PD1 x TGFβR2 bispecifics selectively block TGFβR2 on PD1-positive T cells, promote T cell activation, and elicit an anti-tumor response in solid tumors

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Abstract #714

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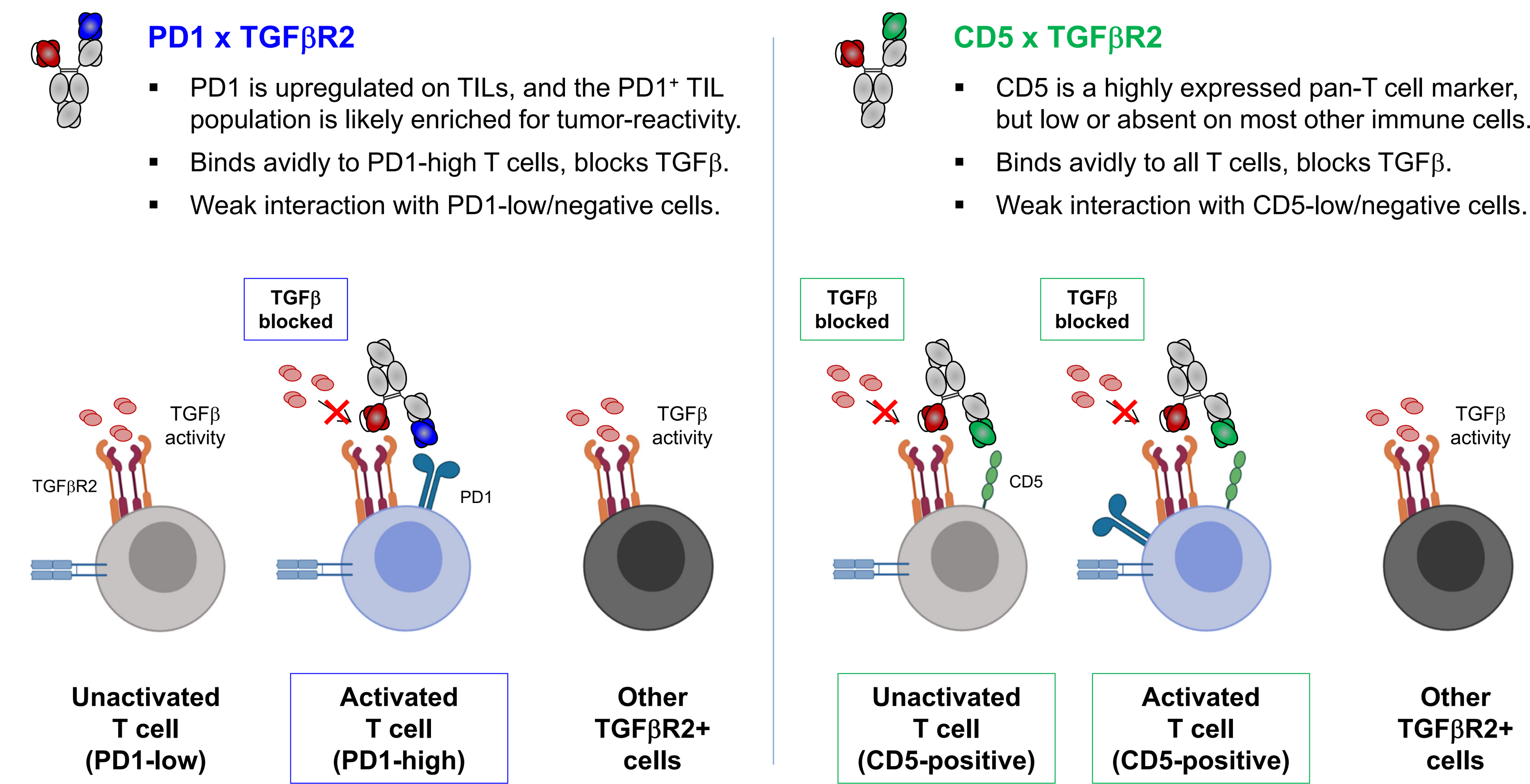
Introduction

- TGFβ production by solid tumors and their microenvironment is a major mechanism used by tumors to avoid immunosurveillance.
- Blockade of TGFβ has been shown to promote an anti-tumor response; however, systemic blockade of TGFβ has also been associated with toxicity.
- We hypothesized that a targeted TGFβR2 bispecific antibody could selectively block the suppressive activity of TGFβ on specific cell populations and enhance their anti-tumor activity while avoiding the toxicity associated with systemic blockade.

