Costimulatory CD28 trispecific antibodies targeting PDL1 and/or PDL2 enhance T cell activation in solid tumors

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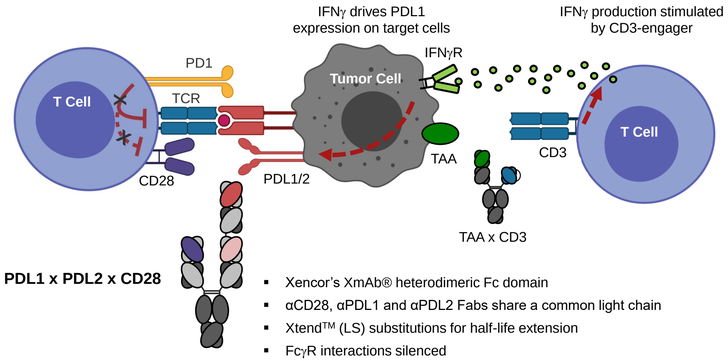
Introduction

- T cells in the tumor microenvironment require TCR/MHC engagement and costimulatory receptor engagement to achieve optimal activation.
- CD28 is a classical costimulatory receptor expressed on T cells, and tumor cells lack expression of CD28 ligands, so we hypothesized that activation of CD28 signaling at the T cell/tumor cell interface would enhance anti-tumor activity.
- We designed a PDL1 x PDL2 x CD28 XmAb® trispecific antibody that provides CD28 costimulation in the presence of PDL1 or PDL2 antigen expression, and TCR engagement.
- Since PDL1 can inhibit CD28 costimulation, this trispecific has the potential to costimulate CD28 while simultaneously blocking the PD1/PDL1 axis which can suppress activity.
- Furthermore, since CD3 bispecific T cell engagers are known to indirectly promote PDL1 and PDL2 expression via IFNγ, PDL1 x PDL2 x CD28 XmAb® trispecific antibodies can potentially be applied in combination to CD3-bispecific T cell engagers to enhance their activity.

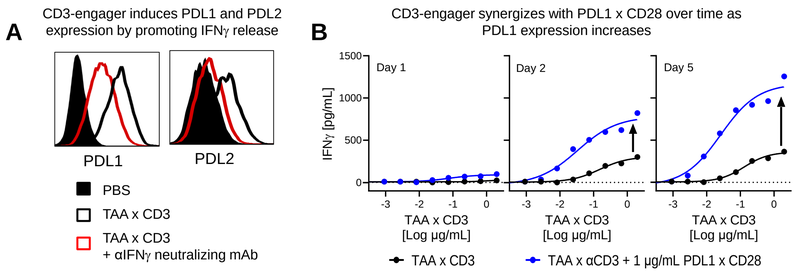
PDL1 x PDL2 x CD28 provides costimulation to T cells while also blocking PD1-mediated suppression



PDL1 x PDL2 x CD28

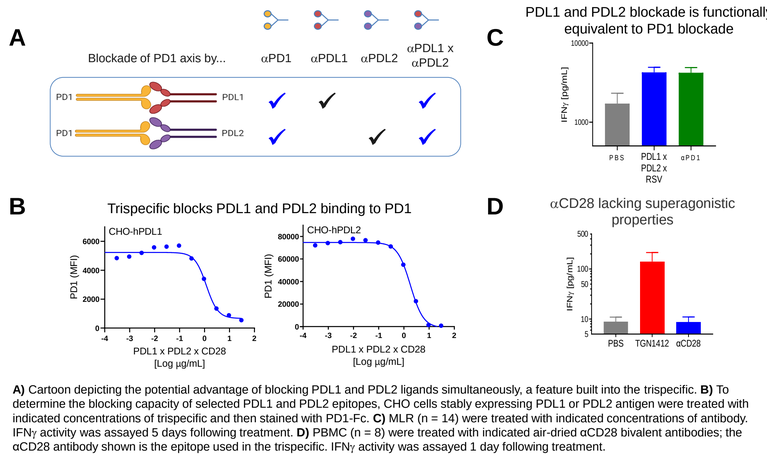
- Xencor's XmAb® heterodimeric Fc domain
- αCD28, αPDL1 and αPDL2 Fabs share a common light chain
- Xtend™ (LS) substitutions for half-life extension
- FcγR interactions silenced

PDL1 and PDL2 expression is inducible with a CD3-engager, highlighting PDL1 and PDL2 as targets for CD28 costimulation



