

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

SITC 2023
Abstract #: 1396



Meghan M. Verstraete, Maya C. Poffenberger, Matteo Zago, Veronica Luu, Brenda Ma, Nichole K. Escalante, Janessa Li, Diego Perez Escanda, Siran Cao, Edward Meier, Sifa Arrafi, Yun Peng, Anna Von Rossum, Genevieve Desjardins, Nina E. Weisser, Thomas Spreter von Kreudenstein

Author Affiliations: Zymeworks BC Inc., Vancouver, BC, Canada

Introduction

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

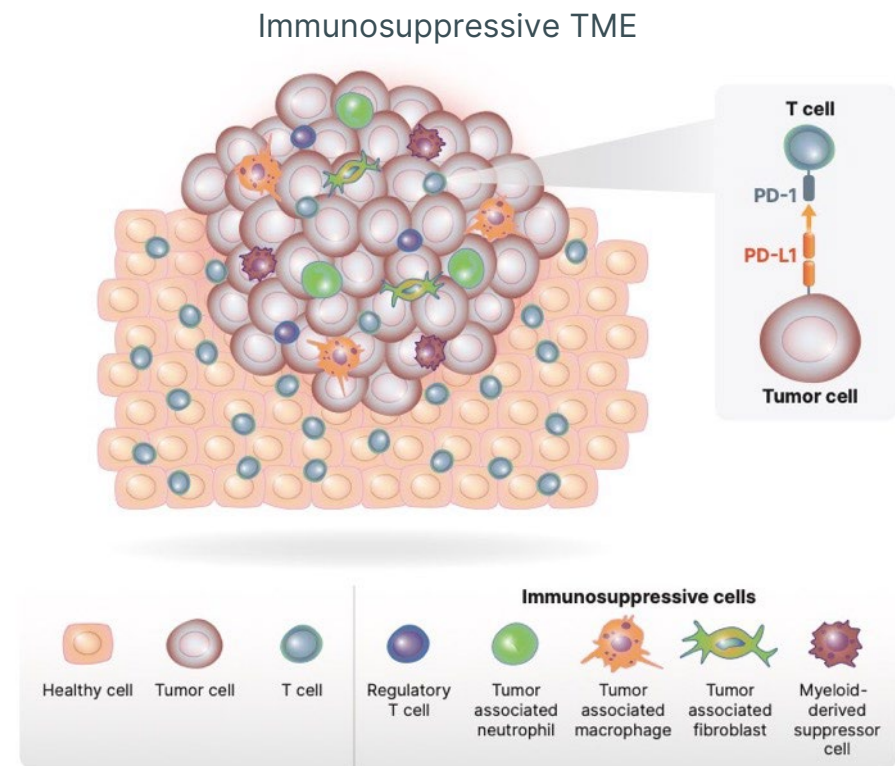
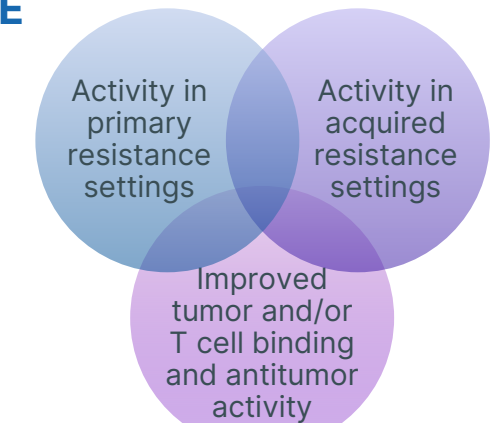


Figure 1. Immunosuppression in the tumor microenvironment (TME) can limit the cytotoxic potential of T cells in solid tumors. Immunosuppression is facilitated by primary resistance mechanisms, such as the expression of checkpoint inhibition proteins like PD-L1, and the presence of immunosuppressive immune 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.

