TriTCE CPI, Next Generation Trispecific T Cell Engagers with Integrated Checkpoint Inhibition (CPI) for the Treatment of Solid Tumors

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Introduction

CD3-bispecific T cell engager (TCE) therapies have exhibited clinical utility against hematological malignancies, but successes in solid tumor indications have been limited. Compared to hematological malignancies, treatment of solid tumors is hindered by immunosuppressed microenvironments that can be refractory to traditional CD3-bispecific TCEs. Immunosuppression in the tumor microenvironment limits treatment responses in part due to the expression of inhibitory immune checkpoints, such as PD-1 on exhausted T cells and PD-L1 on tumor cells. To improve T cell responses and anti-tumor activity in immunosuppressed solid tumors, we generated trispecific TCE antibodies (Abs) that target a tumor associated antigen (TAA), CD3 and PD-L1 (via a PD1 moiety) to stimulate tumor-directed T cell killing and checkpoint blockade at the tumor site (TriTCE CPI).

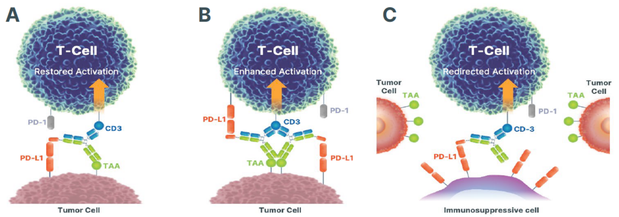

Figure 1. Proposed mechanisms of action for TriTCE CPI therapeutics. (A) TCE activity with concurrent blockade of PD-L1/PD-1 interactions between T cells and tumor cells. **(B)** Avidity-driven binding to tumor cells and/or exhausted T cells overexpressing PD-L1 in the tumor microenvironment (TME). **(C)** Activation and/or elimination of suppressive immune cells in the TME.

TriTCE CPIs Contain a PD-1 Domain Engineered to have Increased Affinity for PD-L1 Relative to Wild Type PD-1

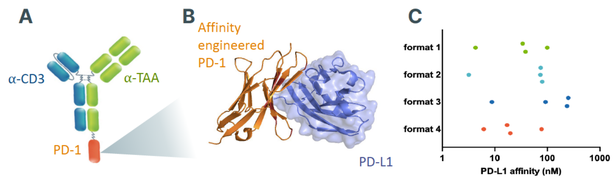

Figure 2. Modular PD-1 domain appended to TCEs is engineered to have increased affinity for PD-L1. (A) Representative PD-1 TriTCE CPI format diagram. TriTCE CPIs are composed of anti-CD3 targeting and anti-TAA targeting paratopes and affinity engineered PD-1 domain. **(B)** PD-1 domain is engineered to have increased affinity for native PD-L1. **(C)** PD-1 domain library affinity for PD-L1 in the context of different TriTCE CPI formats was measured by surface plasmon resonance. A panel of PD-1 domains were engineered with a range of binding affinities for PD-L1 (2-650 nM). Wild type PD-1 binds PD-L1 with 8 μM affinity[1].

TriTCE CPIs Were Screened for Activity using T Cell Cytotoxicity and PD-1/PD-L1 Checkpoint Blockade Assays

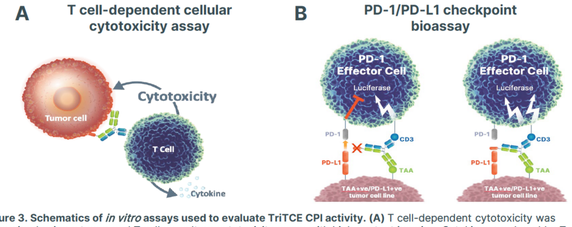

Figure 3. Schematics of *in vitro* assays used to evaluate TriTCE CPI activity. (A) T cell-dependent cytotoxicity was determined using a tumor and T cell co-culture cytotoxicity assay with high content imaging. Cytokines produced by T cells following co-culture were also measured by electrochemiluminescence immunoassay (Meso Scale Discovery). **(B)** The affinity engineered PD-1 domain on TriTCE CPI were evaluated for PD-1/PD-L1 checkpoint blockade by a T cell activation reporter gene assay where production of luciferase was contingent on both PD-1 effector cell (via CD3) and tumor cell cross-linking (via TAA, PD-L1) and PD-1/PD-L1 checkpoint blockade by the TriTCE CPI. Tumor cells are pretreated with IFNγ 24 hours prior to assay to induce upregulation of PD-L1.

