IL-15 by PDL1-ISA to CD8 or NK cells. The presentation of IL-15 on the PD-L1 IgM can occur on both antigen presenting cells (APCs) and/or PD-L1-expressing tumor cells.

Characterization of anti-PDL1 IgG and IgM (mAb 3C5)

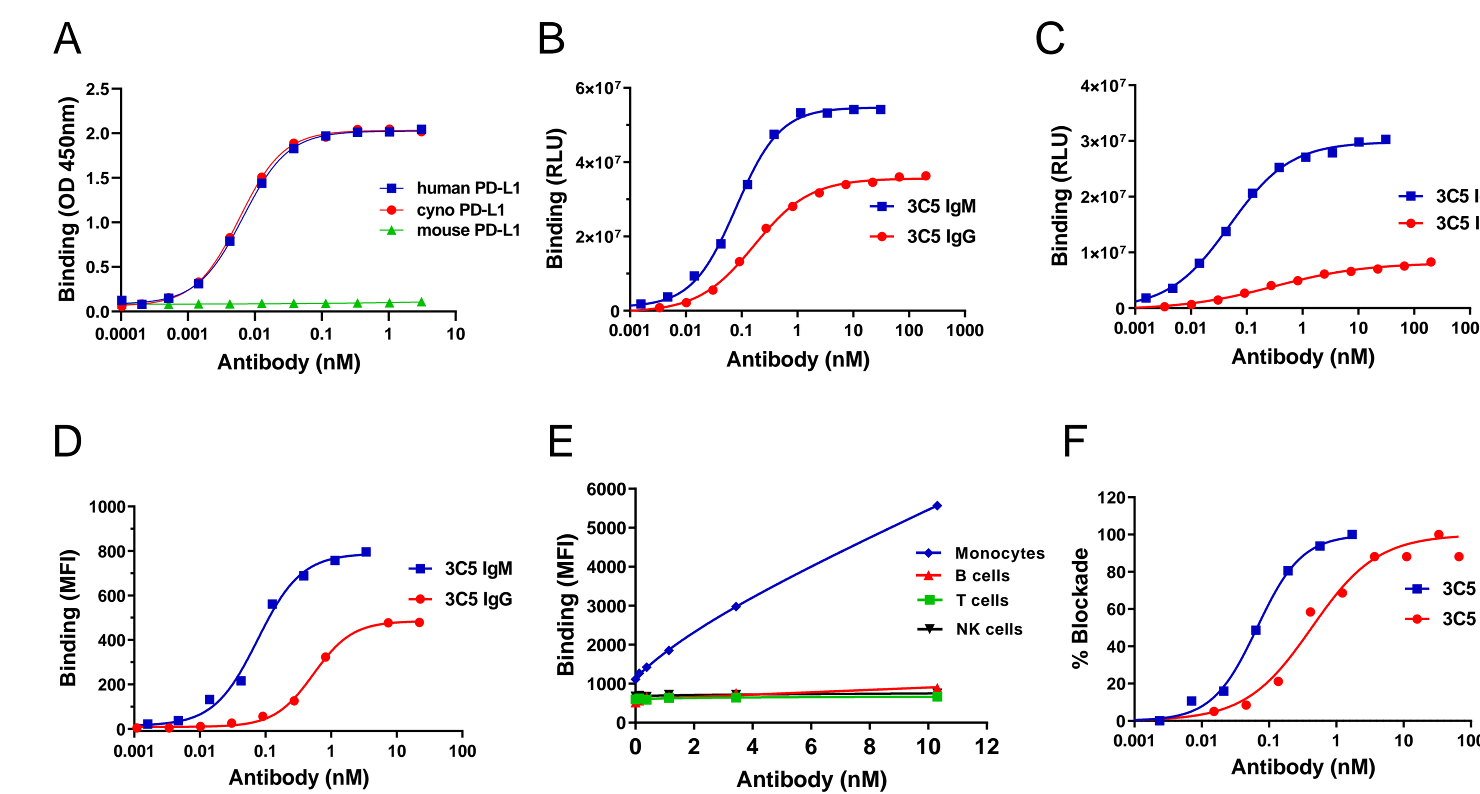


Figure 2. The 3C5 anti-PD-L1 antibody was generated from an hybridoma campaign. **A)** Species specificity was tested by ELISA with 3C5 IgG and recombinant PD-L1 from human, cynomolgus and mouse was coated at 1 µg/mL. Binding was measured using an anti-human kappa antibody secondary antibody. **B & C)** Binding of 3C5 IgG or IgM on human PD-L1-His coated at **B)** 1µg/mL and **C)** 0.1 µg/mL was measured by ELISA using an anti-human kappa antibody secondary antibody conjugated to HRP. **D)** Binding to PD-L1 positive SUP-HD1 cells was performed by flow cytometry using directly AF488-labeled 3C5 IgG and IgM. **E)** Binding to healthy human donor PBMC was evaluated using a directly AF488-labeled 3C5 IgM and a cocktail of anti-CD3, anti-CD4, anti-CD19, anti-CD56 and anti-CD14 antibodies. The cells were analyzed on a Cytek DxP8 flow cytometer, data analyzed in FlowJo (TreeStar) and MFI graphed against the 3C5 IgM antibody concentration for each cell subset. **F)** PD-1/PD-L1 blockade was performed using the Promega blockade reporter assay.

