immune function is unclear, with both costimulatory and proapoptotic roles described. CD30 is transiently upregulated following activation of memory T cells and expression has been linked to highly activated/suppressive IRF4+ effector Tregs.

Methods Here we evaluated the activity of BV on CD30-expressing T cell subsets in vitro and in vivo.

Results Treatment of enriched T cell subsets with clinically relevant concentrations of BV drove selective depletion of CD30-expressing Tregs > CD30-expressing CD4+ T memory cells, with minimal effects on CD30-expressing CD8+ T memory cells. In a humanized xeno-GVHD model, treatment with BV selectively depleted Tregs resulting in accelerated wasting and robust T cell expansion. The observed differential activity on Tregs is likely attributable to significant increases in CD30 expression and reduced efflux pump activity relative to other T cell subsets. Interestingly, blockade of CD25 signaling prevents CD30 expression on T cell subsets without impacting proliferation, suggesting a link between CD25, the high affinity IL-2 receptor, and CD30 expression.

Conclusions Together, these data suggest that BV may have an immunomodulatory effect through selective depletion of highly suppressive CD30-expressing Tregs.

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Ethics Approval Animals studies were approved by and conducted in accordance with Seattle Genetics Institutional Care and Use Committee protocol #SGE-024.

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697

TUMOR-TARGETED CD28 COSTIMULATORY BISPECIFIC ANTIBODIES ENHANCE T CELL ACTIVATION IN SOLID TUMORS

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Background T cells in the tumor micro-environment require TCR/MHC engagement and co-stimulatory receptor engagement to achieve complete activation. Solid tumors often lack expression of CD28 ligands, so we hypothesized that activation of CD28 signaling could be beneficial in solid tumors. We designed tumor-associated-antigen (TAA) x CD28 bispecific antibodies that conditionally costimulate CD28 only in the presence of TAA and TCR engagement. 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.

Methods We designed a stability and affinity optimized anti-CD28 antibody that can be paired with TAA of choice to engage CD28 monovalently using Xencor's XmAb 2+1 and 1+1 platforms. In vitro T cell activation with these bispecifics was measured by T cell proliferation, cytokine production, and cytotoxicity, in co-cultures of human cancer cell lines mixed with primary human CD3-stimulated T cells. In vitro activity was validated in a CMV recall assay measuring CMV+ T cell proliferation of CMV+ PBMC co-cultured with cancer cell lines ectopically treated with pp65-derived NLV-peptide. In vivo anti-tumor and T cell proliferative activity of B7H3 x CD28 bispecific antibodies were determined in tumor-bearing huPBMC-NSG mice treated simultaneously with TAA x CD3 bispecific antibody. In vivo activity of PDL1 x CD28 antibodies was determined with hCD28 KI mice inoculated with

MC38 tumors expressing hPDL1-antigen. Finally, safety and tolerability of B7H3 x CD28 and PDL1 x CD28 was determined in cynomolgus monkeys.

Results B7H3 x CD28 and PDL1 x CD28 antibodies enhanced T cell degranulation, cytokine secretion, and cancer cell cytotoxicity in concert with CD3 stimulation only in the presence of target antigen. B7H3 x CD28, alone or in combination with anti-PD1 antibody, enhanced proliferation of CMV+ T cells recognizing cancer cells loaded with pp65-derived NLV peptide. PDL1 x CD28 also enhanced CMV+ cell expansion but did not synergize with anti-PD1 antibody treatment. B7H3 x CD28 significantly enhanced in vivo anti-tumor activity of TAA x CD3 antibodies while also promoting greater T cell expansion. In hCD28 mice inoculated with MC38 tumors expressing hPDL1, PDL1 x CD28 antibody inhibited tumor growth greater than an anti-PDL1 antibody alone. B7H3 x CD28 and PDL1 x CD28 were well tolerated in cynomolgus monkeys.

Conclusions B7H3 x CD28 and PDL1 x CD28 bispecific antibodies show promising anti-tumor activity and warrant further development.

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698

TARGETING HLA-G-MEDIATED IMMUNOSUPPRESSION WITH A FIRST-IN-CLASS ANTAGONIST ANTIBODY

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Background Human leukocyte antigen-G (HLA-G) is an immune checkpoint molecule that belongs to the non-classical HLA-class I family of receptors. HLA-G restrains immune cell activation and effector function by engaging with inhibitory receptors ILT2 and ILT4. While expression of HLA-G is highly restricted under normal healthy conditions, we have demonstrated that its expression in cancer is aberrantly upregulated and broadly detected across a variety of tumor types. Tizona Therapeutics has generated a novel, fully human antibody that specifically targets HLA-G and reverses HLA-G-mediated immunosuppression. Here we present in vitro and in vivo data demonstrating the functional impact of HLA-G blockade on immune cells and evidence to support the use of TTX-080 in the clinic to treat patients with advanced solid tumors.

Methods Evaluation of HLA-G expression in cancer was performed using immunohistochemistry, flow cytometry, and gene profiling. Expression of ILT2 and ILT4 was assessed on tumor infiltrating leukocytes by flow cytometry. To demonstrate the suppressive function of HLA-G, primary human NK cells, T cells, and monocyte-derived macrophages were cultured with target cells expressing HLA-G. TTX-080 was then evaluated for its ability to reverse this suppression. In addition, TTX-080 was investigated in vivo using a disseminated xenograft tumor model.

Results Expression of HLA-G was detected on tumor cells and tumor infiltrating leukocytes across a variety of solid tumor types. TTX-080 blocked interaction of HLA-G with both ILT2 and ILT4 and restored cytotoxicity in multiple assays using either primary NK cells or NK cell lines. Monocyte-derived macrophages expressing ILT2 and ILT4 exhibited decreased phagocytosis of HLA-G⁺ target cells; this inhibition was reversed with an antigen-binding fragment of TTX-080. TTX-