Concept: Avid binding of 1+1 bispecific antibodies enables selective TGFβR2 blockade



1. XmAb® heterodimeric Fc platform allows for well-behaved and easily manufactured bispecific antibodies

Fc substitutions promote heterodimer formation and facilitate purification by standard methods such as Protein A + ion-exchange chromatography.
Modified Fc domain eliminates FcγR interactions while preserving FcRn affinity for antibody-like half-life.

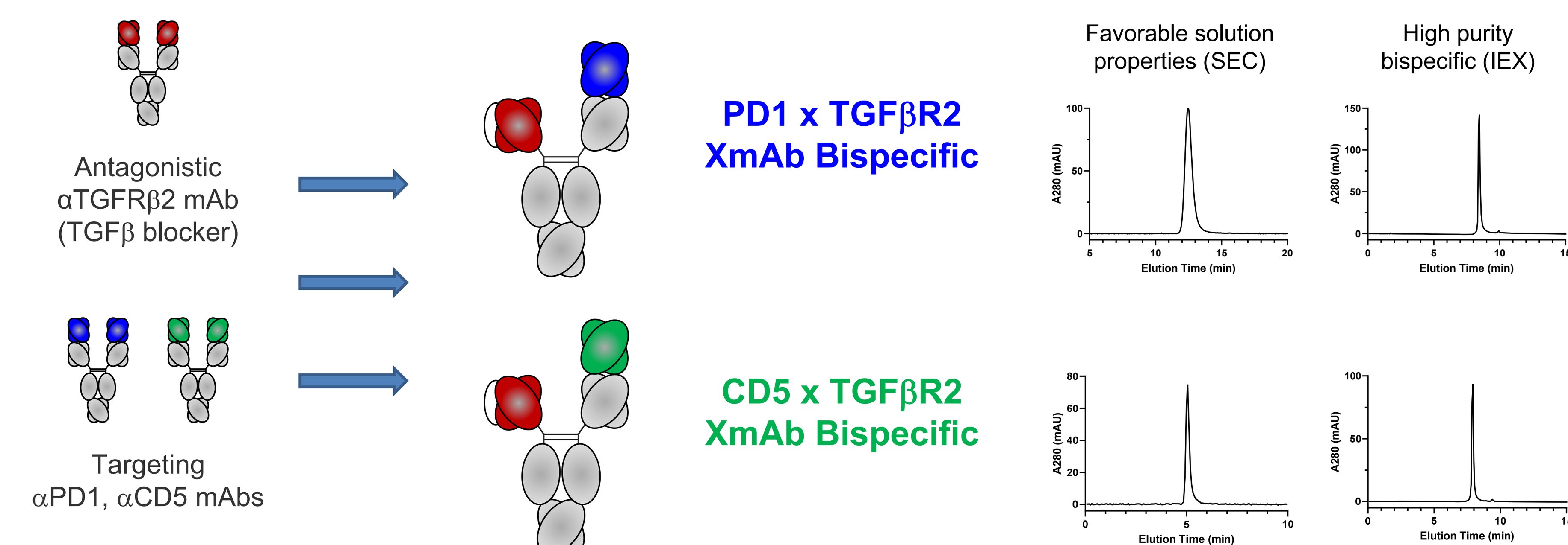


Figure 1. Engineering schematics of PD1 x TGFβR2 and CD5 x TGFβR2 bispecifics and analytical characterization.