PDL1-ISA IgM binds to monocytes and promotes NK and CD8 T cell proliferation

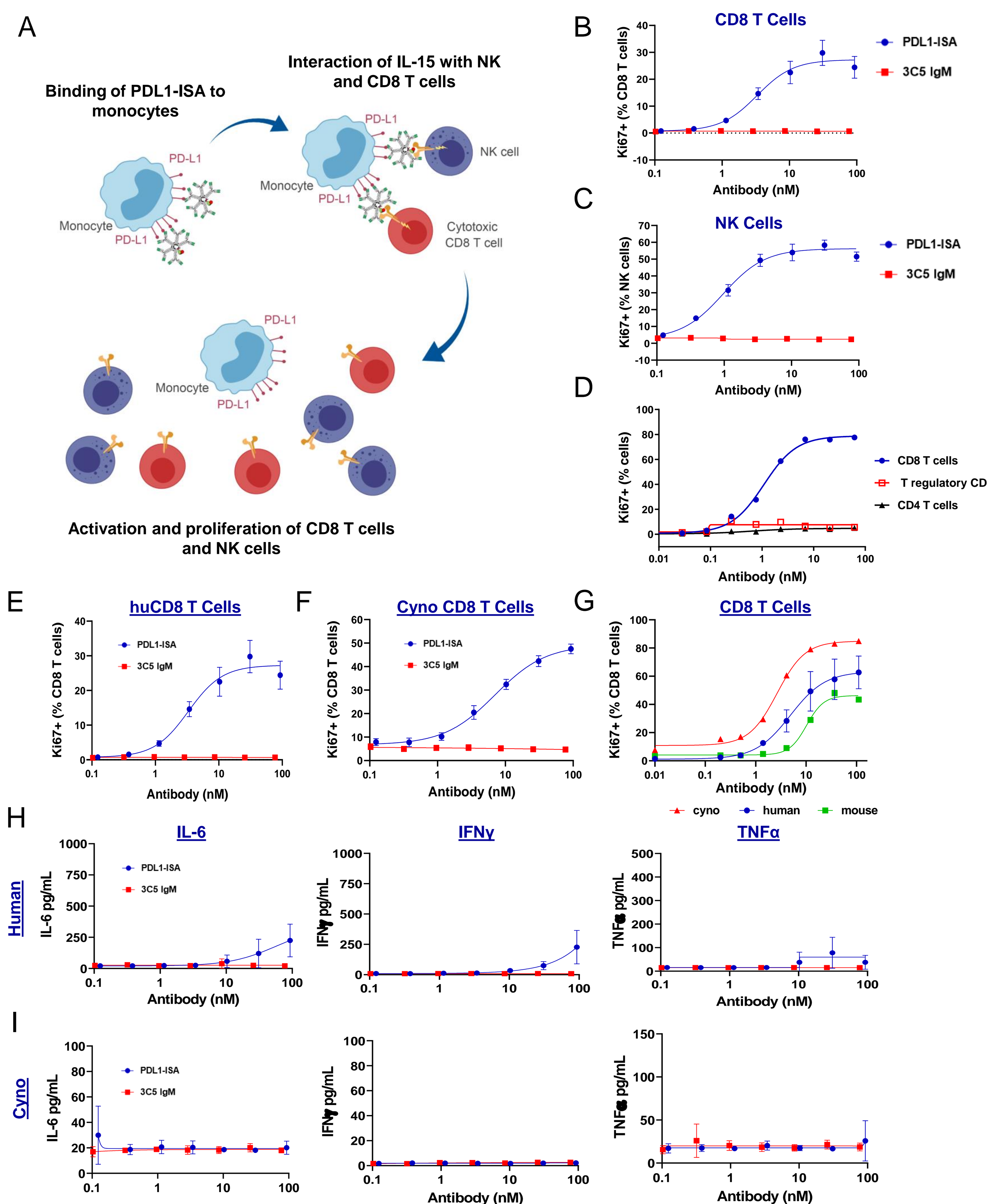


Figure 3. Potency of PDL1-ISA in PBMC cultures. **A)** Proposed mechanism of action of PDL1-ISA. **B and C)** Proliferation assay with PBMCs to measure the potency of PDL1-ISA: Healthy donor PBMCs (n=5) were incubated with dose titrations of PDL1-ISA and controls for 3-4 days. Cells were then stained for CD3, CD4, CD8, CD56, NKp46 and intracellularly for Ki-67. Stained cells were evaluated on DxP8 (Cytek) or Intellicyt iQue3 (Sartorius) and the percentage of NK and CD8 T cells expressing Ki-67 was graphed. The PDL1-ISA induced proliferation of CD8 and NK cells had EC_{50}'s of 3.3 and 1nM respectively **D)** The proliferative effect of PDL1-ISA on CD8, CD4 and T regulatory cells (FoxP3+) was evaluated. Proliferation of CD4 and T regs was not induced by PDL1-ISA. **E and F)** PDL1-ISA-induced proliferation of cynomolgus CD8 T cells (n=4) was similar to that of huCD8 T cells (n=5), with EC_{50} of 6.8 and 3.3nM respectively. **G)** We tested the effect of PDL1-ISA on mouse splenic CD8 T (n=2) cells side by side with human (n=2) and cyno (n=1) CD8 T cells. Proliferation EC_{50} were 10.2, 4.9 and 2.6nM for mouse, human and cyno CD8 T cells respectively. **H and I)** Cytokine concentrations in culture supernatants from **E** and **F** were evaluated by Cytometric Bead Array (CBA) assays using the human Th1/Th2 and non-human Primate Th1/Th2 kits (BD Biosciences). Shown are average values for IL-6, IFNγ and TNFα. All other cytokines tested (IL-2, IL-4, IL-5 and IL-10) were below the limits of detection.

PDL1-ISA enhances T and NK dependent cytotoxicity of breast carcinoma line MDA-MB-231 in vitro

