

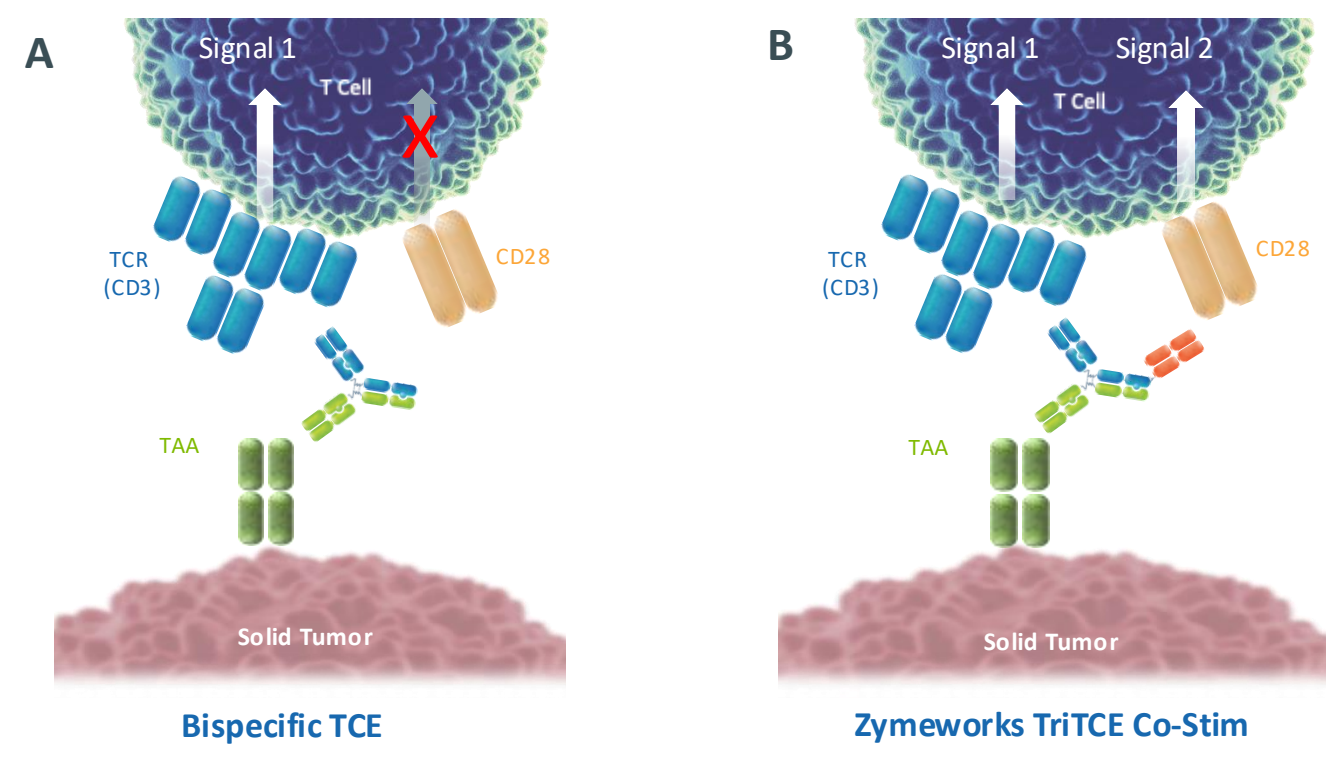


# TriTCE Co-Stim, Next Generation Co-Stimulatory Trispecific T cell Engagers for the Treatment of Solid Tumors

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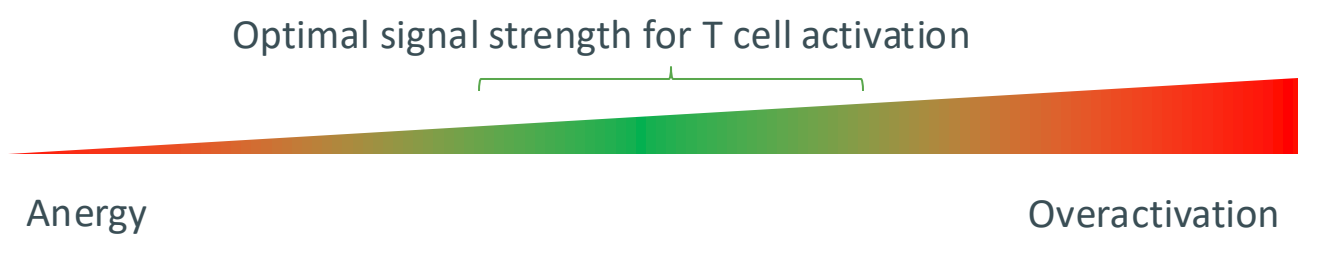
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## Introduction



**Figure 1. Schematic of T cell engager (TCE)-mediated T cell activation in solid tumors.** Lack of co-stimulatory ligand engagement in solid tumors can limit the activity and durability of bispecific TCE responses. (A) Activation of the T cell receptor (TCR) in the absence of co-stimulation can result in T cell anergy, limiting the activity and durability of bispecific TCE anti-tumor responses. (B) Activation of TCR with concomitant co-stimulation may enhance T cell activation, metabolism and fitness, cytokine production, and sustained proliferation.

