TriTCE Co-Stim: A trispecific T cell engager platform with integrated CD28 co-stimulation to improve T cell function and anti-tumor responses in hard-to-treat cancers

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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)

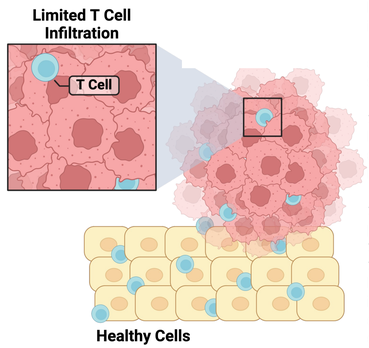

Figure 1. Schematic of T cell infiltration in solid tumors. T cell density positively correlates with prognosis in several solid tumor indications. The ability of conventional tumor-targeting, CD3-engaging, bispecific TCEs to induce T cell expansion relies on high baseline T cell infiltration[1]. Treatment of solid tumors with these TCEs can result in limited proliferation and recruitment to the tumor site, suggesting that treatment with conventional bispecific TCEs may be insufficient to inhibit the growth of poorly infiltrated, rapidly growing tumors.

Co-stimulatory trispecific TCEs (TriTCE Co-stim) have the potential to provide more durable responses and activate T cell responses in 'cold' tumors with lower T cell infiltration

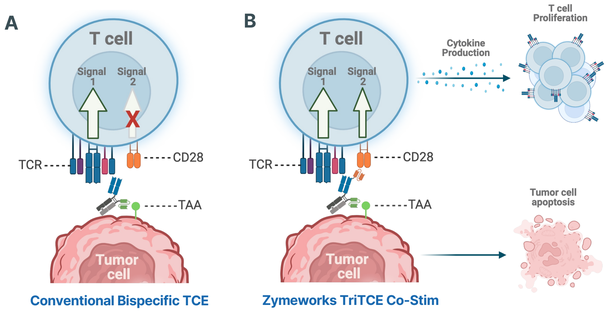

Figure 2. Enhancing T cell responses and anti-tumor activity with TriTCE Co-Stim, engineered with conditional CD28 co-stimulation and obligate *cis* T cell engagement. Lack of co-stimulatory ligand engagement in solid tumors can limit the activity and durability of conventional bispecific TCE responses. **(A)** Activation of the T cell receptor (TCR; signal 1) in the absence of co-stimulation limits T cell activation and leads to T cell anergy, thus limits conventional bispecific TCE anti-tumor responses. **(B)** Target-dependent TCR activation with concomitant conditional CD28 co-stimulation (signal 2) in *cis* enhances T cell activation, metabolism, fitness, cytokine production, sustained proliferation and avoids T cell fratricide[2, 3].

TriTCE Co-stim Antibodies Exhibit Enhanced T cell Mediated Cytotoxicity

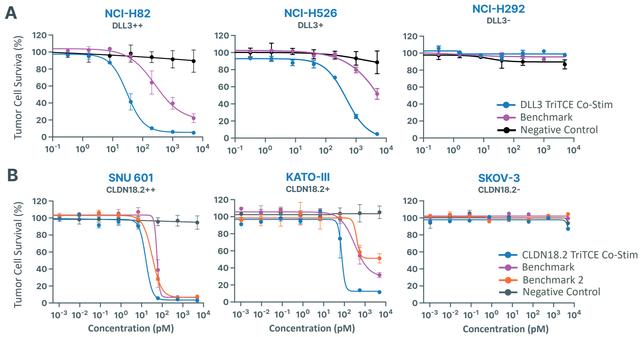

Figure 3. TriTCE Co-stim molecules display superior cytotoxic potency in long term low effector to target cell ratio (E:T) co-cultures compared to bispecific benchmarks. (A) DLL3- or **(B)** CLDN18.2-targeting TCE molecules or negative control were incubated with human T cells co-cultured with tumor cell lines for 7 days at low E:T and evaluated for cytotoxicity of target cells. TriTCE Co-Stim display superior cytotoxicity in a long-term low E:T ratio cytotoxicity assay and demonstrate no activity on tumor-associated antigen negative (TAA-) cells. (A) DLL3 TriTCE Co-Stim (ZW209), Benchmark (AMG 757). (B) CLDN18.2 TriTCE Co-Stim (ZW239), Benchmark (AMG 910), Benchmark 2 (ASP 2138).

DLL3 TriTCE Co-stim Molecule Exhibits Superior *in vivo* Activity in an Admixture Xenograft model

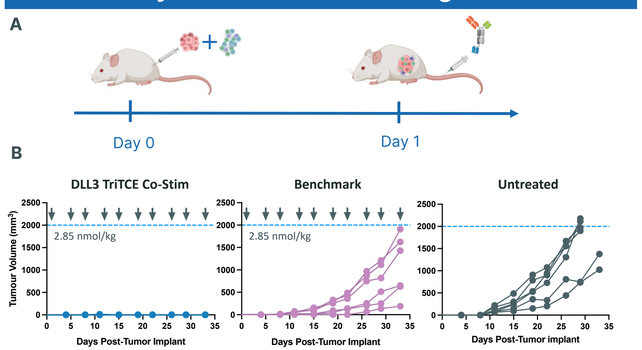

Figure 4. *in vivo* anti-tumor activity following treatment with DLL3 TriTCE Co-stim (ZW209) in an admixture model. (A) Schematic representation of an admixture tumor model protocol. **(B)** NCI-H82 cells were co-injected with isolated T cells SC in NCG mice. After implantation, mice were treated IP with DLL3 TriTCE Co-stim (ZW209) or Benchmark (AMG 757) (arrowheads indicated dosing). Full tumor growth inhibition was observed in 6/6 mice treated with DLL3 TriTCE Co-Stim.

