Tumor-targeted CD28 costimulatory bispecific antibodies enhance T cell activation in solid tumors

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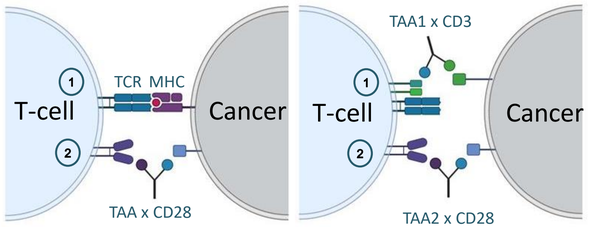
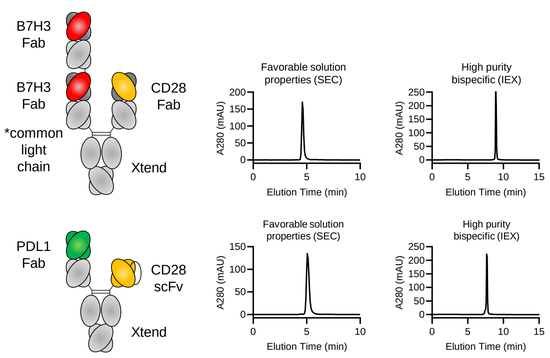
Introduction

- T cells in the tumor micro-environment require TCR/MHC engagement and co-stimulatory receptor engagement to achieve full activation.
- Targeted TAA x CD28 bispecific antibodies have the potential to provide conditional co-stimulation to T cells recognizing neoantigens, or in concert with CD3 bispecific T cell engagers.
- Conditional co-stimulation can enhance T cell activation and proliferation, potentiating T cell-directed therapeutics particularly in cold tumors.
- We designed a conditionally agonistic αCD28 antibody that was paired using Xencor's XmAb bispecific platform to derive B7H3 x CD28 and PDL1 x CD28 bispecific antibodies.
- Clinical application of this class of antibodies has potential to enhance activity of either anti-PD(L)1 antibodies or TAA x CD3 T cell engagers.
- B7H3 x CD28 and PDL1 x CD28 were both well tolerated in cynomolgus monkeys and display compelling preclinical activity warranting further investigation.



1. B7H3 x CD28 and PDL1 x CD28 Antibody Design



- Modified Fc domain to eliminate FcγR interactions and contain Xtend Fc technology to promote longer half-life and extended pharmacodynamics.
- Fc substitutions promote heterodimer formation and facilitate purification by standard methods such as Protein A + ion-exchange chromatography.

Figure 1. Engineering schematics and analytical characterization of B7H3 x CD28 and PDL1 x CD28 bispecifics.

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2. B7H3 x CD28 activates T cells only in the presence of B7H3 antigen

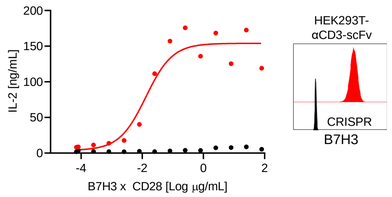

Figure 2: HEK293T cells stably expressing an anti-CD3-scFv (red) had B7H3 expression removed with CRISPR (black) and were then mixed with T cells and indicated concentrations of B7H3 x CD28 bispecific antibody. IL2 was measured 24 h post treatment.

3. B7H3 x CD28 is not a superagonist

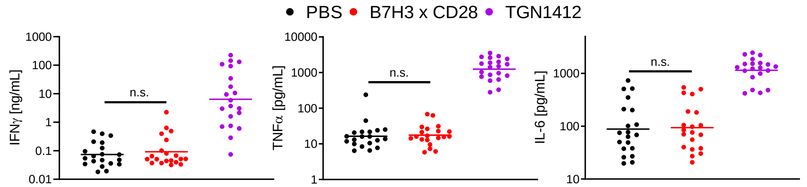

Figure 3: PBMC from 20 healthy donors were treated with indicated air-dried antibodies for 24 h. TGN1412 is an αCD28 superagonistic antibody.

4. B7H3 x CD28 combines with αPD1 mAb

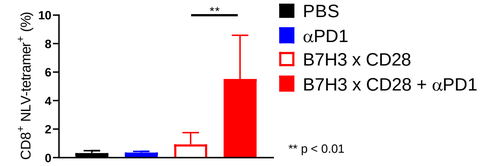

Figure 4: MCF-7 cancer cells were ectopically loaded with pp65-derived NLV-peptide for 24 h. The following day T cells from a CMV+ donor were added with indicated antibodies. Expansion of CMV+ T cells was measured with an NLV-specific tetramer 6 days post antibody treatment.

