TriTCE CPI: a novel trispecific T cell engager platform with integrated PD-1/PD-L1 checkpoint inhibition engineered for the treatment of immunosuppressed tumors

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Introduction

Immunosuppression in the tumor microenvironment limits antitumor responses of conventional CD3-engaging bispecific T cell engagers (TCEs) in solid tumors

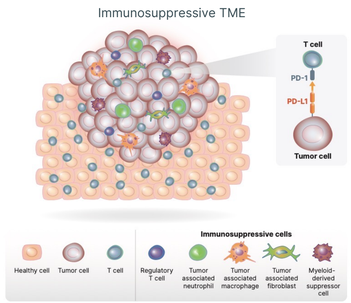
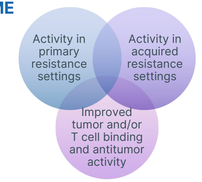


Figure 1. Immunosuppression in the tumor microenvironment (TME) can limit the cytotoxic potential of T cells in solid tumors. Immunosuppression is facilitated by **primary resistance** mechanisms, such as the expression of checkpoint inhibition proteins like PD-L1, and the presence of immunosuppressive immune cells. Though conventional TCEs can direct T cell cytotoxicity towards tumors, T cell activation and resultant inflammation can induce an **acquired resistance** mechanism of PD-L1 upregulation on tumor and T cells. This treatment-related increase in immune suppression in the tumor microenvironment (TME) can limit clinical responses.

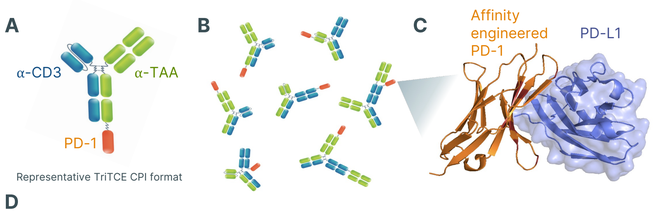
Trispecific TCEs with checkpoint inhibition (TriTCE CPI) are designed to increase T cell responses to address primary and acquired resistance mechanisms in the TME

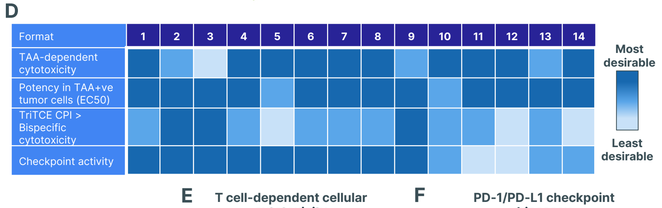
TriTCE CPIs with integrated CD3 and PD-L1 engagement (via an engineered PD-1 domain) have the potential to enhance T cell responses in immunosuppressed and exhausted T cell microenvironments.



TriTCE CPI design is optimized for format and affinity

TriTCE CPI formats are screened for increased antitumor activity and T cell responses, PD-1/PD-L1 checkpoint blockade, and avidity-driven binding





TriTCE CPIs were tested with multiple tumor-associated antigen (TAA) targets

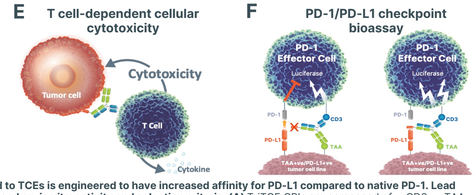


Figure 2. PD-1 domain appended to TCEs is engineered to have increased affinity for PD-L1 compared to native PD-1. Lead TriTCE CPI formats identified based on *in vitro* activity and selection criteria. (A) TriTCE CPIs are composed of α-CD3, α-TAA targeting paratopes, and an affinity engineered PD-1 domain. **(B)** Representative TriTCE formats screened. **(C)** PD-1 domain is engineered to have increased affinity for native PD-L1 compared to native PD-1. **(D)** TriTCE CPI selection criteria used for lead selection. **(E-F)** Schematics of the *in vitro* assays used to evaluate TriTCE CPI activity. **(E)** T cell-dependent cytotoxicity was determined using a tumor and T cell co-culture cytotoxicity assay with high content imaging. **(F)** TriTCE CPIs were evaluated for PD-1/PD-L1 checkpoint blockade using a reporter gene assay. Tumor cells are pretreated with IFNγ 24 hours prior to assay to induce upregulation of PD-L1.