2. PD1 x TGFβR2 selectively inhibits pSMAD induction in PD1-positive T cells

PD1-targeting enables a 100-fold increase in blocking potency compared to an untargeted anti-TGFβR2 control

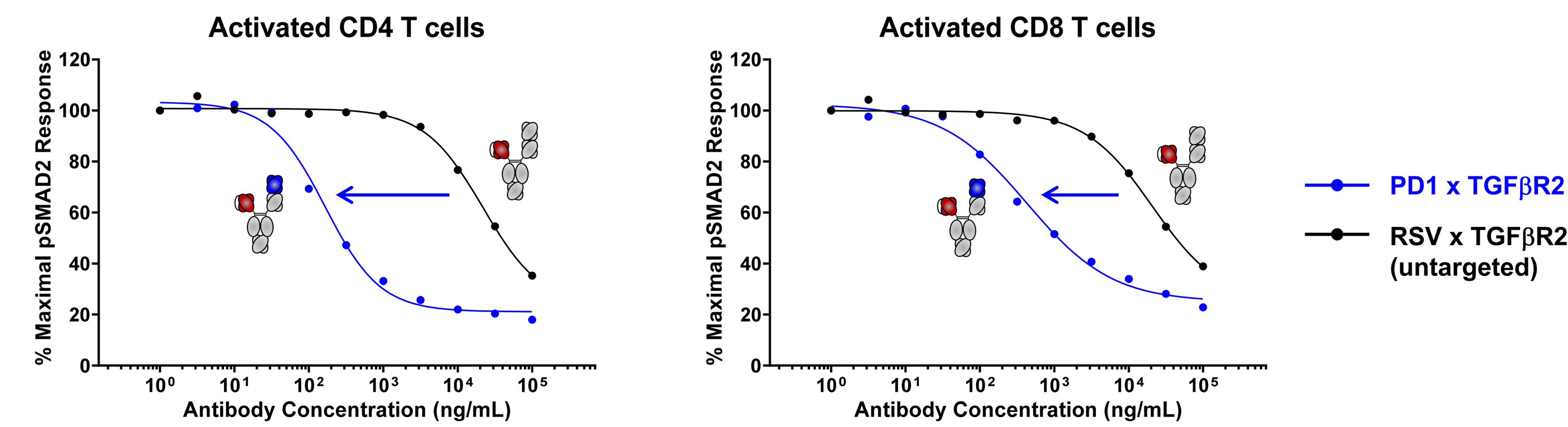


Figure 2. PBMC were activated by seeding on 0.5 μg/mL anti-CD3 for 48 h, then serum deprived for 16 h (0.1% FBS). The PBMC were then incubated with bispecific antibodies for 30 min at RT followed by incubation with bispecific antibodies + TGFβ1 at 1 ng/mL for 30 min at 37 °C. Intracellular phospho-flow cytometry was then performed to measure pSMAD2.

PD1 x TGFβR2 blocking activity is highly selective for activated (PD1-high) vs. unactivated (PD1-low) T cells

