Background T cells in the tumor micro-environment require TCR/MHC engagement and co-stimulatory receptor engagement to achieve optimal activation. Tumor cells lack expression of CD28 ligands, so we hypothesized that activation of CD28 signaling at the T cell/tumor cell interface could enhance anti-tumor activity. We designed PDL1 x PDL2 x CD28 trispecific antibodies that conditionally costimulate CD28 only in the presence of PDL1 or PDL2 expression, and TCR engagement. As PD1 signaling has been shown to directly inhibit CD28 costimulation, this novel bispecific modality has potential to promote CD28 costimulation while simultaneously preventing the suppression of the same signal. Furthermore, since CD3 bispecific T cells engagers are known to indirectly promote PDL1 expression, PDL1 x PDL2 x CD28 trispecific antibodies can potentially be applied in combination to CD3-bispecific T cell engagers to enhance their activity.

Methods We developed a CD28 antibody that is agonistic only under the context of TCR engagement. We paired this CD28 binding domain with PDL1 and PDL2 Fab binding domains that share a common light chain to generate PDL1 x PDL2 x CD28 trispecifics monovalent for each of the three antigens. We optimized the affinity for each of the antigens for potent blockade of PDL1 and PDL2 and enable CD28 costimulation across a range of PDL1 and PDL2 cell surface densities. Cell based assays with different TCR engagement modalities were utilized to describe the function of trispecifics including, CD3-stimulated T cells cocultured with cancer cell lines, CMV+ T cells recognizing cancer cells expressing pp65-derived NLV peptide, and Mixed Lymphocyte Reactions. Cell based assays measured IL2 and IFNγ release, T cell proliferation and cancer-cell death as readouts for activity.

Results PDL1 x PDL2 x CD28 trispecifics block PDL1 and PDL2 binding to PD1 in both solid phase and cell-based assays. PDL1 and PDL2 blockade led to enhanced cytokine secretion from human mixed lymphocyte reactions. Target cell expression of either PDL1 or PDL2 was shown to be sufficient to promote CD28 costimulation leading to acute IL2 release of cocultures of CD3-stimulated PBMC with cancer cell lines. In a more physiologically relevant model of TCR engagement, PDL1 x PDL2 x CD28 trispecifics enhanced T cell activation in a CMV-recall assay. When in combination with CD3-targeted T cell engagers, PDL1 x PDL2 x CD28 trispecifics enhanced T cell proliferation and TDCC activity.

Conclusions Data warrant further development of PDL1 x PDL2 x CD28 trispecifics for treatment of solid tumors.