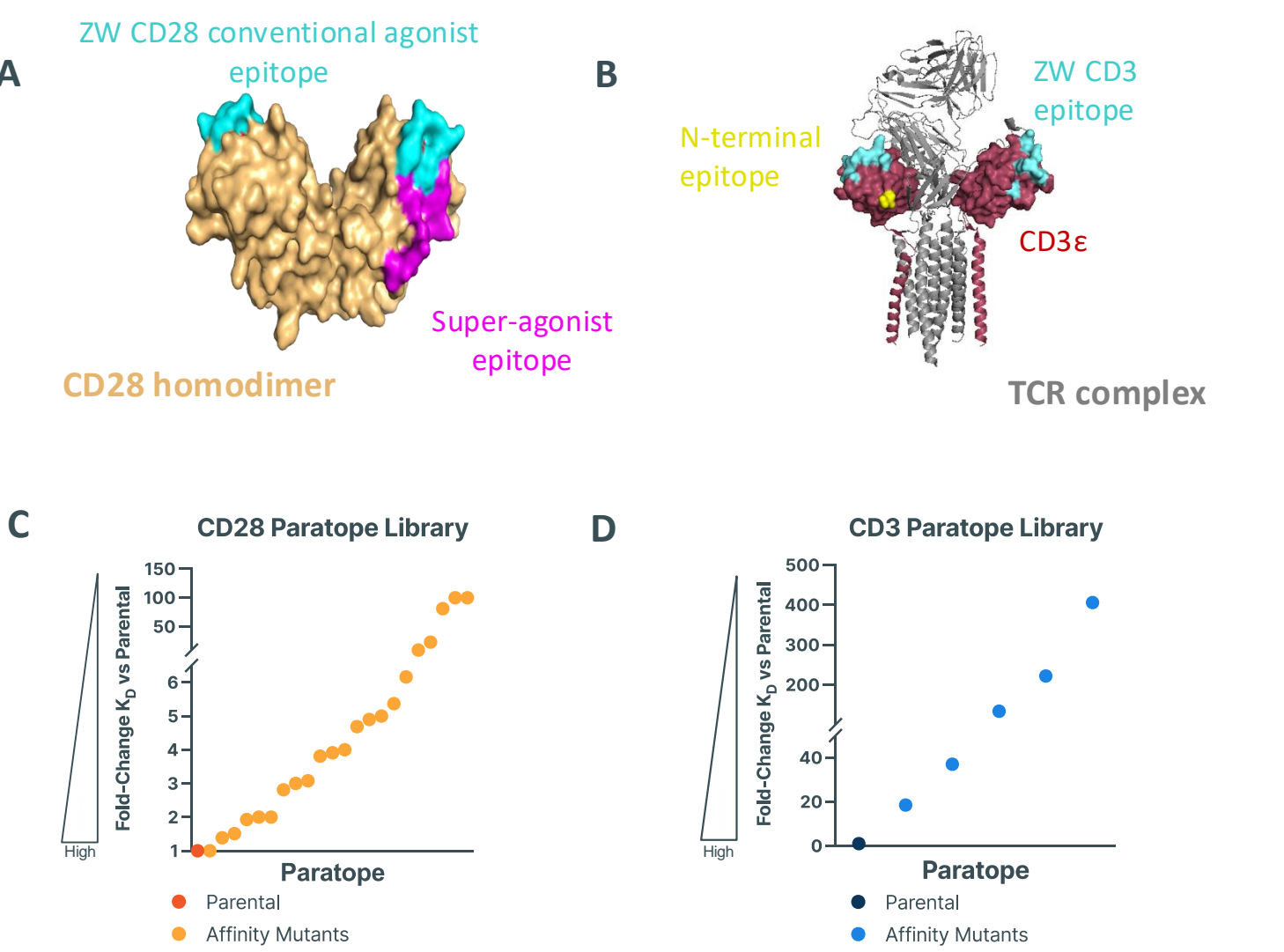
**Co-stimulatory trispecific TCEs (TriTCE Co-Stim) have the potential to provide more durable responses and re-invigorate 'cold' tumors with lower T cell infiltration**



**Figure 2. Activation requires a balance of "Signal 1" and "Signal 2".** Lack of Signal 2 co-stimulation leads to T cell anergy and no sustained T cell proliferation. Overactivation leads to T cell dysfunction and excessive cytokine release.