Different TriTCE CPI Geometries Were Screened to Identify Formats Where Addition of Affinity-engineered PD-1 Domain Potentiated Increased T cell-dependent Cytotoxicity

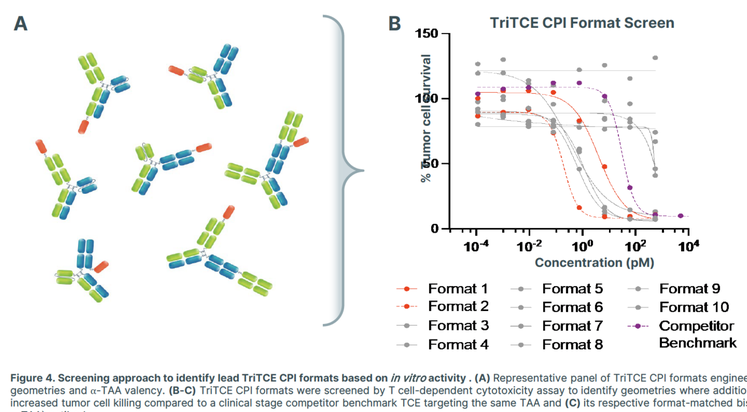

Figure 4. Screening approach to identify lead TriTCE CPI formats based on *in vitro* activity . (A) Representative panel of TriTCE CPI formats engineered with different geometries and α-TAA valency. **(B-C)** TriTCE CPI formats were screened by T cell-dependent cytotoxicity assay to identify geometries where addition of PD-1 increased tumor cell killing compared to a clinical stage competitor benchmark TCE targeting the same TAA and **(C)** its respective format-matched bispecific (α-CD3 x α-TAA) antibody.

Lead TriTCE CPI Formats were Identified by TAA-dependent Cytotoxicity and PD-1/PD-L1 Checkpoint Blockade Activity

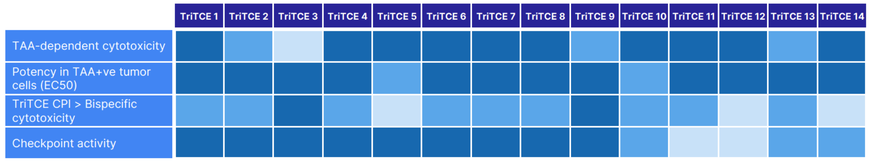

Figure 5. Lead TriTCE CPI selection criteria. T cell-dependent cellular cytotoxicity assay and PD-1/PD-L1 checkpoint bioassay data in both TAA+ and TAA- tumor cell lines are evaluated to select candidate formats and tune affinity of the engineered PD-1 domain for *in vivo* anti-tumor response studies.

TriTCE CPIs Induce TAA-dependent T Cell Cytotoxicity and PD-1/PD-L1 Checkpoint Blockade

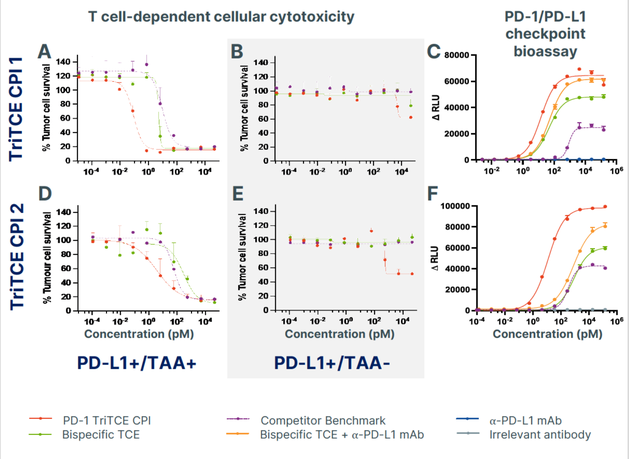

Figure 6. TriTCE CPI formats were evaluated *in vitro* to determine the effect of PD-L1 affinity on cytotoxicity, PD-1/PD-L1 checkpoint blockade, and potency on PD-L1+/TAA- cells. Cytotoxicity assays were used to identify TriTCE CPIs that activate T cell-dependent tumor killing of **(A, D)** PD-L1+/TAA+ cell lines but not **(B,E)** PD-L1+/TAA- cell lines. **(C, F)** PD-1/PD-L1 checkpoint assay was used to identify formats which can block PD-1 and PD-L1 interaction while stimulating T cells in a TAA dependent manner.