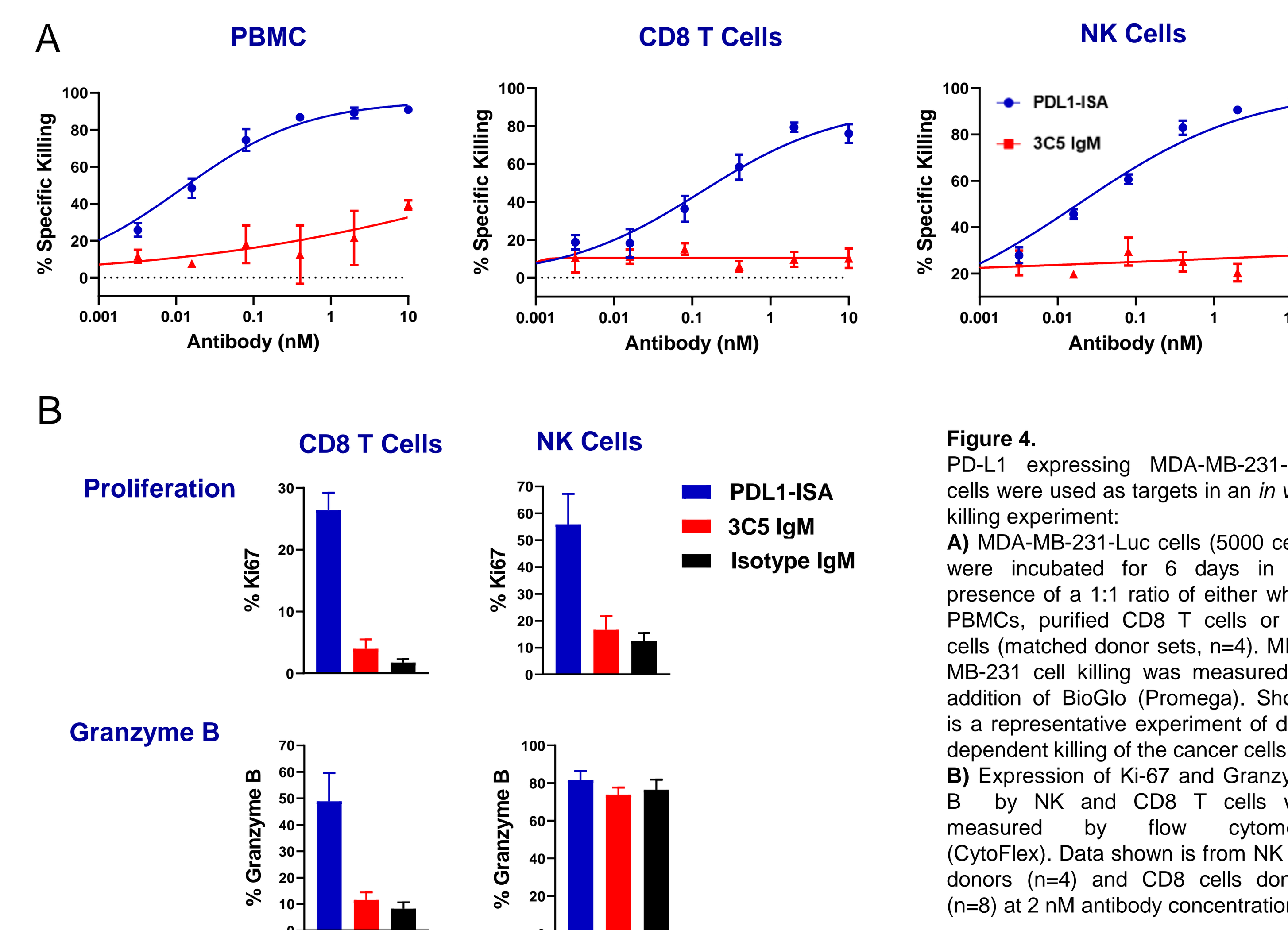


Figure 4.
PD-L1 expressing MDA-MB-231-Luc cells were used as targets in an *in vitro* killing experiment:
A) MDA-MB-231-Luc cells (5000 cells) were incubated for 6 days in the presence of a 1:1 ratio of either whole PBMCs, purified CD8 T cells or NK cells (matched donor sets, n=4). MDA-MB-231 cell killing was measured by addition of BioGlo (Promega). Shown is a representative experiment of dose dependent killing of the cancer cells.
B) Expression of Ki-67 and Granzyme B by NK and CD8 T cells was measured by flow cytometry (CytoFlex). Data shown is from NK cell donors (n=4) and CD8 cells donors (n=8) at 2 nM antibody concentration.