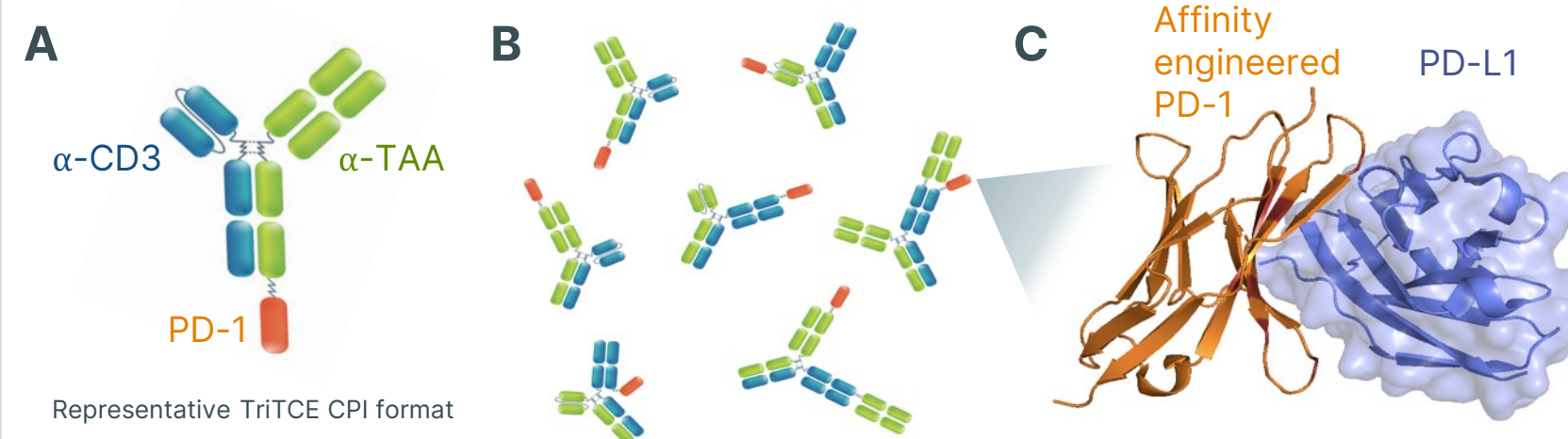
Trispecific TCEs with checkpoint inhibition (TriTCE CPI) are designed to increase T cell responses to address primary and acquired resistance mechanisms in the TME

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 anti-tumor activity and T cell responses, PD-1/PD-L1 checkpoint blockade, and avidity-driven binding



Format	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Most desirable
TAA-dependent cytotoxicity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Color scale from light blue (Least desirable) to dark blue (Most desirable)
Potency in TAA+ve tumor cells (EC50)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
TriTCE CPI > Bispecific cytotoxicity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Checkpoint activity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	

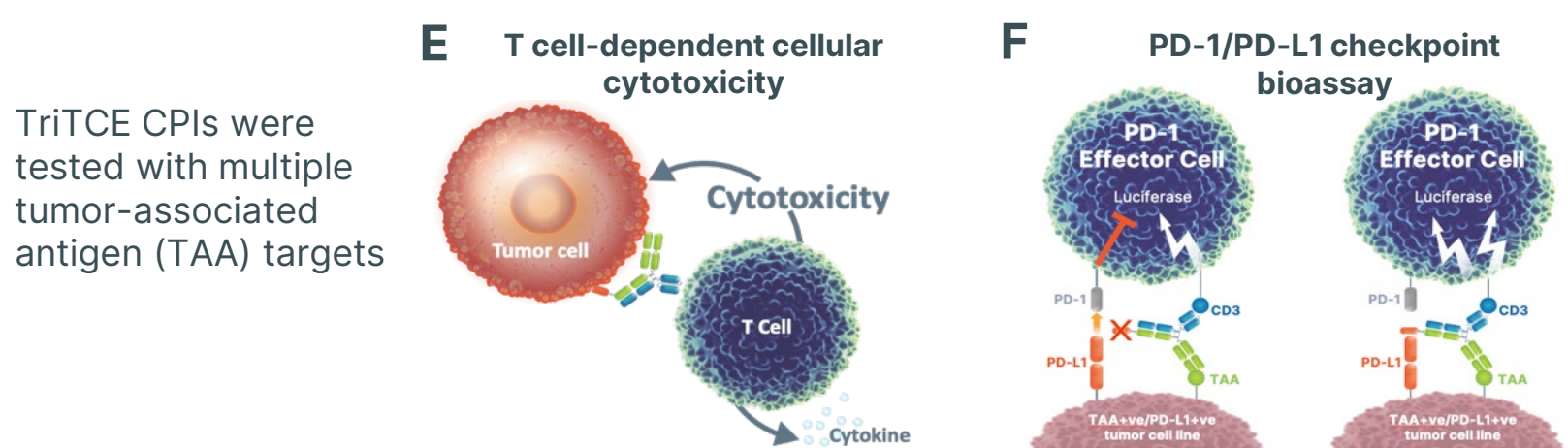


Figure 2. PD-1 domain appended to TCEs is engineered to have increased affinity for PD-L1 compared to native PD-1. Lead TriTCE CPI formats identified based on *in vitro* activity and selection criteria. (A) TriTCE CPIs are composed of α -CD3, α -TAA targeting paratopes, and an affinity engineered PD-1 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 IFN γ 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

