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TRITCE CO-STIM: A NOVEL TRISPECIFIC T CELL ENGAGER PLATFORM, WITH INTEGRATED CD28 COSTIMULATION, ENGINEERED TO WIDEN THE THERAPEUTIC WINDOW FOR TREATMENT OF POORLY INFILTRATED TUMORS

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Background Bispecific T cell engagers (TCEs) have shown clinical benefit in treating hematological cancers, but limited success in solid tumors. Overcoming the immunosuppressive environment and low T cell infiltration remain some of the key challenges limiting the activity of traditional CD3-engaging bispecific TCEs. Conventional T cell activation requires signaling via CD3 (signal 1) and costimulatory molecules (signal 2), such as CD28. Superagonist anti-CD28 antibodies activate T cells but resulted in clinical toxicities with severe cytokine release. Therefore, the balance between signals 1 and 2 is critical for optimal T cell activation and proliferation. Using our Azymetric™ and EFECT™ technologies, we generated heterodimeric costimulatory trispecific TCE (TriTCE Co-stim) antibodies with silenced Fc gamma function to optimally engage CD3, CD28, and CLDN18.2. We previously identified a lead trispecific format with improved in vitro cytotoxicity and in vivo anti-tumor activity compared to traditional bispecific TCEs. Here, we further characterize the safety profile, anti-tumor properties, and the mechanism of action of our lead TriTCE Co-stim.

Methods To understand safety, we investigated cytokine production by monocultures of T cells or PBMCs as well as using predictive in vitro and in vivo models of cytokine release syndrome (CRS). We further assessed the ability of our lead format to induce cytotoxicity of T cells. Human PBMC-engrafted CLDN18.2-expressing xenograft models were used to assess in vivo anti-tumor activity and T cell infiltration following treatment with TriTCE Co-stim. Upregulation of effector and central memory and exhaustion markers were interrogated in vitro. To understand the impact of co-engagement of CD3 and CD28 with a trispecific molecule, we evaluated cytokine production and tumor cytotoxicity in vitro compared to a combination of CD3 and CD28-engaging bispecific TCEs.

Results We observed minimal induction of cytokine by TriTCE Co-stim in monocultures of PBMCs and T cells and similarly with in vitro and in vivo models of CRS. Furthermore, our lead TriTCE Co-stim induced no cytotoxicity of T cells. TriTCE Co-stim exhibited enhanced antitumor activity and T cell infiltration in vivo compared to bispecific TCE with increased effector and memory subsets in vitro. Finally, our lead TriTCE Co-stim exhibited enhanced cytotoxicity with reduced cytokine production by T cells compared to a combination of CD3- and CD28-engaging bispecific TCEs.

Conclusions These data suggest TriTCE Co-stim may provide tolerable and more durable anti-tumor responses and re-invigorate poorly infiltrated tumors. Taken together, our lead TriTCE Co-stim demonstrates favorable characteristics that may contribute to improved clinical outcomes.

Ethics Approval The protocol and procedures involving the care and use of animals in these studies was conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and were reviewed and approved by the Institutional Animal

Care and Use Committee (IACUC; CrownBio, Jackson ImmunoResearch).

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.1372>