TriTCE CPI mediate enhanced activity against PD-L1 positive tumor models

TriTCE CPI may improve responses in tumor settings of primary resistance

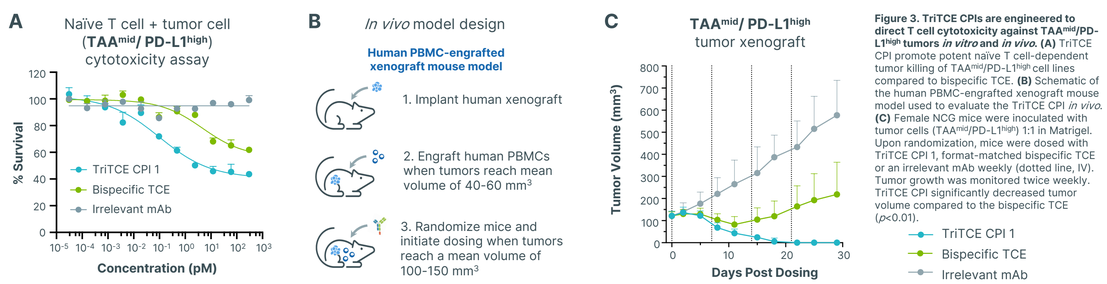


Figure 3. TriTCE CPIs are engineered to direct T cell cytotoxicity against TAA^{mid}/PD-L1^{high} tumors *in vitro* and *in vivo*. (A) TriTCE CPI promote potent naïve T cell-dependent tumor killing of TAA^{mid}/PD-L1^{high} cell lines compared to bispecific TCE. **(B)** Schematic of the human PBMC-engrafted xenograft mouse model used to evaluate the TriTCE CPI *in vivo*. **(C)** Female NCG mice were inoculated with tumor cells (TAA^{mid}/PD-L1^{high}) 1:1 in Matrigel. Upon randomization, mice were dosed with TriTCE CPI 1, format-matched bispecific TCE or an irrelevant mAb weekly (dotted line, IV). Tumor growth was monitored twice weekly. TriTCE CPI significantly decreased tumor volume compared to the bispecific TCE ($p<0.01$).

TriTCE CPI exhibit higher binding and cytotoxicity with exhausted T cells

T cell exhaustion occurs in settings of primary and acquired resistance
TriTCE CPI may improve T cell responses in resistant settings

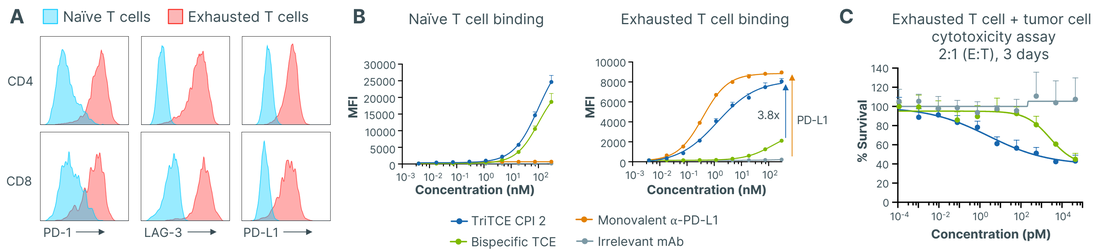


Figure 4. TriTCE CPI formats can direct tumor cell killing by exhausted T cells *in vitro*. (A) Naïve T cells were exhausted by repeat α-CD3/α-CD28 stimulation every second day for a total of 8 days. Expression of exhaustion markers was measured by flow cytometry. **(B)** TriTCE CPI and bispecific TCE show comparable binding to naïve T cells. Binding measurement to exhausted T cells resulted in increased binding of the TriTCE CPI compared to bispecific TCE due to increased PD-L1 expression. **(C)** Cytotoxicity assays demonstrate that the TriTCE CPI can direct tumor cell killing with exhausted T cells.