TriTCE CPIs Promote Strong *In Vivo* Anti-tumor Responses

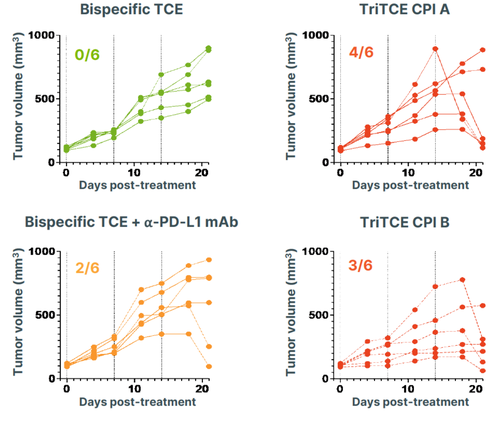

Figure 7. TriTCE CPI therapeutics display superior anti-tumor activity compared to bispecific TCE in preliminary *in vivo* assessment. Female NCG mice were inoculated with 5x10⁶ tumor cells (TAA^mid/PD-L1^lo) 1:1 in Matrigel. Tumors were grown to 100-120mm³, randomized and PBMCs injected IV. Upon randomization, mice were dosed with 0.01mg/kg TriTCE CPI A, TriTCE CPI B or format matched bispecific TCE (IV – QW; dotted line) and 5mg/kg α-PD-L1 mAb (IP - BIW) as indicated. Tumor growth was monitored twice weekly. Treatment with TriTCE CPI A and TriTCE CPI B significantly inhibited tumor growth compared to bispecific TCE control treatment ($p < 0.05$). Score indicate the number of mice per group with tumor growth inhibition. These data supports the utility of affinity engineered PD-1 in TCE and trispecific advantage over the bispecific TCE treatment.

TriTCE CPI Formats Can be Fine-tuned to Optimize Dendritic Cell (DC)-Dependent T Cell Activation

Many types of immune cells (e.g., myeloid-derived suppressor cells, DCs, and Tregs) create an immunosuppressive TME by providing co-inhibitory signals via PD-L1 that can inhibit the function of T cells[2]. Furthermore, IFNγ released after T cell engagement can upregulate PD-L1 expression[3]. TriTCE CPIs were evaluated for their ability to modulate T cell agonism in the presence of PD-L1 expressing DCs in co-culture assay.

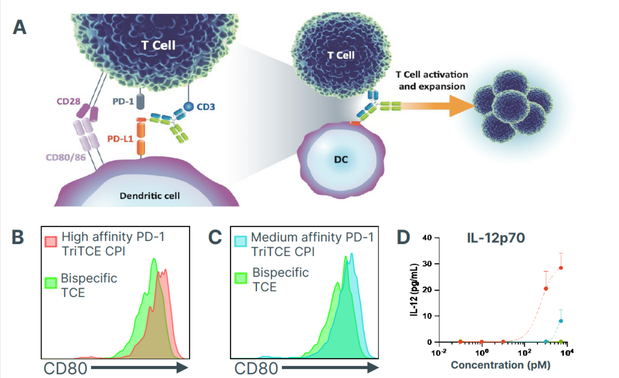

Figure 8. Bridging between DCs and T cells induces DC activation and T cell proliferation. (A) TriTCE CPIs can induce T cell activation by CD3 engagement on the T cells while blocking the PD-1/PD-L1 inhibitory interactions which has been shown to aid co-stimulatory interactions between CD80 and CD28[2]. **(B-H)** Autologous mature DC (CD14-CD11c+CD80+CD86+PDL1+) and T cells were co-cultured for 5 days with TriTCE CPIs, bispecific TCE control or combination treatments. **(B-C)** CD80 expression was elevated on DCs at 5 days post-treatment with 10 pM high affinity PD-1 TriTCE CPI **(B)** and 1000 pM medium affinity PD-1 TriTCE CPI **(C)** compared to bispecific TCE control at matched concentration in co-culture. **(D)** TriTCE CPI treatment induced production of the DC-produced T cell stimulatory cytokine, IL-12p70. **(E-H)** Dose-dependent increase in CD4+ and CD8+ T cell counts and activation (CD25+) was observed following TriTCE CPI treatment.

Conclusions

We have generated multiple TriTCE CPI antibodies that combine **tumor-dependent T cell cytotoxicity with checkpoint blockade**, which may translate to improved T cell responses in immunosuppressed solid tumors. Zymeworks' Azymetric™ technology serves as a versatile platform to generate multispecific TCEs with different molecular architecture to identify antibody formats with **enhanced avidity-driven tumor cell binding, TAA-dependent anti-tumor activity, and PD-1/PD-L1 checkpoint blockade**. These novel molecular design characteristics may widen the TCE therapeutic index and lead to improved clinical outcomes.

References
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