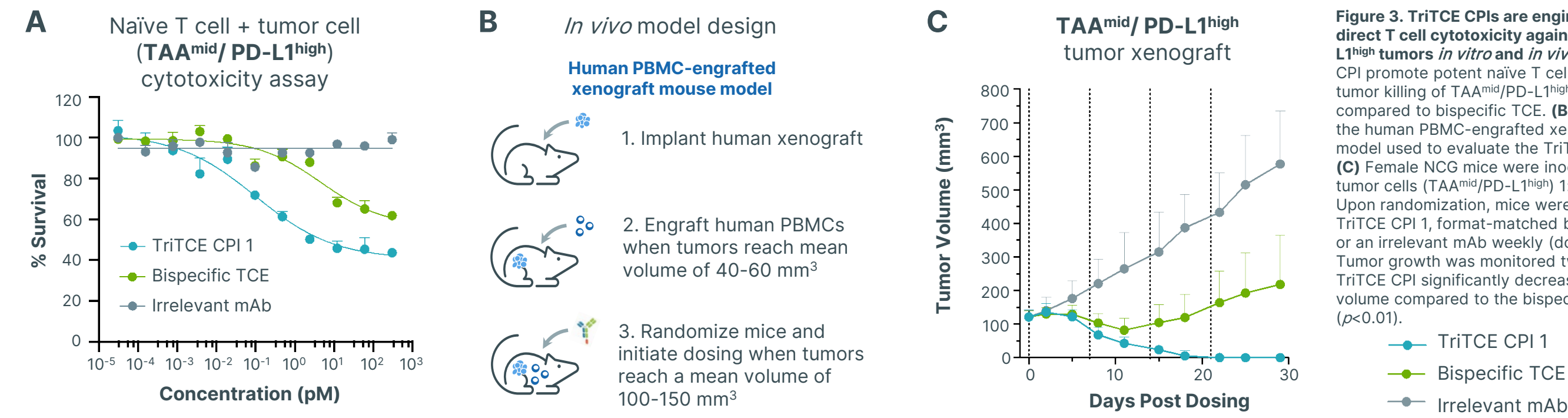


Figure 3. TriTCE CPIs are engineered to direct T cell cytotoxicity against TAA^{mid}/PD-L1^{high} tumors *in vitro* and *in vivo*. (A) TriTCE CPI promote potent naive T cell-dependent tumor killing of TAA^{mid}/PD-L1^{high} cell lines compared to bispecific TCE. (B) Schematic of the human PBMC-engrafted 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

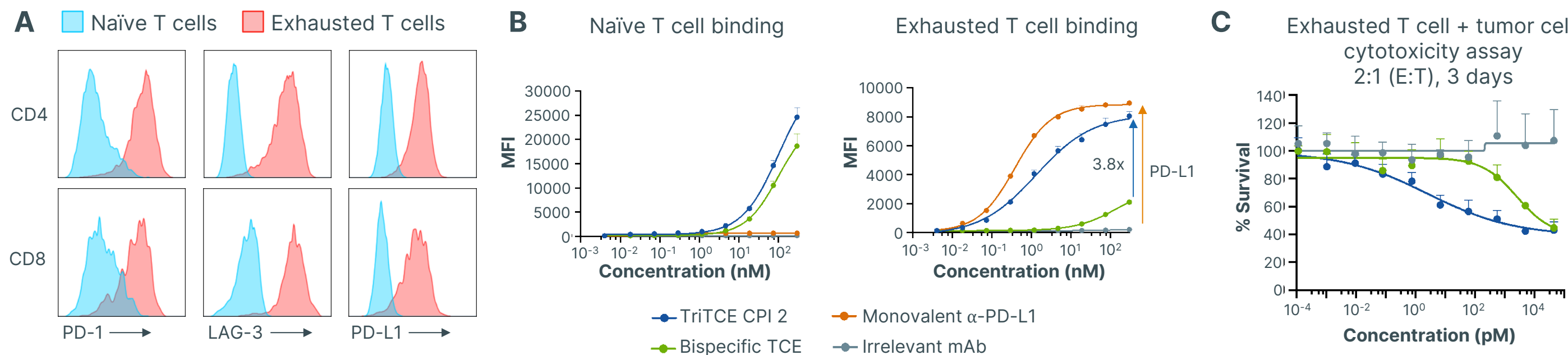


Figure 4. TriTCE CPI formats can direct tumor cell killing by exhausted T cells *in vitro*. (A) Naive T cells were exhausted by repeat α -CD3/ α -CD28 stimulation every second day for a total of 8 days. Expression of exhaustion markers was measured by flow cytometry. (B) TriTCE CPI and bispecific TCE show comparable binding to naive 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 anti-tumor 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

