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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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Background 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 PD1 x TGFβR2 bispecific antibody could selectively block the suppressive activity of TGFβ on tumor T cells and enhance their anti-tumor activity while avoiding the toxicity associated with systemic blockade.

Methods We engineered bispecific antibodies that simultaneously engage PD1 and TGFβR2 using Xencor's XmAb platform. The anti-TGFβR2 arm was tuned for optimal activity by introducing affinity-modulating amino acid substitutions. The activity of PD1 x TGFβR2 bispecifics was evaluated in vitro using a signaling assay to measure phosphorylated SMAD (pSMAD) by flow cytometry with exogenous TGFβ in unactivated and activated PBMC. In vivo activity was evaluated by monitoring the engraftment of human PBMC in NSG mice (huPBMC-NSG). Anti-tumor activity was assessed in huPBMC-NSG mice engrafted with established human cancer cell lines. Antibodies against other T cell targets were also incorporated into TGFβR2 bispecifics, and similarly evaluated in vitro and in vivo.

Results PD1 x TGFβR2 bispecifics were confirmed to bind PD1 and block binding of TGFβ to TGFβR2. In vitro, we found that T cells from activated, serum-deprived PBMC exhibited robust induction of pSMAD in response to TGFβ, and PD1 x TGFβR2 bispecifics selectively inhibited pSMAD induction in PD1-positive T cells as demonstrated by over a 100-fold potency increase compared to an untargeted anti-TGFβR2 control. Additionally, we saw an enhancement of potency when evaluating blocking activity in activated (PD1-high) vs. unactivated (PD1-low) T cells. Similar selectivity was measured when comparing inhibition of pSMAD induction for activated T cells versus other PD1-negative, TGFβ-responsive immune cells. Intriguingly, TGFβR2 bispecifics incorporating antibodies against other T cell targets allowed for the targeting of a broader population of T cells while still conferring potent selectivity against target-negative cells. In vivo, treatment of huPBMC-NSG mice with TGFβR2 bispecifics promoted superior T cell engraftment and combined additively with PD1 blockade. Furthermore, TGFβR2 bispecific treatment of huPBMC-NSG mice containing established MDA-MB-231 triple-negative breast cancer tumors promoted an anti-tumor response that was also augmented with PD1 blockade.

Conclusions Multiple PD1 x TGFβR2 bispecifics were engineered to selectively block TGFβR2 on PD1-positive T cells and evaluated in vitro and in vivo. Compelling activity, including additivity with PD1 blockade, suggests that clinical development is warranted for the treatment of human malignancies.

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FS118, A TETRAVALENT BISPECIFIC ANTIBODY TARGETING LAG-3 AND PD-L1, INDUCES LAG-3 SHEDDING RESULTING IN RECEPTOR DOWNREGULATION BY T CELLS VIA A NOVEL MECHANISM OF ACTION

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Background Upregulation of immune checkpoints, such as LAG-3, plays an important role in promoting resistance to anti-PD-(L)1 therapy. FS118, currently being evaluated in a Phase 1 clinical trial in patients with advanced malignancies, is a tetravalent bispecific antibody targeting LAG-3 and PD-L1 that can overcome immune suppressive signals with greater preclinical activity than a combination of monoclonal antibodies.¹ Here, we demonstrate a novel mechanism of action for FS118 in shedding of LAG-3 from the surface of T cells that is not observed with the combination of PD-L1 and LAG-3 antibodies.

Methods Human ex vivo assays were performed by co-culturing activated CD4+ T cells with iDCs in the presence of Staphylococcal enterotoxin B and FS118, or control reagents. Soluble LAG-3 was measured by ELISA from day 4 to 13. A mouse tumor model used MC38 cells implanted subcutaneously into C57Bl/6 mice. Expression of surface markers was measured on tumor-infiltrating lymphocytes (TILs) from disaggregated tumors and soluble LAG-3 was measured in serum following dosing of mice intraperitoneally with FS118 surrogate or control reagents. Soluble LAG-3 in the serum of patients treated with FS118 was measured by ELISA (Phase 1 trial NCT03440437).

Results In an ex vivo T cell assay, FS118 resulted in an increase in the concentration of soluble LAG-3 in the cell culture medium, an effect that was greater than with the combination of the individual bispecific components. Addition of inhibitors of either ADAM10 or ADAM17 to the FS118-treated cells resulted in a decrease in the levels of soluble LAG-3 in the cell culture medium. In MC38 tumor-bearing mice, a mouse surrogate of FS118 decreased the levels of surface LAG-3 expressed by TILs, in contrast to the combination of the bispecific components where an increase in surface LAG-3 was observed. This corresponded with an increase in soluble LAG-3 in the serum following treatment with a mouse surrogate of FS118. Finally, in patients receiving treatment with FS118, a dose dependent increase in soluble LAG-3 was detected in the blood.

Conclusions FS118 mediates LAG-3 shedding from the surface of immune cells via a mechanism that is dependent upon simultaneous binding to both PD-L1 and LAG-3. This shedding was mediated by ADAM10 and ADAM17 metalloproteinases. Removing LAG-3 from the surface of TILs via shedding may be an important mechanism by which FS118 overcomes compensatory upregulation of LAG-3 induced by PD-L1