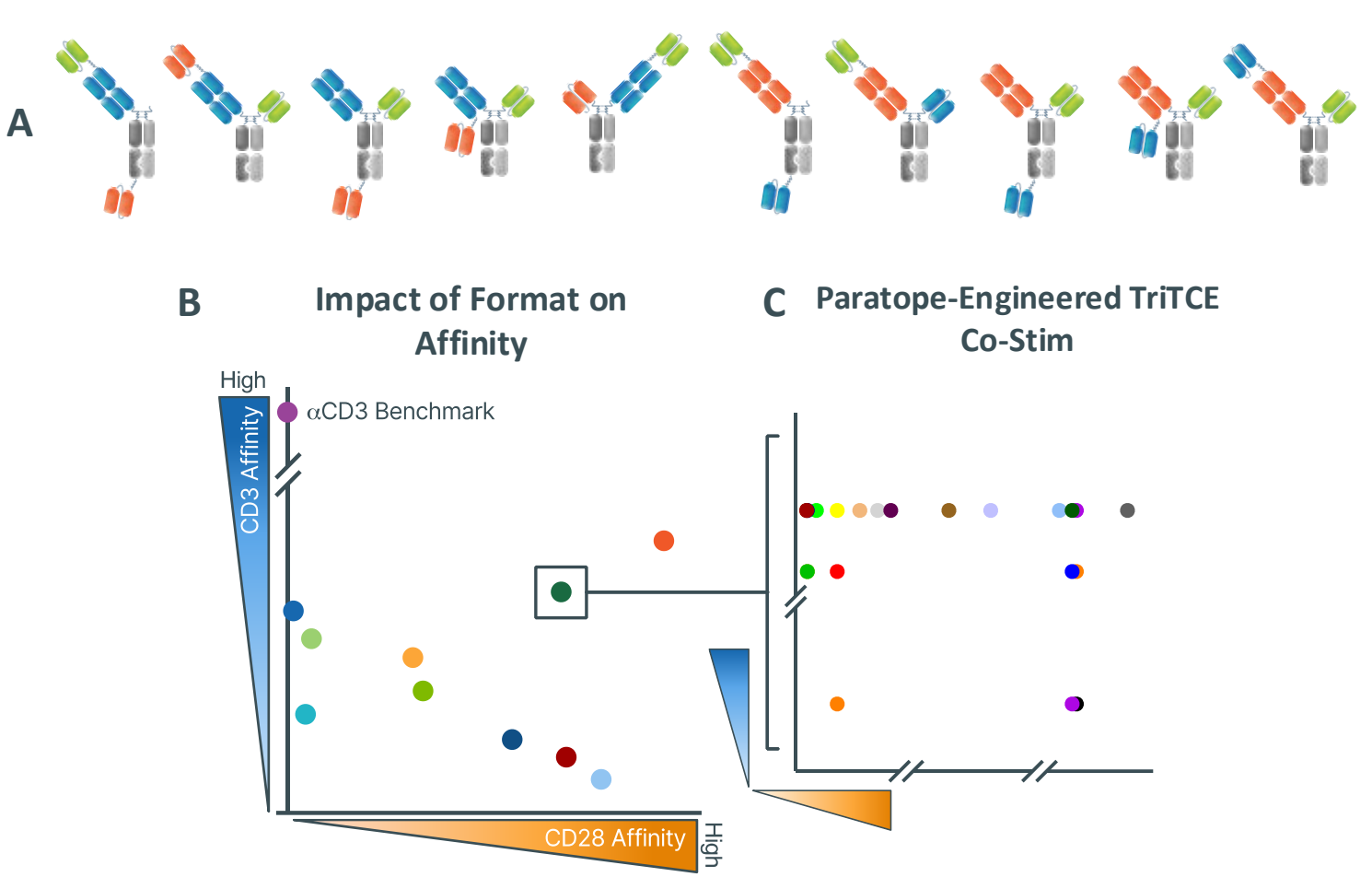
## Paratope Engineering for Therapeutic Window Optimization

**Engineering solutions employed to optimize signal strength for T cell activation and anti-tumor activity**



**Figure 3. CD3 and CD28 paratope engineering.** (A) Surface representation of CD28 homodimer structure (modelled using 1YJD) highlighting epitopes for Zymeworks' (ZW) conventional agonist vs super-agonist antibodies. (B) Cartoon representation of the full TCR complex (modelled using 7FJD) with surface representation of CD3-ε domain, highlighting for ZW (conformational) vs the N-terminal (linear) epitopes. (C) A library of conventional agonist paratope variants with a range of CD28 binding affinities determined by surface plasmon resonance (SPR). (D) A library of agonist paratope variants with a range of CD3 binding affinities determined by SPR.