TriTCE Co-stim Antibodies Display Enhanced In Vivo Anti-tumor Activity in Established PBMC-Engrafted Xenograft Models

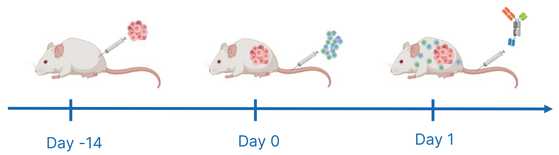

Figure 5. Schematic representation of an established PBMC-engrafted tumor model protocol. Tumor cells are injected SC in NCG mice. Mice are randomized when the average tumor size reaches 70-100mm^3 and injected IV with human PBMCs. Following humanization, mice are treated IV with test article Q1W.

DLL3 TriTCE Co-stim Displays Superior *in vivo* Anti-tumor Activity Relative to Bispecific TCE in a Humanized SHP-77 Xenograft Model

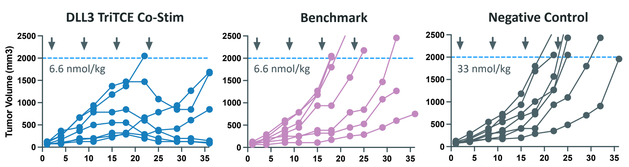

Figure 6. *in vivo* efficacy following treatment with DLL3 TriTCE Co-stim molecule. SHP-77 cells were injected SC in NCG mice. When tumors reached the target size, mice were humanized with PBMCs, and treated IV with DLL3 TriTCE Co-stim, Benchmark (AMG 757) or irrelevant mAb. Full or partial tumor regression is observed in 4/7 mice treated with DLL3 TriTCE Co-Stim when IV treated q1wx4.

CLDN18.2 TriTCE Co-stim Displays Superior *in vivo* Anti-tumor Activity Compared to Bispecific TCE in a SNU620 PBMC-Engrafted Xenograft Model

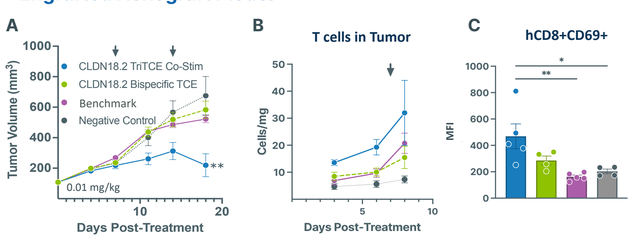

Figure 7. *in vivo* efficacy of CLDN18.2 TriTCE Co-stim (ZW239) molecule. (A) Following humanization with PBMCs, mice were treated IV with test article q1w (arrowheads indicate dosing) and monitored for tumor volume (mean +/- SEM, ** p<0.01). Benchmark (AMG 910) **(B)** Tumor T cells (CD3+) were assessed by flow cytometry on day 3, 6 and 8 after treatment. **(C)** An increase of activated T cells (CD8+CD69+) in the tumor was observed on day 8 after treatment.

CLDN18.2 TriTCE Co-stim Exhibits Favorable Safety Profile *in vivo*

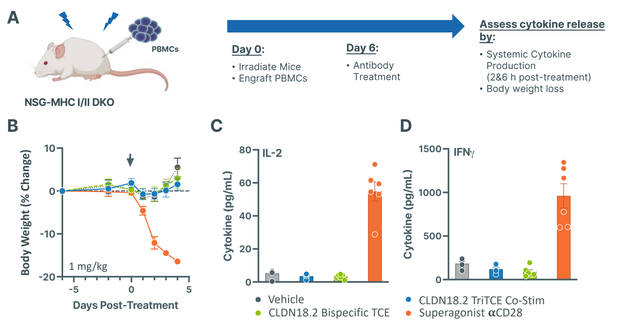

Figure 8. No body weight loss or systemic cytokine production is observed in an *in vivo* model for cytokine release. (A) Schematic representation of a cytokine release *in vivo* model. **(B)** Percent body weight change after treatment with 1 mg/kg of test article. Body weight loss was observed following superagonist αCD28 treatment, but not CLDN18.2 TriTCE Co-Stim (ZW239). Treatment with CLDN18.2 TriTCE (ZW239) or Bispecific control resulted in lower serum IL-2 **(C)** and IFNγ **(D)** at 6 h post-treatment when compared to superagonist αCD28 treatment. CLDN18.2 TriTCE Co-Stim is cross-reactive with mouse CLDN18.2 (data not shown).

Conclusions

Zymeworks' TriTCE Co-stim molecules targeting DLL3 (**ZW209**) and CLDN18.2 (**ZW239**) show:

- **Greater *in vitro* cytotoxicity at low E:T ratios** compared to bispecific TCEs
- **Enhanced *in vivo* anti-tumor activity in humanized admixture xenograft model** relative to clinical benchmark bispecific
- **Enhanced anti-tumor activity and intratumoral T cell count in an established PBMC-humanized xenograft model** relative to clinical benchmark bispecific
- **No systemic cytokine release** in an *in vivo* cytokine release model

By providing target-dependent T cell activation with integrated CD28 co-stimulation in *cis*, TriTCE Co-stim molecules provide more effective and tolerable anti-tumor responses[2, 3,4]

References

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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 tumor. [Poster Presentation] SITC. San Diego, CA
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