TriTCE CPI mediate enhanced binding and antitumor activity against tumor models with inducible PD-L1 expression

Upregulation of PD-L1 expression occurs following TCE treatment and is a mechanism of acquired resistance
TriTCE CPI may show enhanced antitumor activity in settings of acquired resistance

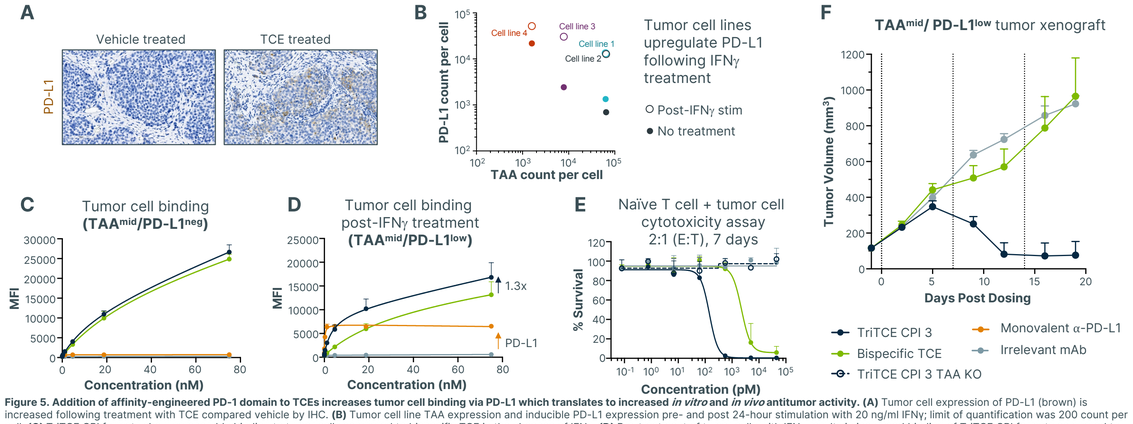


Figure 5. Addition of affinity-engineered PD-1 domain to TCEs increases tumor cell binding via PD-L1 which translates to increased *in vitro* and *in vivo* antitumor activity. (A) Tumor cell expression of PD-L1 (brown) is increased following treatment with TCE compared vehicle by IHC. **(B)** Tumor cell line TAA expression and inducible PD-L1 expression pre- and post 24-hour stimulation with 20 ng/ml IFNγ; limit of quantification was 200 count per cell. **(C)** TriTCE CPI formats show comparable binding to tumor cells compared to bispecific TCE in the absence of IFNγ. **(D)** Pre-treatment of tumor cells with IFNγ results in increased binding of TriTCE CPI formats compared to bispecific TCE due to upregulation of PD-L1 on the tumor cells. **(E)** TriTCE CPI show increased T cell directed tumor cell killing in cytotoxicity assay compared to bispecific TCE. No TAA-independent activity is observed following treatment with a TriTCE TAA KO (knockout) control deficient in TAA binding. **(F)** In PBMC engrafted xenograft model, TriTCE CPIs dosed weekly (dotted line, IV) show superior tumor growth inhibition compared to bispecific TCE ($p<0.005$).