## TriTCE Co-Stim Antibodies Generated Using the Azymetric™ and EFECT™ Platforms



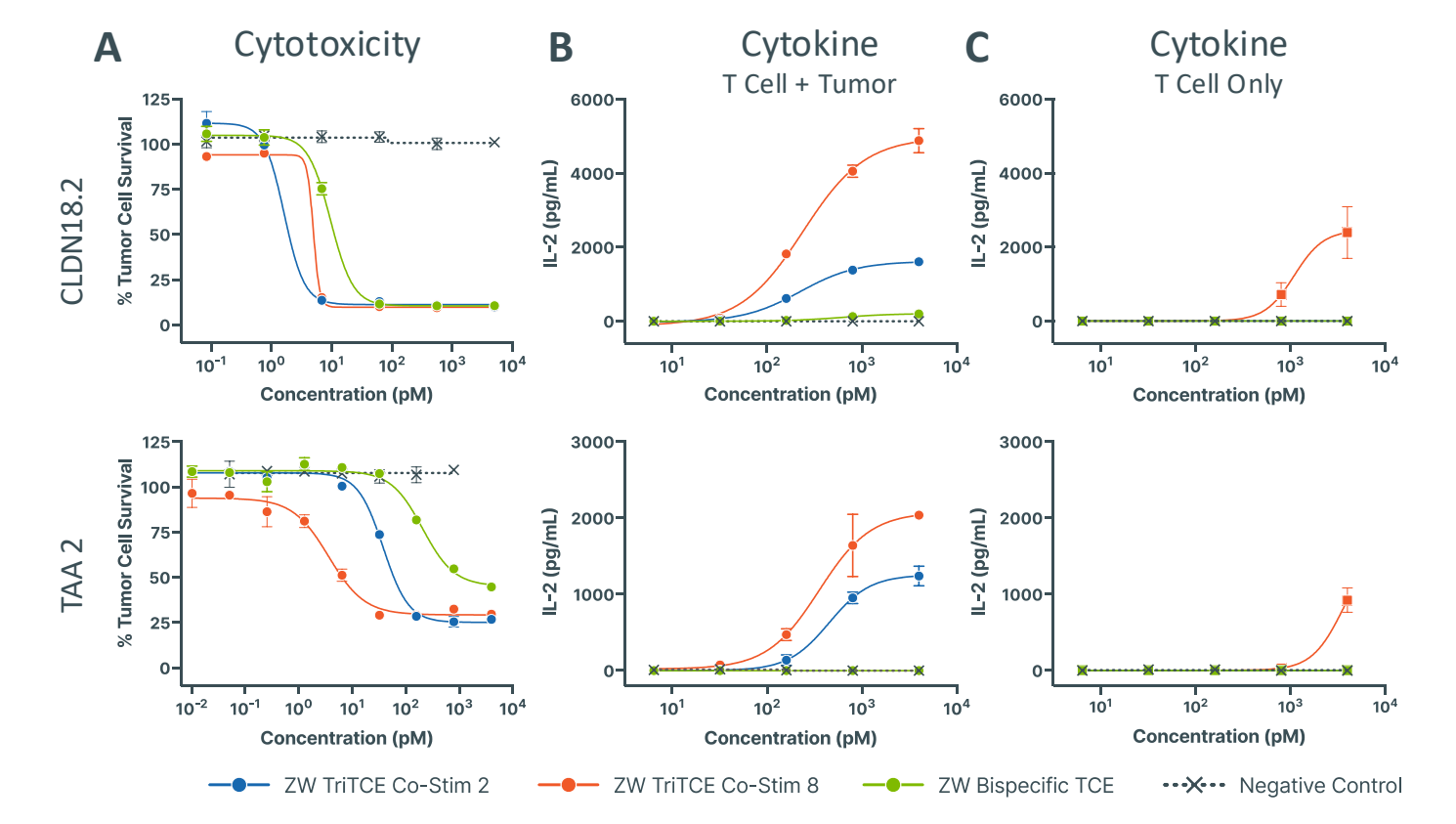
**Figure 4. TriTCE Co-Stim antibodies with various paratope formats and geometries are engineered using the Azymetric™ and EFECT™ platforms.** (A) Schematic representation of a subset of TriTCE Co-Stim antibody formats. (B) Representation of the impact of paratope format (scFv vs. Fab) and geometry on the binding affinities to CD3 and CD28 for a subset of formats with the same CD3 and CD28 paratopes. (C) Representation of affinities following CD3 and CD28 paratope engineering for one TriTCE Co-Stim format, which can be transferred among formats to create a large panel of TriTCE Co-Stim Abs.

