1396 TRITCE CPI: A NOVEL TRISPECIFC T CELL ENGAGER PLATFORM WITH INTEGRATED PD-1/PD-L1 CHECKPOINT INHIBITION ENGINEERED FOR THE TREATMENT OF IMMUNOSUPPRESSED TUMORS

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Background Immunosuppression in the solid tumor microenvironment (TME) is a critical obstacle that has limited the efficacy of T cell engager (TCE) immunotherapies. Though TCEs can direct T cell cytotoxicity towards tumors, T cell activation and inflammation can induce tumor cell and T cell expression of immune checkpoint proteins, such as PD-L1. This treatment-related increase in immune suppression in the TME further limits clinical responses.

We have previously presented screening data on a panel of trispecific T cell engagers (TriTCEs). Preliminary mechanistic data showed enhanced antitumor activity where TriTCEs can concurrently direct T cell activity towards tumor cells while eliciting PD-1/PD-L1 checkpoint inhibition (CPI) by the addition of an affinity-engineered PD-1 domain to the TCE. Here, we present data that further characterizes and differentiates the lead TriTCE CPI formats.

Methods Lead TriTCE CPI formats were screened for potency *in vitro*. Co-engagement of CD3, tumor-associated antigen (TAA), and PD-L1 by TriTCE CPIs was determined using on-cell binding measurements to exhausted T cells and tumor cells stimulated with IFNγ to upregulate PD-L1 and recapitulate cellular phenotypes expected to be found in an immunosuppressed TME. TriTCE CPI mediated T cell activation in the presence of PD-L1 expressing dendritic cells was assayed in co-culture. *In vivo* activity was determined using humanized PBMC and syngeneic mouse models.

Results Lead TriTCE CPI formats were compared by assaying TAA-dependent cytotoxicity where addition of affinity-engineered PD-1 increased potency *in vitro*. Format-dependent differences in PD-1 location on the TriTCE and the presence of one or two α-TAA binding arms was found to enhance avidity-driven binding, which translated into increased T cell-dependent cytotoxicity compared to clinical benchmark controls. Lead TriTCE CPIs had broad anti-tumor activity across tumor cell lines with varying TAA and PD-L1 expression levels. *In vivo* testing of lead TriTCE CPI formats demonstrated tumor growth inhibition and enabled preliminary assessment of toxicity. A threshold for tolerability of affinity-engineered PD-1 was identified in humanized syngeneic mouse model.

Conclusions Our next-generation TriTCE CPIs aim to leverage PD-L1-mediated immunosuppression for enhanced avidity-driven tumor cell targeting and CPI in the TME to improve T cell responses in solid tumors. Addition of an affinity-engineered PD-1 to the TCE enhanced activity across multiple tumor cell lines compared to a bispecific TCE, supporting that TriTCE CPIs can be active against primary and acquired PD-L1 resistance mechanisms. Humanized immunocompetent syngeneic mouse models suggest a widened therapeutic window based on measures of tumor regression with a favorable safety profile.

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).

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