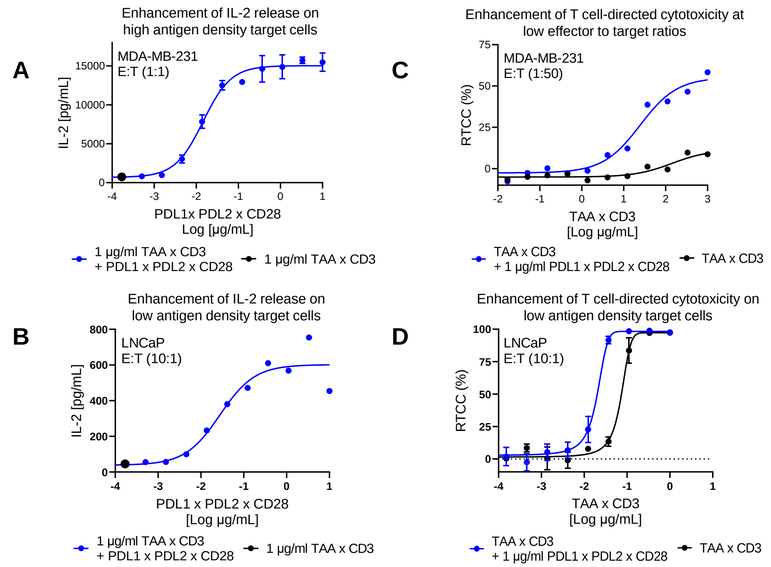
PC3 cell coculture with T cells and then treated with indicated antibodies for indicated times. **A)** FACS analysis of PDL1 expression on PC3 cells cultures with T cells after treatment with TAA x CD3 and IFNγ neutralizing mAb. **B)** IFNγ was measured in culture supernatants at indicated times after antibody treatment.

PDL1 x PDL2 x CD28 is comprised of αPDL1/αPDL2 antagonists and an αCD28 lacking superagonistic properties



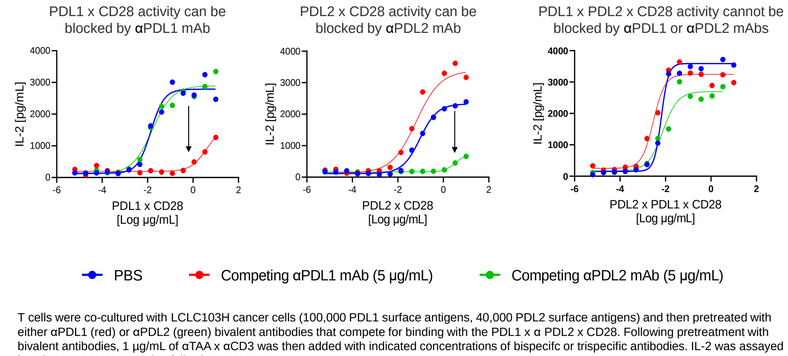
A) Cartoon depicting the potential advantage of blocking PDL1 and PDL2 ligands simultaneously, a feature built into the trispecific. **B)** To determine the blocking capacity of selected PDL1 and PDL2 epitopes, CHO cells stably expressing PDL1 or PDL2 antigen were treated with indicated concentrations of trispecific and then stained with PD1-Fc. **C)** MLR (n = 14) were treated with indicated concentrations of antibody. IFNγ activity was assayed 5 days following treatment. **D)** PBMC (n = 8) were treated with indicated air-dried αCD28 bivalent antibodies; the αCD28 antibody shown is the epitope used in the trispecific. IFNγ activity was assayed 1 day following treatment.

PDL1 x PDL2 x CD28 enhances the activity of a CD3-engager



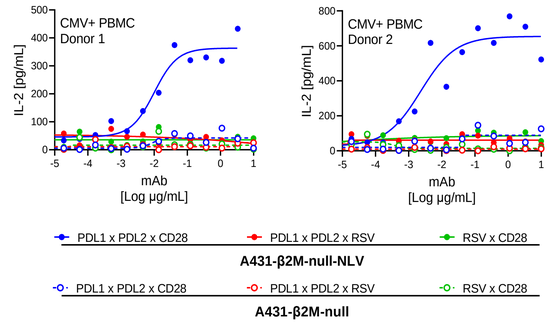
T cells were co-cultured with (A & C) MDA-MB-231 (130,000 PDL1 antigens) or (B & D) LNCaP (13,000 PDL1 antigens) and treated with indicated concentrations of antibodies. IL-2 activity was assayed 1 day following treatment. RTCC activity was measured by luminescence 5 days following treatment.

PDL1 x PDL2 x CD28 requires either PDL1 or PDL2 expression for activity



T cells were co-cultured with LCLC103H cancer cells (100,000 PDL1 surface antigens, 40,000 PDL2 surface antigens) and then pretreated with either αPDL1 (red) or αPDL2 (green) bivalent antibodies that compete for binding with the PDL1 x α PDL2 x CD28. Following pretreatment with bivalent antibodies, 1 μg/mL of αTAA x αCD3 was then added with indicated concentrations of bispecifc or trispecific antibodies. IL-2 was assayed in culture supernatants1 day following treatment.

PDL1 x PDL2 x CD28 enhances a T cell recall response in a CD28- and HLA-dependent manner



A CMV recall assay derived with A431-β2M-null cells (40,000 PDL1 antigen) stably expressing a fusion of HLA-A2, β2M and NLV-peptide, a peptide presented by CMV-infection (*Carreno et. al., J Imm. 188:5839-5849, 2012*). CD3+ enriched T cells from two unique CMV+ PBMC donors were mixed with indicated cell lines and antibodies. IL-2 was measured in culture supernatants 24 h following treatment.

Summary

- PDL1 x PDL2 x CD28 XmAb® trispecific antibodies engineered to costimulate CD28 while simultaneously blocking PDL1/PDL2 were evaluated in vitro.
- PDL1 x PDL2 x CD28 enhanced the activity of TAA x CD3 bispecifics and native TCR/MHC-I interaction in vitro.
- CD3 bispecific T cell engagers can indirectly promote PDL1 and PDL2 expression via IFNγ, highlighting these antigens as targets for CD28 costimulation.