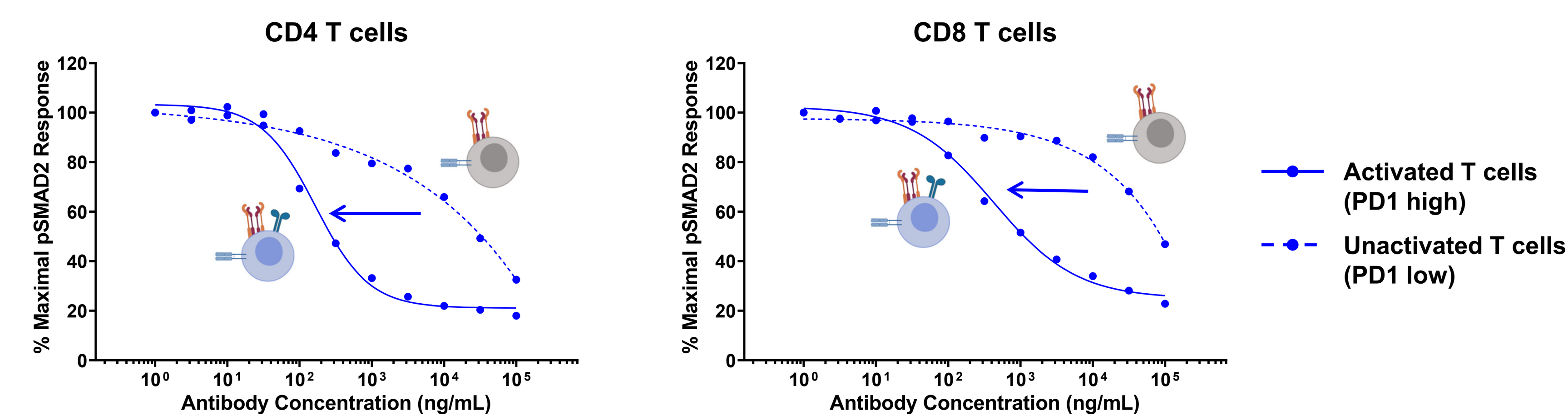


Figure 3. PBMC were recovered overnight for 24 h, then serum deprived for 16 h (0.1% FBS). The PBMC were then incubated with bispecific antibodies for 30 min at RT followed by incubation with bispecific antibodies + TGFβ1 at 1 ng/mL for 30 min at 37 °C. Intracellular phospho-flow cytometry was then performed to measure pSMAD2.

3. CD5 x TGFβR2 selectively inhibits pSMAD induction in a broader T cell population

Targeting with CD5, a pan-T cell marker, enables potent and highly selective (over 1000-fold increase vs. control) TGFβR2 blockade on T cells without regard to activation status

