TriTCE Co-Stim: A next generation trispecific T cell engager platform with integrated CD28 co-stimulation, engineered to improve responses in the treatment of solid tumors

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Lisa Newhook, Purva Bhojane, Kurt Stahl, Nichole K. Escalante, Polly Shao, Diego Perez Escanda, Kesha Patel, Marylou Vallejo, Bing Catherine Wu, Gavin Storoschuk, Peter Repenning, Alexandra Livernois, Chayne L. Piscitelli, Nicole Afacan, Paul A. Moore, Nina E. Weisser, Thomas Spreter von Kreudenstein

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

Introduction

- **Low T cell infiltration and T cell anergy are challenges for the treatment of solid tumors with conventional CD3-engaging bispecific T cell engagers (TCEs)[1]**
- **By optimizing "Signal 1" (CD3) and "Signal 2" (CD28), co-stimulatory trispecific TCEs (TriTCE Co-Stim) have the potential to increase response rates by stimulating T cell proliferation in patients with poorly infiltrated tumors and to provide more durable anti-tumor control by enhancing T cell activation**

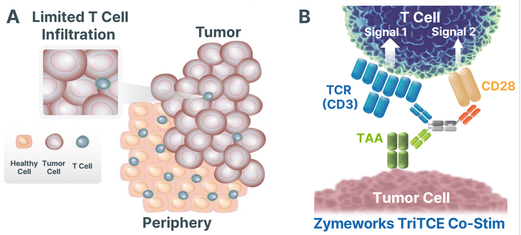
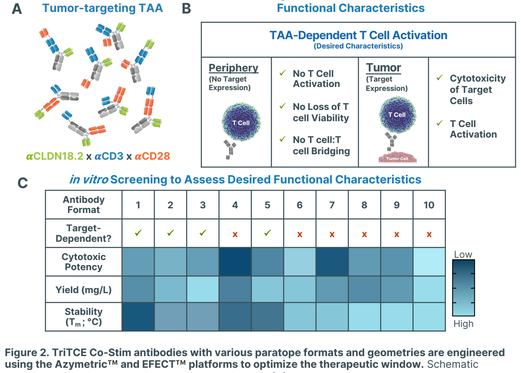


Figure 1. Proposed mechanism of action for Zymeworks' TriTCE Co-Stim. Schematic of limited T cell infiltration in solid tumors **(A)**. Schematic of TriTCE Co-Stim-mediated T cell activation **(B)**. TriTCE Co-Stim is designed to provide tumor-associated antigen (TAA) dependent agonism of Signal 1 (CD3) and Signal 2 (CD28) in a single molecule to increase T cell activation, fitness, and proliferation.

TriTCE Co-Stim: From Concept to Platform

TriTCE Co-Stim formats exhibit antibody-like developability with differential *in vitro* properties[2]



C *in vitro* Screening to Assess Desired Functional Characteristics

Antibody Format	1	2	3	4	5	6	7	8	9	10
Target-Dependent?	✓	✓	✓	x	✓	x	x	x	x	x
Cytotoxic Potency										
Yield (mg/L)										
Stability (T_{m1}; °C)										

Low — High

Figure 2. TriTCE Co-Stim antibodies with various paratope formats and geometries are engineered using the Azymetric™ and EFECT™ platforms to optimize the therapeutic window. Schematic representation of a subset of TriTCE Co-Stim formats **(A)**. Summary of desired target-dependent properties of TriTCE Co-Stim achieved by optimized format design **(B).** TriTCE Co-Stim formats that exhibit potent cytotoxicity of target cells, target-dependency, acceptable yield, and thermal stability are selected through extensive screening *in vitro* **(C)**.

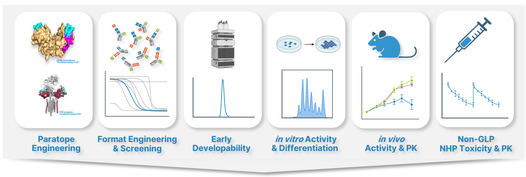


Figure 3. Workflow established for the development of TriTCE Co-Stim platform ¶.