## in vitro Screening of Multivalent Geometries and Affinities Enables Selection of Best-in Class Trispecific TCEs

Antibody Format	1	2	3	4	5	6	7	8	9	10
IC50 (pM)										
TAA-Dependent?	✓	✓	✓	✗	✓	✓	✗	✗	✗	✗

**Figure 5. Lead TriTCE Co-Stim is selected through extensive screening in vitro.** High throughput screening enables rapid assessment of a large panel of multivalent antibodies with various geometries and affinities. TriTCE Co-Stim antibodies are screened for T cell-dependent cytotoxic potency against target-expressing cells. A heatmap indicating cytotoxic potency is represented in the table above. TriTCE Co-Stim are further assessed for target-dependent T cell activation by assessing the induction of cytokine in monocultures of T cells.

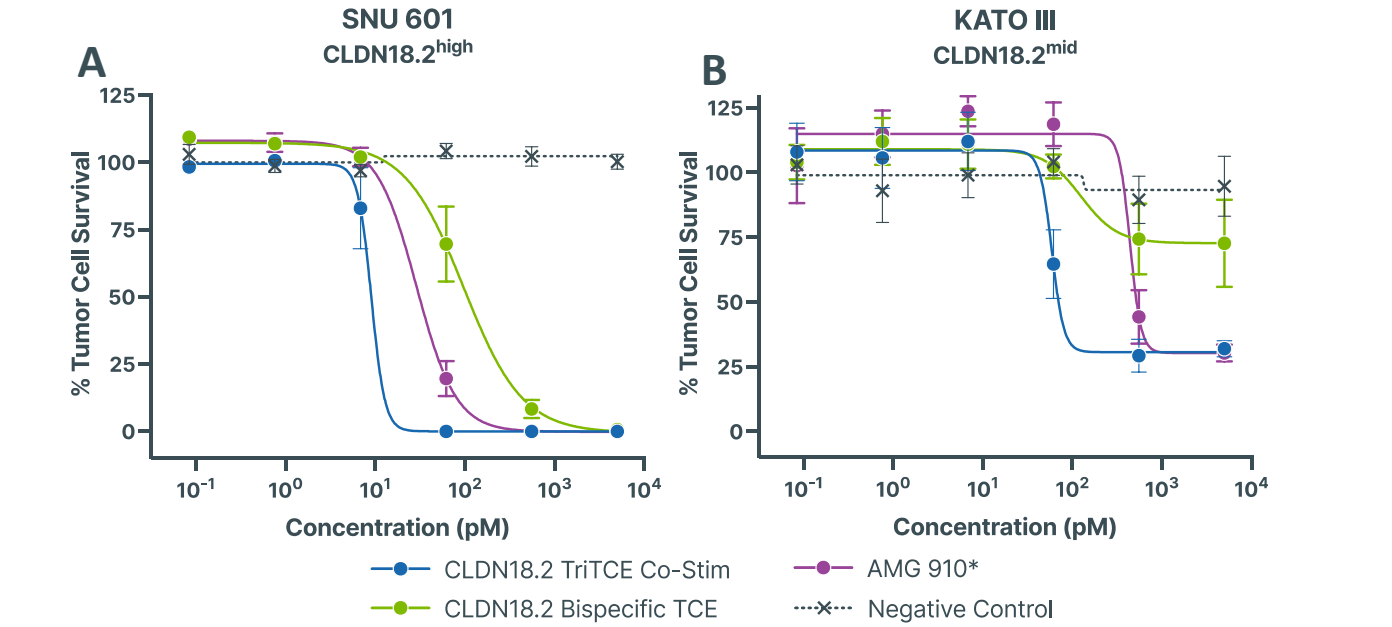
## TriTCE Co-Stim screening process exhibits transferability across different TAA targets



**Figure 6. in vitro high throughput screening to assess TriTCE Co-Stim formats.** Test articles were incubated with T cells co-cultured with TAA-expressing tumor cell lines (A,B) or in a monoculture of T cells (C) and evaluated for cytotoxicity of target cells (A) and IL-2 production by T cells (B,C). Formats 2 and 8 from Figure 5 are depicted to exemplify formats exhibiting TAA-dependent or TAA-independent T cell agonism, respectively.