PDL1-ISA elicits a transient increase in peripheral NK and CD8+ T cells in mice

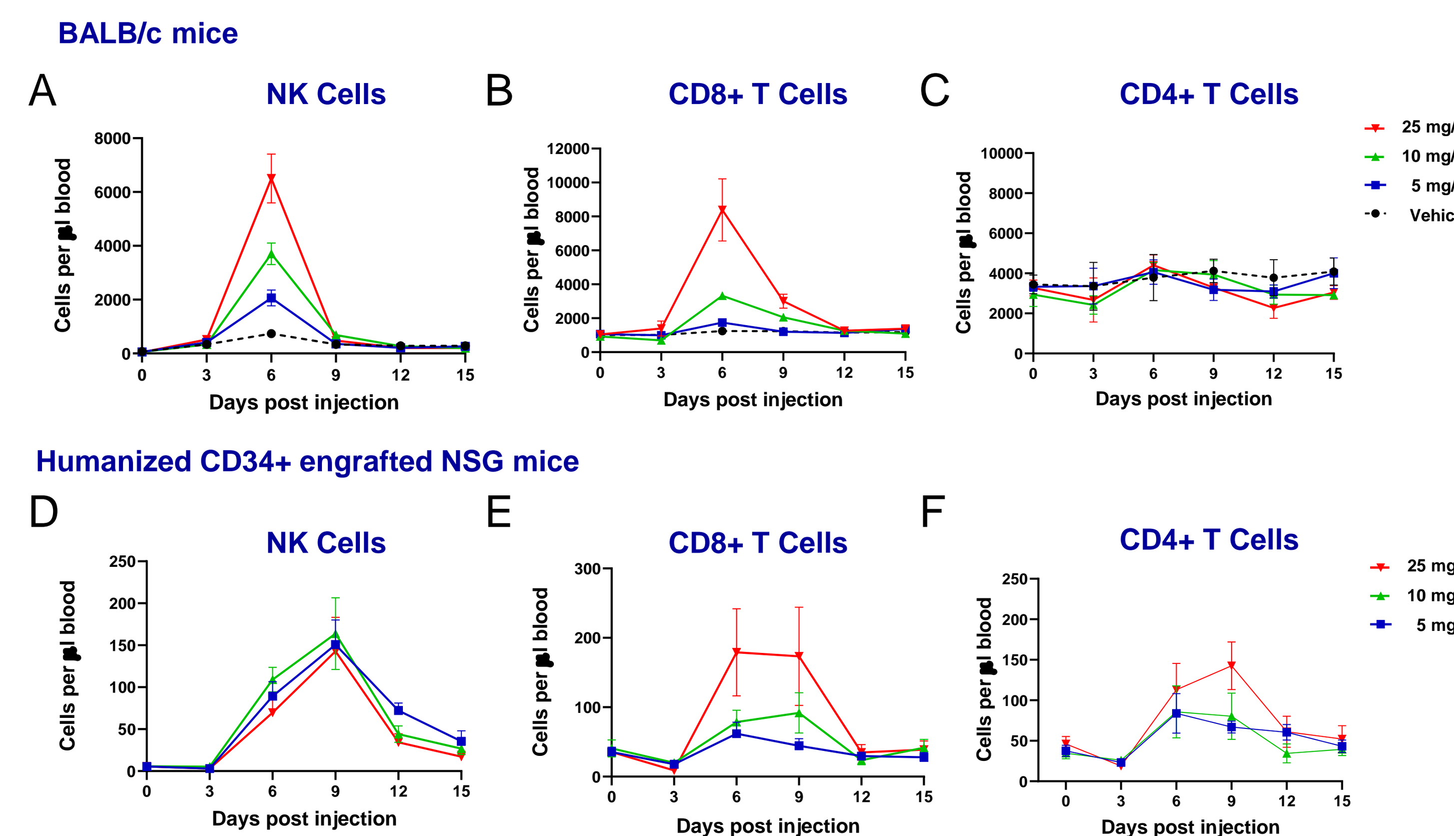
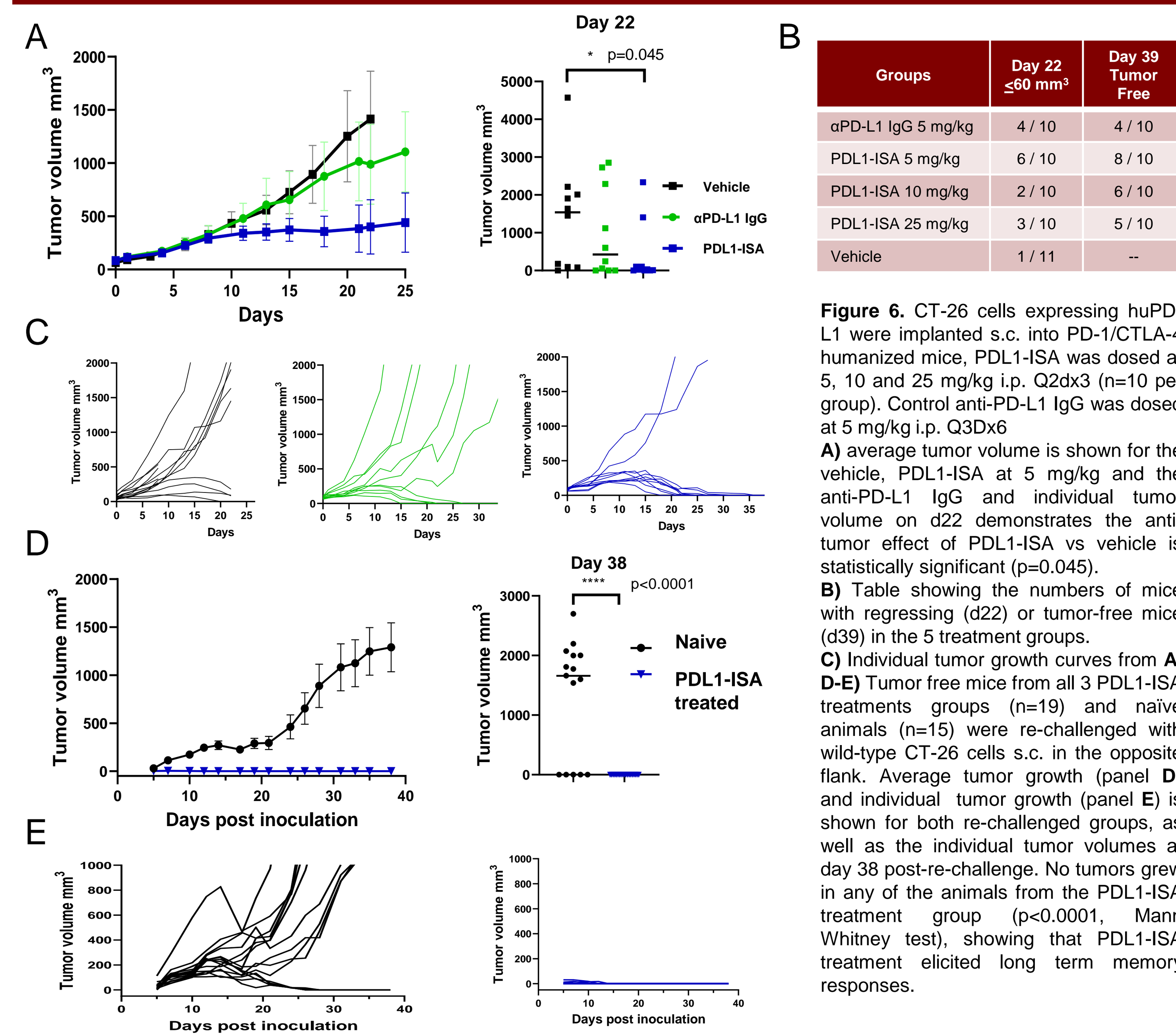