Expanded Validation of TriTCE Co-Stim Platform Using CLDN18.2 As Model Tumor Antigen

- **Building upon previous data, highlighting the mechanistic differentiation of the TriTCE Co-Stim platform[2,3], we aimed to:**
  - **Assess T-cell mediated cytotoxicity *in vitro* and *in vivo* relative to first generation CLDN18.2xCD3 bispecific TCEs (AMG910; ASP2138)†**
  - **Interrogate mechanisms of T-cell engagement relative to an alternative CD28xCD3xTAA modality‡**
  - **Evaluate tolerability, safety, and peripheral cytokine profile in a non-human primate (NHP) toxicology study**

† AMG 910 (CLDN18.2/CD3 BiTE) & ASP2138 (CLDN18.2/CD3 2+1 bsAb) replicas produced in-house.
‡ CD3xCD28xTAA CODV Analog is a CD3xCD28xMSLN trispecific with the same format as the Sanofi Trispecific containing a CD3xCD28 CODV-Fab; produced in-house.

TriTCE Co-Stim Mediates Enhanced T Cell Responses and Anti-tumor Activity Relative to Comparator T Cell Bispecifics

Enhanced long-term, T cell-mediated cytotoxicity at low Effector:Target (E:T) ratios

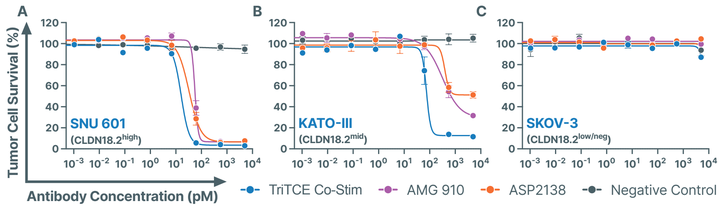


Figure 4. CLDN18.2 TriTCE Co-Stim displays superior cytotoxic potency of CLDN18.2-expressing cell lines in long term, low E:T co-cultures. Test articles were incubated with human T cells co-cultured with SNU 601 (gastric; ~275 000 CLDN18.2/cell) **(A)**, KATO-III (gastric; ~60 000 CLDN18.2/cell) **(B)**, or SKOV-3 (ovarian; <10 000 CLDN18.2/cell) **(C)** cell lines for 7 days at low E:T (1:5) and evaluated for cytotoxicity of target cells. Data are representative of five individual donors and presented as mean ± SEM.

Enhanced T cell proliferation and survival

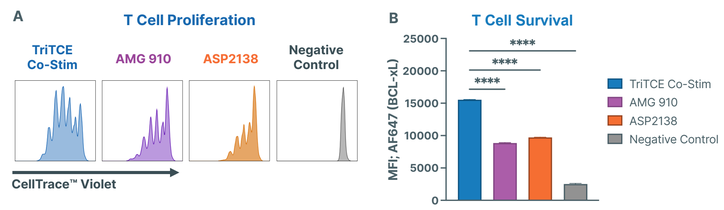


Figure 5. Assessment of T cell proliferation and Bcl-xL upregulation following incubation with CLDN18.2 TriTCE Co-Stim. Test articles (80 pM) were incubated with CellTrace™ Violet-stained T cells co-cultured with SNU 601 cells (5:1 E:T) for 5 days and assessed by flow cytometry **(A)**. Test articles (1 nM) were incubated with T cells co-cultured with SNU 601 cells (2:1 E:T) and evaluated for Bcl-xL expression, an anti-apoptotic marker, by flow cytometry **(B)**. Data are representative of two individual donors and are presented as mean ± SD. **** p<0.0001