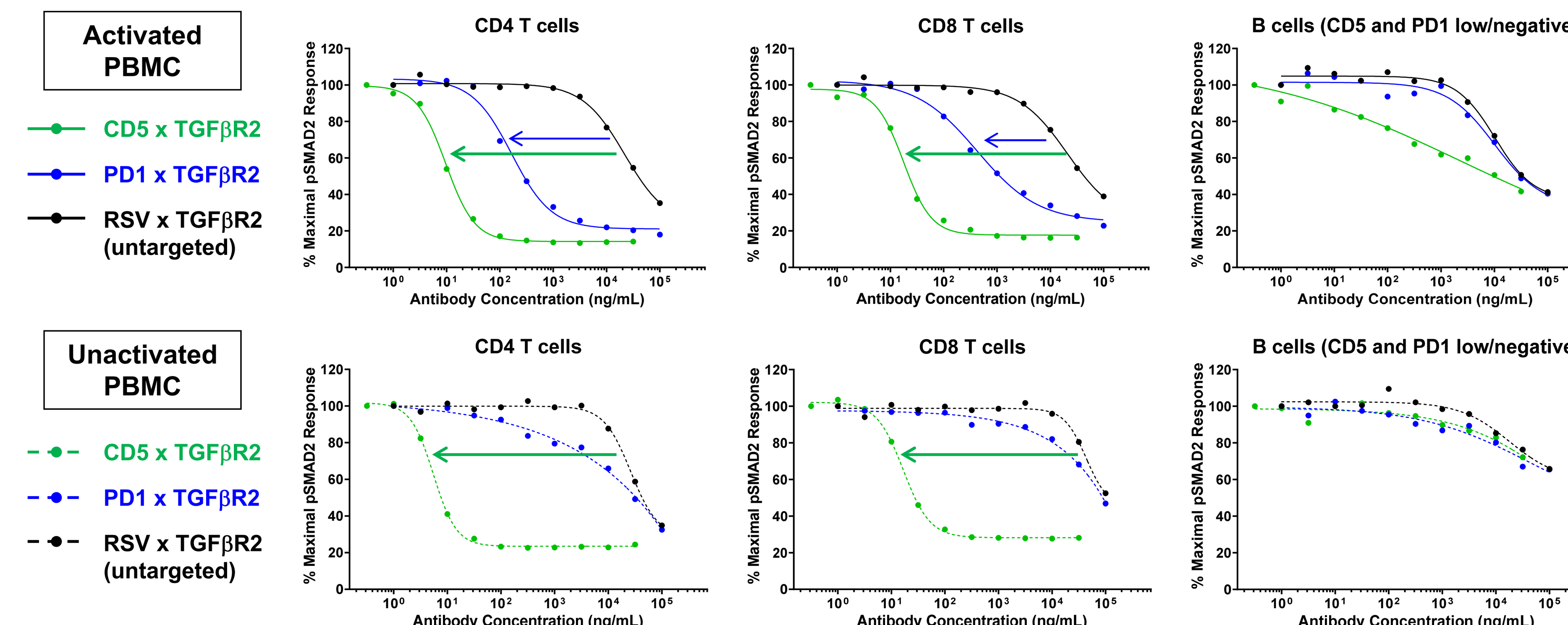


Figure 4. pSMAD2 response assay performed as in Figures 2 and 3.

4. PD1 x TGFβR2 promotes human T cell engraftment in mice

NSG mice engrafted with human PBMC

PD1 x TGFβR2 promoted significantly more GVHD weight loss, T cell expansion, and IFNγ release than vehicle or αPD1 alone

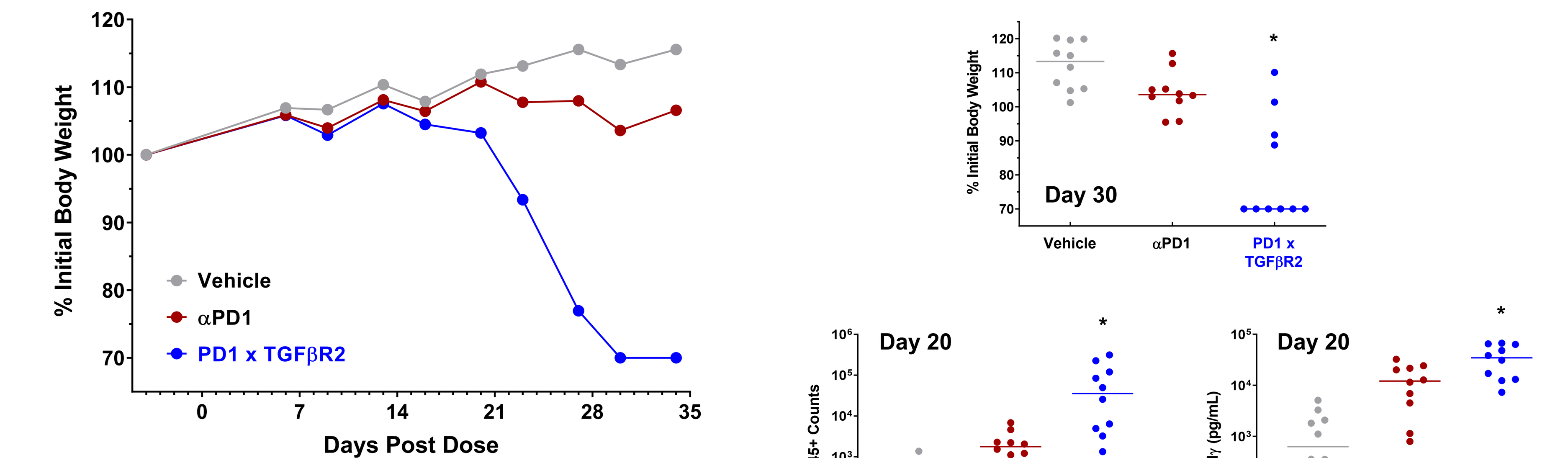


Figure 5. Human PBMC were engrafted in NSG mice along with weekly injections of indicated antibodies. Body weights were measured. CD45+ counts and IFNγ levels shown were from the peripheral blood of the mice at indicated time point.