5. B7H3 x CD28 enhances PSMA x CD3 activity only in the presence of PSMA antigen

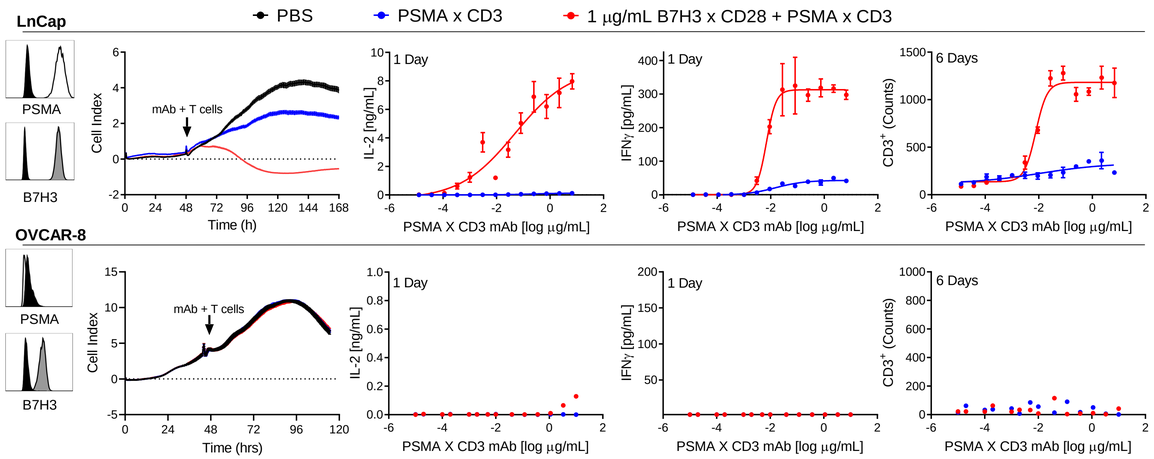

Figure 5: PSMA positive or negative cell lines were mixed with T cells and indicated antibodies. Cancer cell growth was assayed over time with Xcelligence, while cytokine production and T cell counts were measured at indicated times post antibody treatment.

6. B7H3 x CD28 enhances anti-tumor efficacy of a CD3 bispecific T cell engager

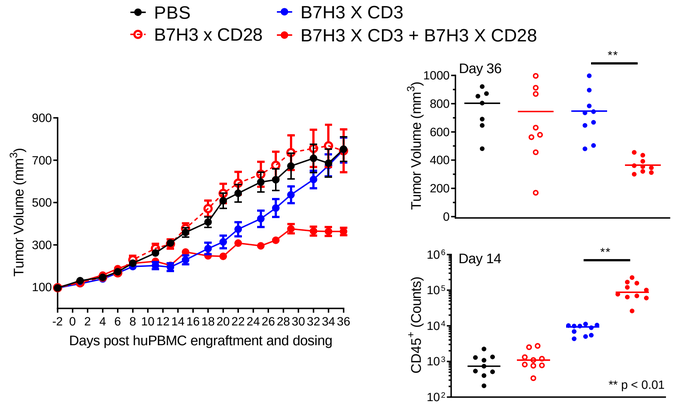

Figure 6: MDA-MB-231 cells were inoculated in NSG-DKO mice and allowed to form palpable tumors. Human PBMC were then engrafted along with weekly injections of indicated antibodies. Tumor measurements were measured with calipers and CD45+ counts shown were from the peripheral blood of the mice at indicated time point. Non-competing B7H3 epitopes were used in B7H3 x CD28 and B7H3 x CD3.

7. PDL1 x CD28 induces expansion of CMV reactive T cells

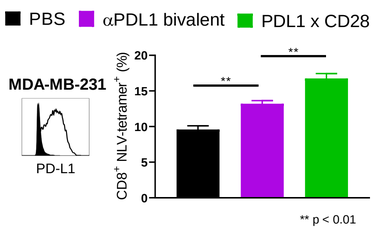

Figure 7: MDA-MB-231 cancer cells were ectopically loaded with pp65-derived NLV-peptide for 24 h. The following day T cells from a CMV+ donor were added with indicated antibodies. Expansion of CMV+ T cells was measured with an NLV-specific tetramer.

8. PDL1 x CD28 enhances activity of a CD3 bispecific T cell engager

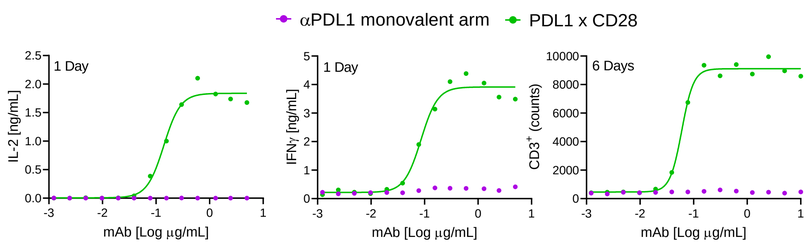

Figure 8: MDA-MB-231 (attached overnight) and T cells were mixed with indicated antibodies in the presence of 1 μg/mL B7H3 x CD3 antibody. Cytokine production and T cell counts were measured at indicated times.

9. PDL1 x CD28 inhibits tumor growth more effectively than an αPDL1 bivalent

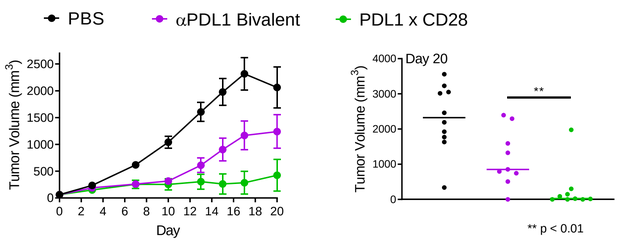

Figure 9: MC38 cells stably expressing hPDL1 antigen were subcutaneously inoculated in hCD28 knock in mice. When tumors were palpable Indicated antibodies were dosed weekly starting at Day 0. Tumor measurements were measured with calipers.