Sustained T cell-mediated cytotoxicity over repeated T cell stimulations

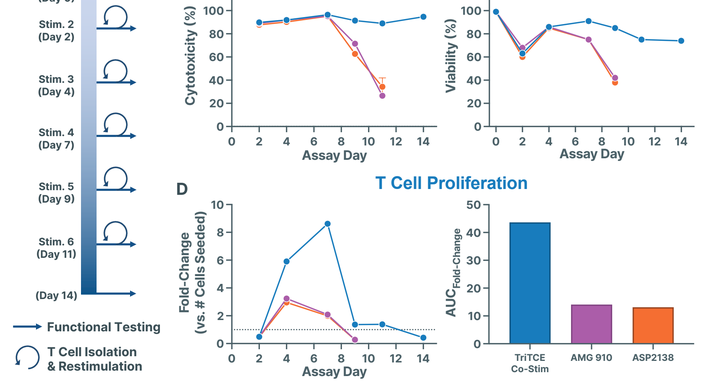


Figure 6.CLDN18.2 TriTCE Co-Stim displays sustained T cell fitness and anti-tumor activity in a serial, repeat challenge assay. T cells were stimulated with SNU 601 cells (5:1 E:T) and test article (1 nM). For each subsequent round of stimulation, T cells are isolated from the T cell:tumor cell co-culture, counted, and re-stimulated with fresh SNU 601 target cells (5:1 E:T) and fresh test article (1 nM). Schematic of T cell restimulation **(A)**. Following each round of stimulation, T cell:tumor cell co-cultures were assessed for tumor cell cytotoxicity **(B)**, T cell viability **(C)**, and T cell proliferation **(D)**. Data are representative of two individual donors and are presented as mean ± SD. Insufficient T cells for continued stimulation with AMG 910 and ASP2138 following stimulation 5. Viability and proliferation data for AMG 910 and ASP2138 were excluded for stimulation 5 due to technical error (tumor cell carryover).

Superior tumor growth control in a humanized model of gastric cancer

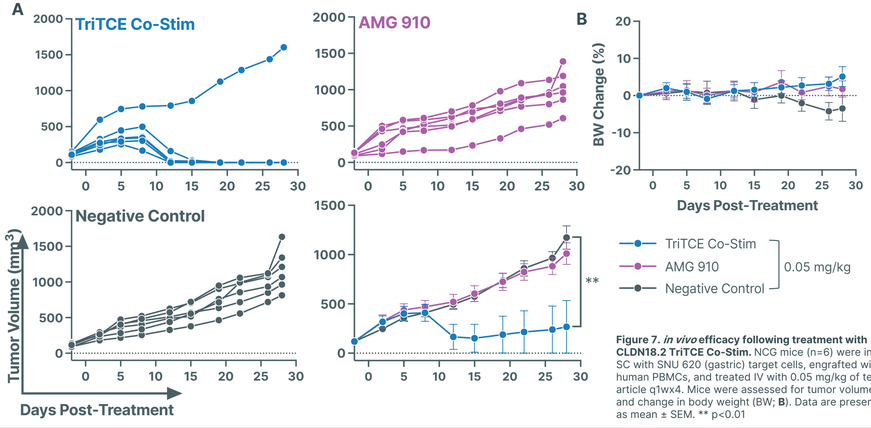


Figure 7. *in vivo* efficacy following treatment with CLDN18.2 TriTCE Co-Stim. NCG mice (n=6) were injected SC with SNU 620 (gastric) target cells, engrafted with human PBMCs, and treated IV with 0.05 mg/kg of test article q1wx4. Mice were assessed for tumor volume **(A)** and change in body weight (BW; **B**). Data are presented as mean ± SEM. ** p<0.01

TriTCE Co-Stim Design Facilitates Desirable T Cell Engagement

Conditional binding of CD28, requiring co-engagement of CD3

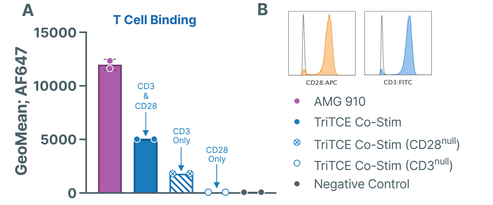


Figure 8. On-cell binding of CLDN18.2 TriTCE Co-Stim and format-matched single-arm binding controls. GeoMean of αIgG:Alexa Fluor 647 (AF647) fluorescence with 25 nM test article **(A)**. T cell expression of CD3 and CD28 **(B)**. AMG 910 included as high affinity CLDN18.2xCD3 bispecific TCE. TriTCE Co-Stim (CD28null) is a CLDN18.2xCD3 bispecific TCE with the same format geometry as the CLDN18.2 TriTCE Co-Stim. Data are representative of three individual donors and are presented as mean ± SD.