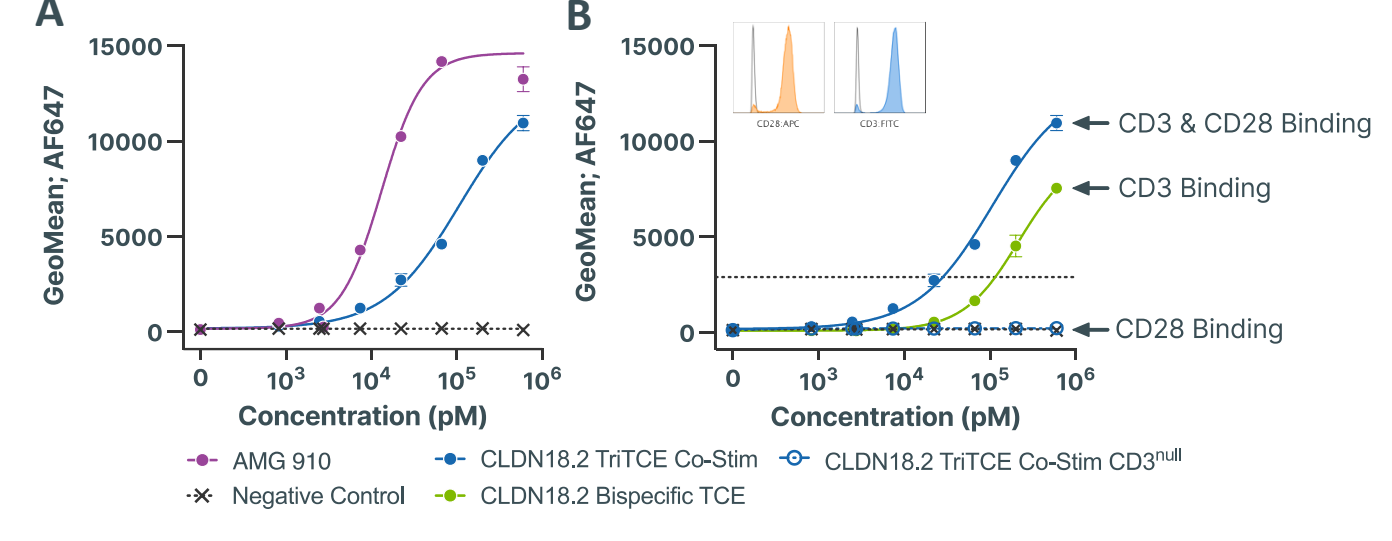
## CLDN18.2 TriTCE Co-Stim (CLDN18.2 x CD3 x CD28) Molecules Support Enhanced T cell Mediated Activity in vitro

**Enhanced long term cytotoxicity of CLDN18.2-expressing target cells co-cultured with T cells at low E:T ratios**



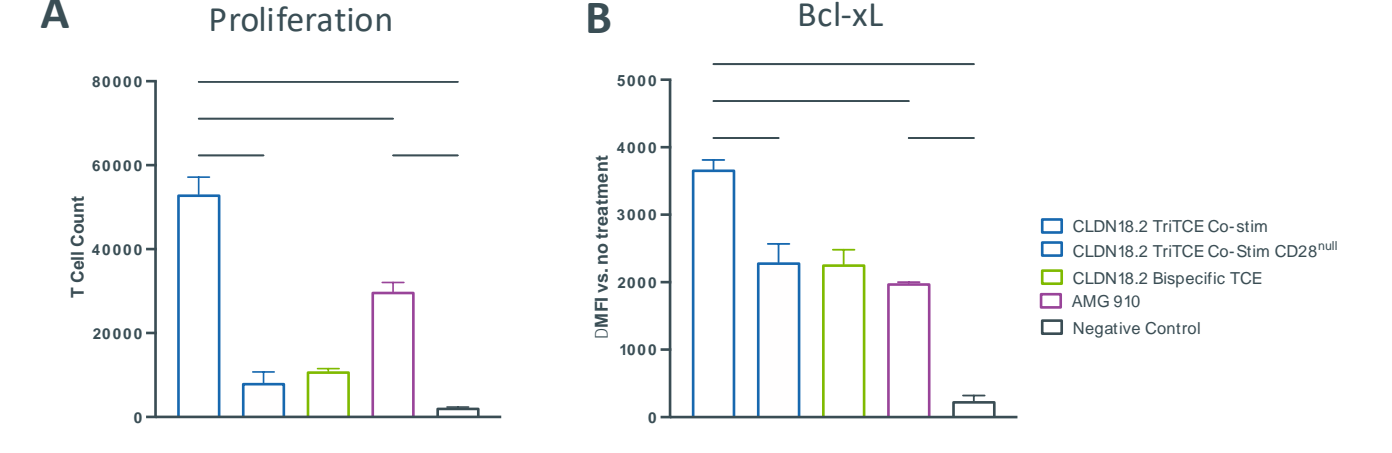
**Figure 7. CLDN18.2 TriTCE Co-Stim displays superior cytotoxic potency of CLDN18.2-expressing cell lines in long term, low E:T co-cultures.** Test articles were incubated with human T cells co-cultured with SNU 601 (A) or KATO III (B) cell lines for 7 days at low E:T and evaluated for cytotoxicity of target cells. \*AMG 910 (CLDN18.2/CD3 BITE) replica produced in-house.