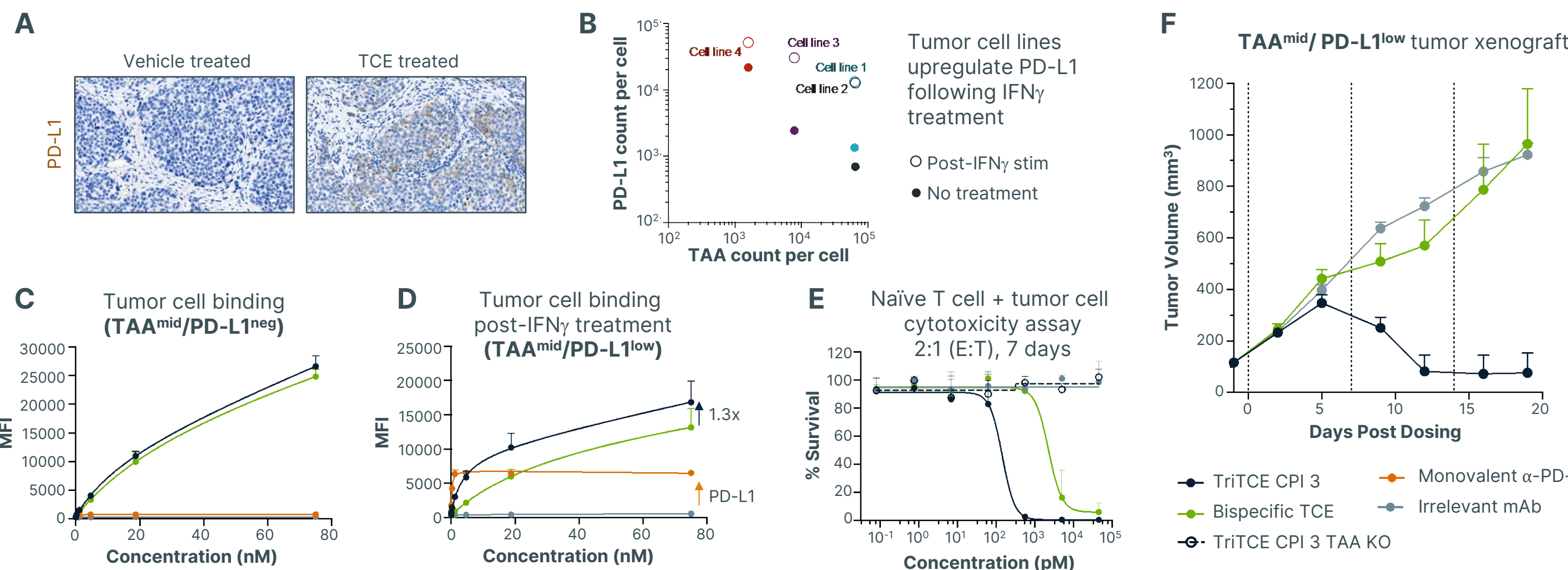


Figure 5. Addition of affinity-engineered PD-1 domain to TCEs increases tumor cell binding by PD-L1 which translates to increased *in vitro* and *in vivo* antitumor activity. (A) Tumor cell expression of PD-L1 (brown) is increased following treatment with TCE compared vehicle by IHC. (B) Tumor cell line TAA expression and inducible PD-L1 expression pre- and post 24-hour stimulation with 20 ng/ml IFN γ ; 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 IFN γ . (D) Pre-treatment of tumor cells with IFN γ 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