Obligate *cis* T cell binding of CD3 and CD28

- **Cell bridging by immune cell-engaging antibodies has the potential to mediate effector cell fratricide, ultimately depleting cells required for therapeutic efficacy[4].**

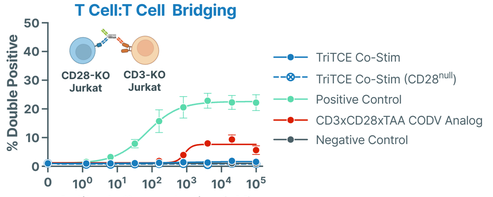


Figure 9. Assessment of T cell:T cell bridging. CD3-Knockout (KO) Jurkat and CD28-KO Jurkat cells were pre-labeled with fluorescent dyes and incubated with test article at a 1:1 ratio (CD3-KO:CD28-KO) for 1 hour. Double positive events, representing cell bridging, were assessed by flow cytometry. Representative schematic of cell bridging (**inset ¶**). Positive control is a CLDN18.2xCD3xCD28 TriTCE Co-Stim format that exhibits target-independent activation of T cells. Data are presented as mean ± SD.

No reduction of the viability of monocultures of T cells

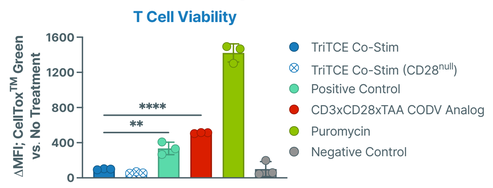


Figure 10. CellTox™ Green T cell viability assay. Test articles (45 nM) were incubated with monocultures of T cells in the presence of CellTox™ Green. After 48h, fluorescence was detected using the Operetta and analyzed for median fluorescence intensity (MFI). Positive control is a CLDN18.2xCD3xCD28 TriTCE Co-Stim format that exhibits target-independent activation of T cells. Puromycin was also included as a positive control for T cell death. Data are representative of three individual donors and presented as mean ± SD. ** p<0.01; **** p<0.0001

Plate-bound TriTCE Co-Stim does not stimulate IL-2 production by PBMCs alone

- **IL-2 production in solid-phase cytokine release assays is correlated with severity of cytokine release syndrome by TGN1412, a superagonist αCD28 antibody[5].**

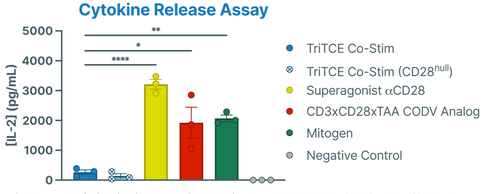


Figure 11. Predictive *in vitro* model for cytokine release syndrome (CRS). Immobilized test articles (1 μg/well) were incubated with PBMCs for 48 hours and assessed for IL-2 production. TriTCE Co-Stim did not exhibit peripheral T cell cytokine release or body weight loss in an *in vivo* model of CRS[3]. Superagonist αCD28 is TGN1412 replica produced in-house. Mitogen is Staphylococcal enterotoxin B. Data are presented are mean ± SEM of three individual PBMC donors. * p<0.05, ** p<0.01, **** p<0.0001

TriTCE Co-Stim is Well-tolerated in Cynomolgus Monkeys

Toxicology findings were mild and associated with the known mechanism of action of TCEs

- **No histopathological changes observed in the stomach, where CLDN18.2 is expressed[6]**
- **Other histopathological changes were secondary to decreased food consumption and body weight loss**