Figure 5. Pharmacodynamic study with PDL1-ISA in BALB/c and humanized CD34+ engrafted NSG mice. BALB/c and NSG mice were injected with PDL1-ISA every 2 days x3 at 5, 10 and 25 mg/kg. Blood samples were collected at days 3, 6, 9, 12 and 15 post first dose and stained either for mouse or human CD45, CD3, CD4, CD8 and CD56. Absolute cell counts were obtained with the use of counting beads added to the samples prior to staining (CountBright Absolute counting beads, Thermo). **A, B and C)** number of mouse NKs, CD8 and CD4 T cells in BALB/c mice; **D, E and F)** number of human NKs, CD8 and CD4 T cells in humanized CD34+ engrafted NSG mice.

PDL1-ISA shows efficacy in hPD-L1 CT-26 tumor model



B

Groups	Day 22 ≤60 mm³	Day 39 Tumor Free
αPD-L1 IgG 5 mg/kg	4 / 10	4 / 10
PDL1-ISA 5 mg/kg	6 / 10	8 / 10
PDL1-ISA 10 mg/kg	2 / 10	6 / 10
PDL1-ISA 25 mg/kg	3 / 10	5 / 10
Vehicle	1 / 11	--

Figure 6. CT-26 cells expressing huPD-L1 were implanted s.c. into PD-1/CTLA-4 humanized mice, PDL1-ISA was dosed at 5, 10 and 25 mg/kg i.p. Q2dx3 (n=10 per group). Control anti-PD-L1 IgG was dosed at 5 mg/kg i.p. Q3Dx6
A) average tumor volume is shown for the vehicle, PDL1-ISA at 5 mg/kg and the anti-PD-L1 IgG and individual tumor volume on d22 demonstrates the anti-tumor effect of PDL1-ISA vs vehicle is statistically significant (p=0.045).
B) Table showing the numbers of mice with regressing (d22) or tumor-free mice (d39) in the 5 treatment groups.
C) Individual tumor growth curves from **A**.
D-E) Tumor free mice from all 3 PDL1-ISA treatments groups (n=19) and naïve animals (n=15) were re-challenged with wild-type CT-26 cells s.c. in the opposite flank. Average tumor growth (panel **D**) and individual tumor growth (panel **E**) is shown for both re-challenged groups, as well as the individual tumor volumes at day 38 post-re-challenge. No tumors grew in any of the animals from the PDL1-ISA treatment group (p<0.0001, Mann Whitney test), showing that PDL1-ISA treatment elicited long term memory responses.

Summary

- Anti-PDL1 IgM antibody bound recombinant and cellular PD-L1 more potently than an IgG antibody with the same binding domains. In functional blocking studies, the anti-PD-L1 IgM was more potent than the IgG.
- PDL1-ISA provided a potent proliferation signal to primary human NK and CD8 T cells *in vitro* with minimal impact on regulatory or CD4 T cells. Limited cytokines were detected. PDL1-ISA had similar potencies for both human and cynomolgus CD8 T cells, and 2-fold lower potency for mouse cells (compared to human).
- Pharmacodynamic studies in humanized and BALB/c mice showed transient and dose-dependent increases in circulating NK and CD8 T cells.
- PDL1-ISA enhanced *in vitro* killing of PD-L1 positive MDA-MB-231 cells by human PBMCs, CD8 T and NK cells compared to the anti-PD-L1 IgM (no IL-15).
- PDL1-ISA demonstrated efficacy in a human PD-L1-CT26 mouse tumor model, with most treated animals having complete tumor regressions. Durable immunity to tumor re-challenge consistent with formation of immune memory response were observed.
- In summary, we have engineered an IL-15 immunostimulatory anti-PD-L1 IgM antibody that binds PD-L1 more potently than an IgG, stimulates NK and CD8 expansion *in vitro* and *in vivo* and induces complete tumor regressions in mouse models. This approach may enhance tumor localization of immunostimulatory cytokine IL-15 through the high affinity and high avidity binding to PD-L1 to improve anti-tumor responses and minimize toxicity.

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