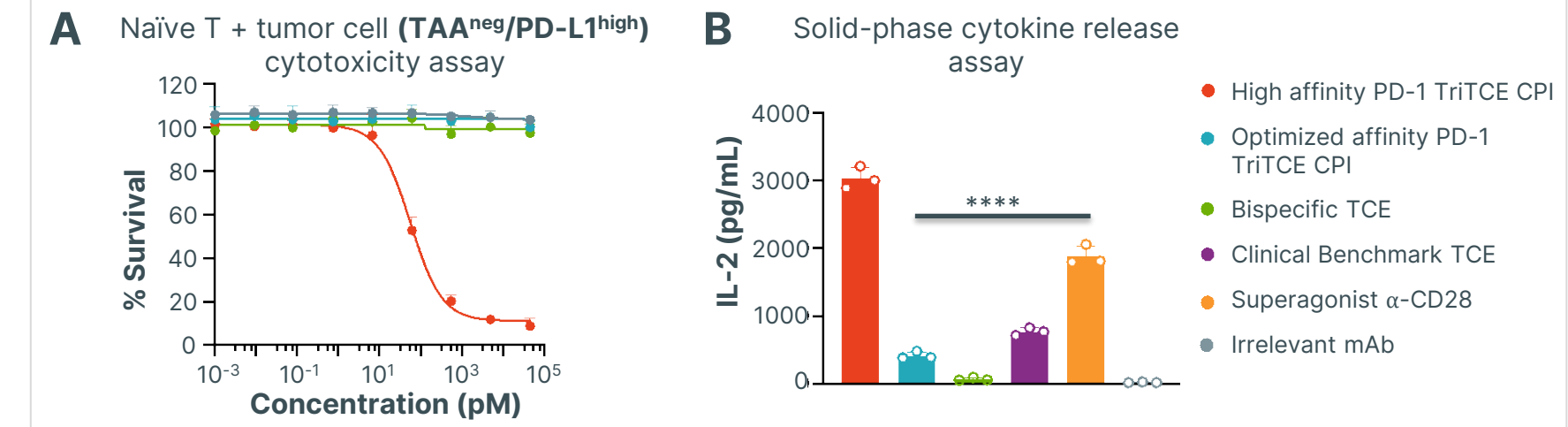


Figure 6. TriTCE CPIs were assessed *in vitro* for toxicity potential. (A) Cytotoxicity assays were used to identify TriTCE CPIs that activate T cell-dependent tumor killing of TAA^{neg}/PD-L1^{high} cell lines. (B) Solid-phase cytokine release assay was conducted where antibodies were immobilized and 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