## Reduced, Avidity-Driven T Cell Binding



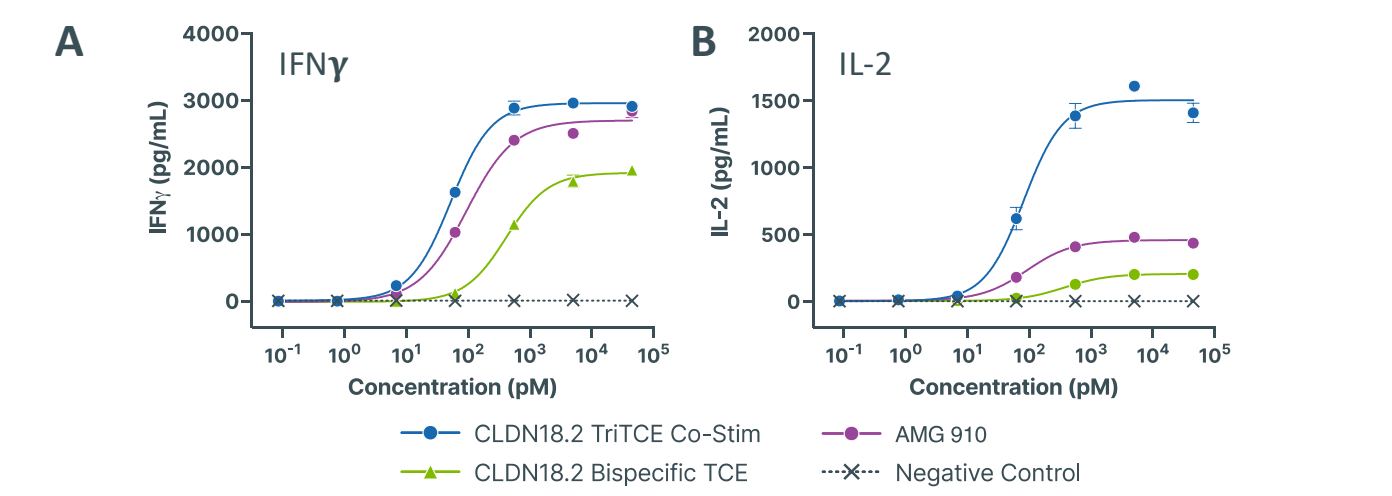
**Figure 8. On-cell binding of TCEs to human CD3+ T cells.** Test articles were incubated with T cells and assessed for binding by flow cytometry. (A) Reduced binding of CLDN18.2 TriTCE Co-Stim compared to the clinical benchmark. Affinity of the clinical benchmark for CD3 is multiple-fold higher than observed for CLDN18.2 TriTCE Co-Stim. (B) The CD28 paratope does not bind T cells without co-engagement of the CD3 paratope. Inset: T cell expression of CD3 & CD28. Dashed line represents B<sub>max</sub> of CD28xCLDN18.2 bispecific.