* $p < 0.05$ vs. Vehicle and αPD1

5. PD1 x TGFβR2 promotes an anti-tumor response

NSG-DKO mice engrafted with human PBMC and MDA-MB-231 triple-negative breast cancer tumors

PD1 x TGFβR2 inhibited tumor growth and promoted more T cell activity than vehicle alone
PD1 x TGFβR2 + αPD1 combination promoted significantly greater anti-tumor response than αPD1 or αPDL1/TGFβ Trap

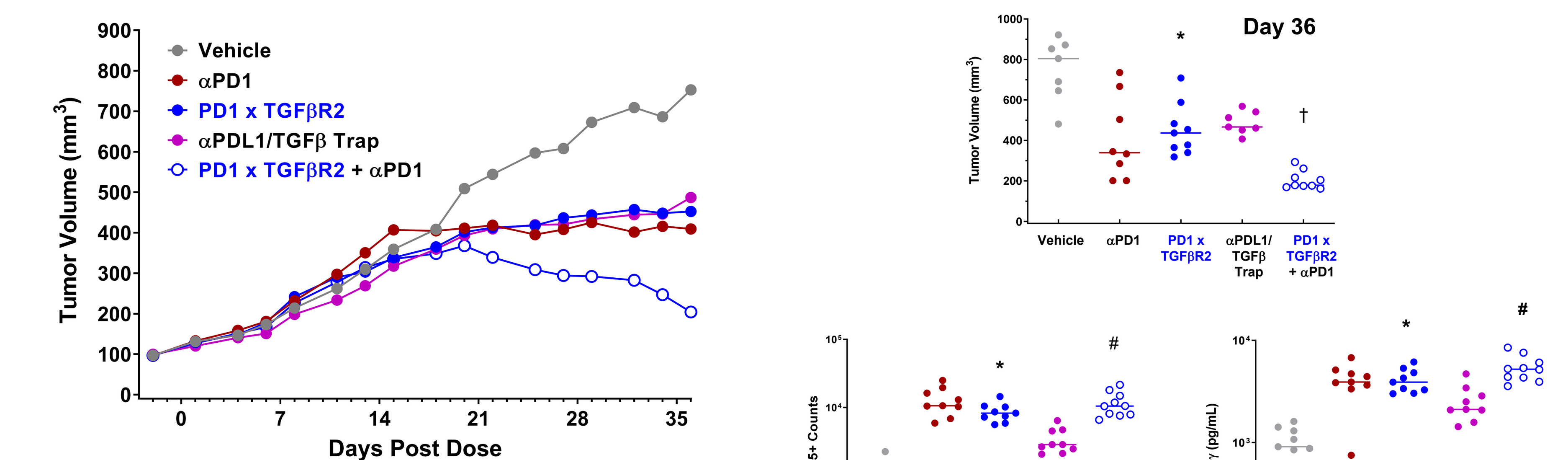


Figure 6. MDA-MB-231 cells were inoculated intradermally in NSG-DKO mice and allowed to form palpable tumors. Human PBMC were then engrafted along with weekly injections of indicated antibodies. Tumor measurements were measured by calipers. CD45+ counts and IFNγ levels shown were from the peripheral blood of the mice at indicated time point.

* $p < 0.05$ vs. Vehicle
# $p < 0.05$ vs. Vehicle and αPDL1/TGFβ Trap
† $p < 0.05$ vs. Vehicle, αPD1, PD1 x TGFβR2, and αPDL1/TGFβ Trap

Summary

- PD1 x TGFβR2 and CD5 x TGFβR2 XmAb® bispecific antibodies were engineered to selectively block TGFβ signaling on targeted cell populations and evaluated in vitro and in vivo.
- PD1 x TGFβR2 and CD5 x TGFβR2 exhibited highly selective blocking of TGFβ-mediated pSMAD2 induction in PD1-high and CD5-high T cell populations, respectively.
- PD1 x TGFβR2 was active in mouse models, promoting T cell engraftment and anti-tumor response. Similar evaluation of CD5 x TGFβR2 is underway.

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