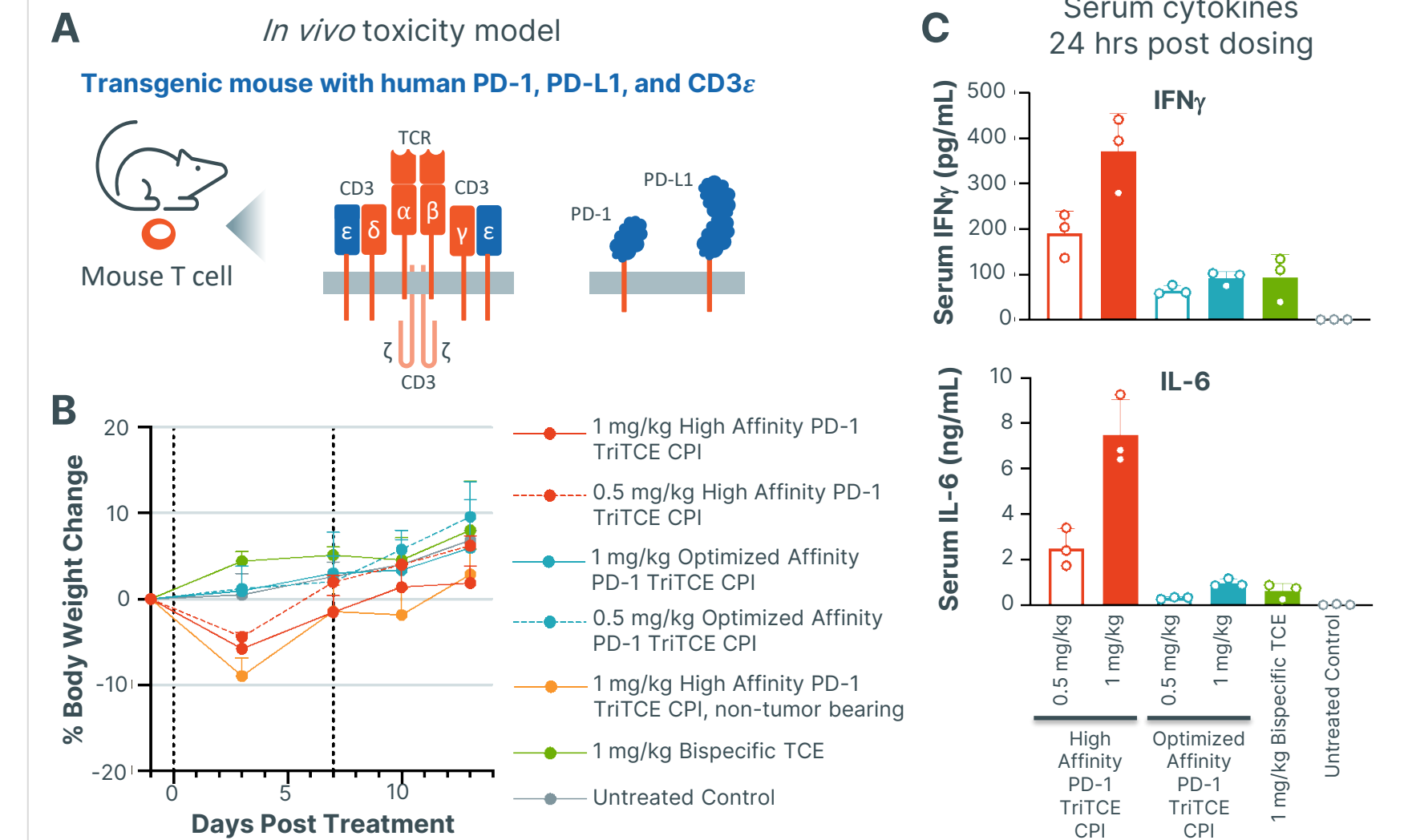


Figure 7. Dose-dependent cytokine production and weight loss observed with high affinity PD-1 domain in transgenic mouse model expressing human PD-1, PD-L1, and CD3_e. (A) Diagram of portion of CD3/TCR complex that is mouse (orange) and human (blue) in transgenic model in addition to human PD-1 and PD-L1. (B) Percent body weight loss and (C) serum IFN γ 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.

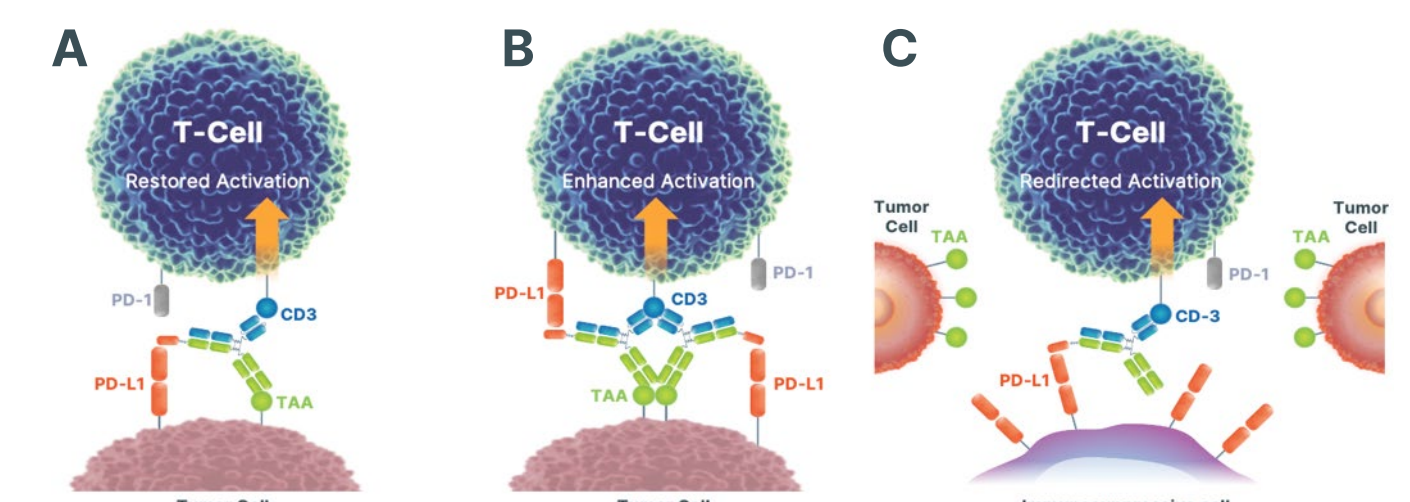


Figure 8. Proposed mechanisms of action for TriTCE CPI therapeutics. (A) TCE activity with concurrent blockade of PD-L1/PD-1 interactions between T cells and tumor cells. (B) Enhanced tumor cell and/or exhausted T cell binding to improve T cell responses. (C) Activation and/or elimination of suppressive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

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

This study was sponsored by Zymeworks BC Inc.