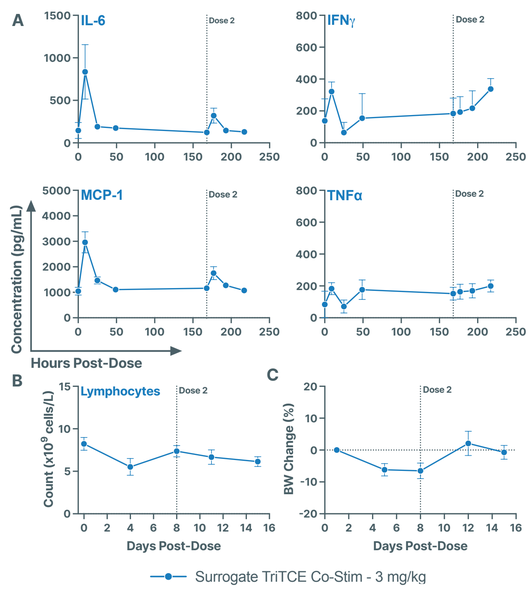


Figure 12. Non-GLP NHP Toxicology. Cynomolgus monkeys (n=3) were given a repeat dose of 3 mg/kg of a cynomolgus surrogate TriTCE Co-Stim on day 0 and day 8. Animals were monitored for serum cytokine levels **(A)**, lymphocyte counts **(B)**, and change in body weight **(C)**. Surrogate TriTCE Co-Stim exhibited ~10-fold increased cytotoxic potency vs. lead TriTCE Co-Stim and ~15-fold reduced cytotoxic potency vs. AMG 910 (AMG 910 dosed up to 0.03 mg/kg in a one-month, repeat dose NHP toxicology study[7]) in cynomolgus T cell-dependent cytotoxicity assays *in vitro* .

Conclusions

- Platform established to generate TriTCE Co-Stim antibodies with optimized CD3 and CD28 binding.
- The lead CLDN18.2 TriTCE Co-Stim molecule:
  - Enhances long-term cytotoxicity at low E:T ratios.
  - Enhances T cell proliferation and survival *in vitro*.
  - Resulted in sustained cytotoxicity, T cell viability and proliferation in serial challenge assays.
  - Exhibits obligate *cis* T cell binding of CD28, requiring co-engagement of CD3.
  - Surrogate is well tolerated in NHP.
- TriTCE Co-Stim has the potential to provide more durable responses, re-invigorate tumors with low T cell infiltration, and avoid potential toxicity liabilities, such as systemic cytokine release, key factors that may contribute to improved clinical outcomes.

References

1. Arvedson T., et al. 2022. Targeting Solid Tumors with Bispecific T Cell Engager Immune Therapy (Vol. 6, pp.17-34).
2. Newhook L., et al. 2023. 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. [Poster Presentation] SITC. San Diego, CA
3. Newhook L., et al. 2023. TriTCE Co-Stim, Next Generation Co-Stimulatory Trispecific T cell Engagers for the Treatment of Solid Tumors. [Poster Presentation] AACR. Orlando, FL
4. Wang Y, et al. 2018. Fratricide of NK Cells in Daratumumab Therapy for Multiple Myeloma Overcome by Ex Vivo Expanded Autologous NK Cells. Clin Cancer Res. (Vol. 24, No 16, pp 4006-4017).
5. Eastwood D., et al. 2013. Severity of the TGN1412 Trial Disaster Cytokine Storm Correlated with IL-2 Release. *Br J Clin Pharmacol.* (Vol. 76, No. 2, pp.299-315).
6. Türeci O., et al. 2011. Claudin-18 Gene Structure, Regulation, and Expression is Evolutionary Conserved in Mammals. Gene. (Vol. 481, No 2, pp 83-92).
7. Bailis J. et al. 2020. Preclinical Evaluation of BiTE® Immune Therapy Targeting MUC17 or CLDN18.2 for Gastric Cancer. [Poster Presentation] AACR.

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