Modulation of PD-1 domain affinity to avoid off-tumor activation and cytotoxicity

PD-1 domain affinity impacts cytotoxicity on TAA^{neg}/PD-L1^{high} cells and cytokine release *in vitro*

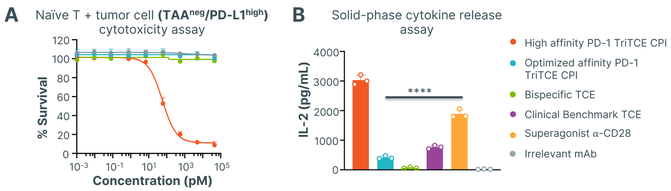


Figure 6. TriTCE CPIs were assessed *in vitro* for toxicity potential. (A) Cytotoxicity assays were used to identify TriTCE CPIs that activate T cell-dependent tumor killing of TAA^{neg}/PD-L1^{high} cell lines. **(B)** Solid-phase cytokine release assay was conducted where antibodies were immobilized and incubated for 48 hours with PBMCs. Treatment with an optimized affinity PD-1 TriTCE CPI resulted in significantly less IL-2 production (assessed by MSD) compared to superagonist α-CD28 treatment ($p<0.0001$).

TriTCE CPI with optimized affinity-engineered PD-1 domain are tolerated in a humanized mouse model

Tuning PD-1 domain affinity limits systemic toxicity and peripheral cytokine release

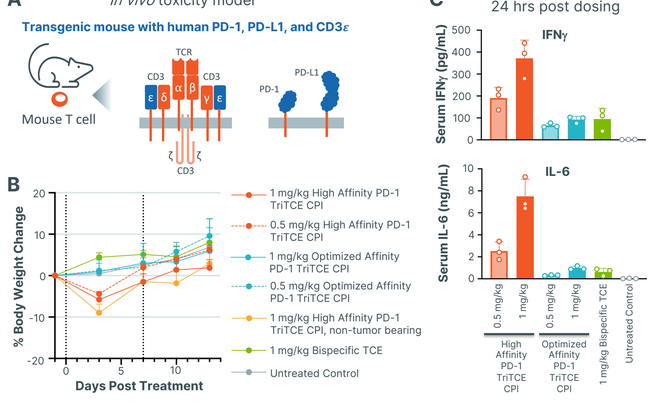


Figure 7. Dose-dependent cytokine production and weight loss observed with high affinity PD-1 domain in transgenic mouse model expressing human PD-1, PD-L1, and CD3ε. (A) Diagram of portion of CD3/TCR complex that is mouse (orange) and human (blue) in transgenic model in addition to human PD-1 and PD-L1. **(B)** Percent body weight loss and **(C)** serum IFNγ and IL-6 increase with high affinity PD-1 TriTCE CPIs in a dose-dependent manner.

Conclusions

TriTCE CPIs have been formatted and designed to:

- Overcome PD-L1 mediated tumor resistance mechanisms that can limit the efficacy of traditional bispecific TCEs.
- Promote increased antitumor activity in PD-L1^{high} tumors and with exhausted T cells and may improve responses in settings of **primary resistance.**
- Promote increased antitumor activity in PD-L1^{low} tumors and in tumors with inflammation-induced PD-L1 upregulation and may improve responses in settings of **acquired resistance.**
- Avoid T cell activation in the absence of tumor cell engagement.

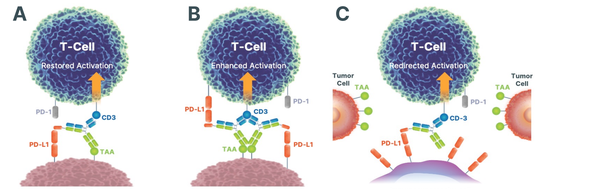


Figure 8. Proposed mechanisms of action for TriTCE CPI therapeutics. (A) TCE activity with concurrent blockade of PD-L1/PD-1 interactions between T cells and tumor cells[1]. **(B)** Enhanced tumor cell and/or exhausted T cell binding to improve T cell responses. **(C)** Activation and/or elimination of suppressive immune cells in the TME[1].

References

1. Poffenberger, M.C., et al. 2023. TriTCE CPI, next generation trispecific T cell engagers with integrated checkpoint inhibition (CPI) for the treatment of solid tumors. [Poster Presentation] AACR. Orlando, FL

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