## Improved T cell proliferation and survival



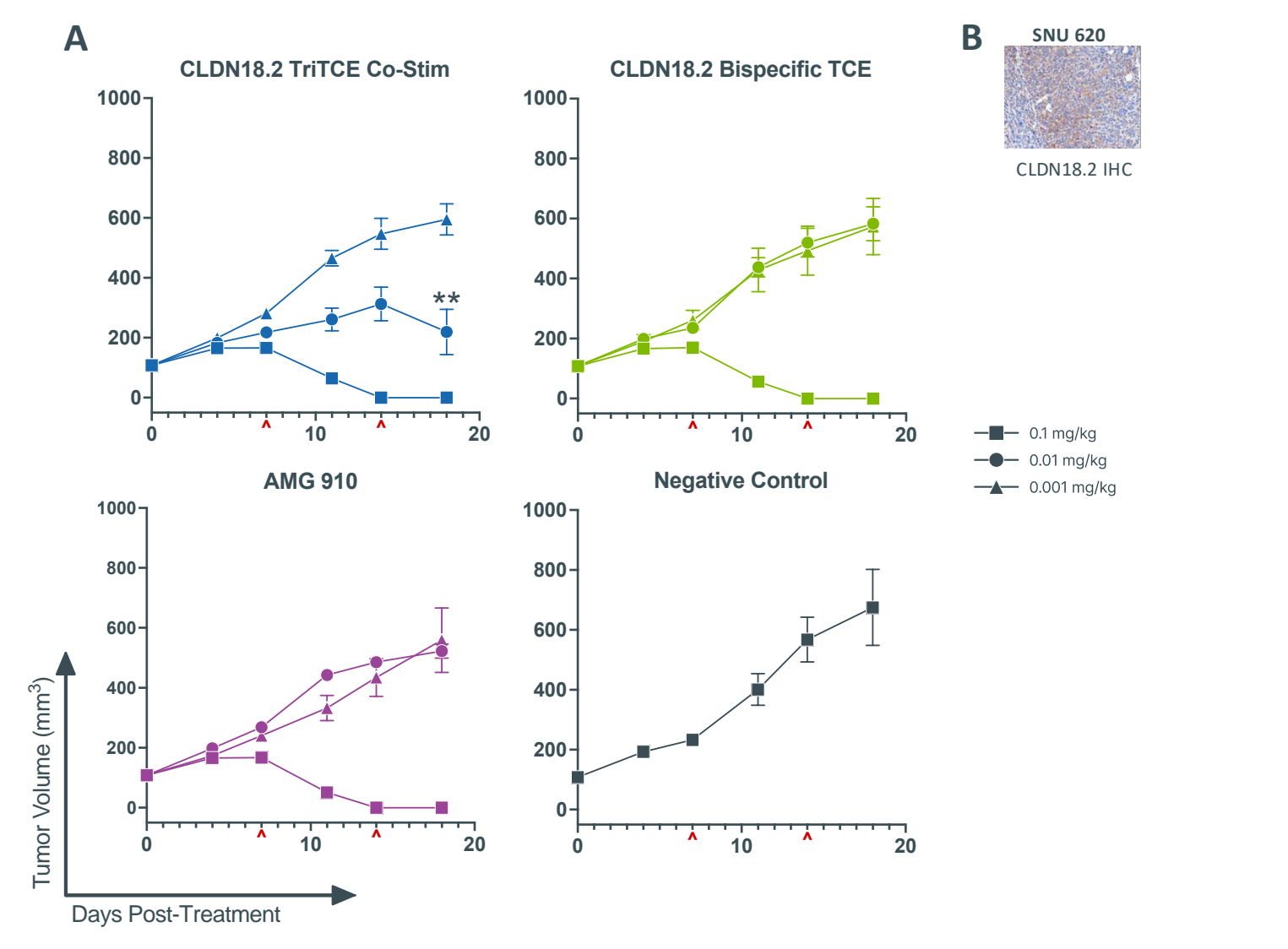
**Figure 9. Assessment of T cell proliferation and Bcl-xL upregulation following incubation with CLDN18.2 TriTCE Co-Stim.** (A) Test articles (200 pM) were incubated with T cells co-cultured with SNU 601 cells for 7 days and quantified by flow cytometry. (B) Test articles (20 nM) were incubated with T cells co-cultured with SNU 601 cells and evaluated for Bcl-xL expression by flow cytometry. (\*\*\*\* p<0.0001; \*\*\* p<0.001)

## Greater CD28-mediated cytokine production



**Figure 10. Assessment of IFNγ and IL-2 production following incubation with CLDN18.2 TriTCE Co-Stim.** Test articles were incubated with T cells co-cultured with SNU 601 cells and assessed for IFNγ (A) or IL-2 (B) production. Cytokine release was not observed following incubation of TriTCE Co-Stim with a monoculture of T cells.

## CLDN18.2 TriTCE Co-Stim Molecule Exhibits Superior in vivo Anti-Tumor Activity in a PBMC-Engrafted Xenograft Model



**Figure 11. in vivo efficacy of CLDN18.2 TriTCE Co-Stim.** (A) SNU 620 cells were injected s.c. in NCG mice. Following humanization with PBMCs, mice were treated IV with test article q1w (\* indicates dosing) and monitored for tumor volume (mean +/- SEM, \*\* p<0.01). (B) IHC of CLDN18.2 expression in established SNU 620 xenograft tumors. (C) Tumor growth inhibition constants.

## Conclusions

Using our Azymetric™ and EFECT™ platforms, we have generated a panel of TriTCE Co-Stim Ab formats. The evaluation of multiple formats, geometries, and paratope affinities allowed **optimization of selectivity and activity to promote maximal therapeutic index and efficacy.**

Our lead CLDN18.2-targeting TriTCE Co-Stim exhibited **CLDN18.2-dependent T cell agonism**, with enhanced IL-2 – but similar IFNγ – production compared to bispecific TCEs. TriTCE Co-Stim induced **greater in vitro cytotoxicity of CLDN18.2-expressing tumor cells** and exhibited **improved T cell proliferation and survival** compared to bispecific TCEs. Furthermore, our lead TriTCE Co-Stim demonstrated avidity-driven T cell binding. Finally, TriTCE Co-Stim mediated **improved tumor regression in vivo** compared to bispecific TCE.

Taken together, these data suggest TriTCE Co-Stim has the potential to provide **more durable responses, to re-invigorate 'cold' tumors with lower T cell infiltration, while avoiding potential toxicity liabilities such as systemic cytokine release.** Taken together, TriTCE Co-Stim demonstrates key factors that may contribute to **improved clinical outcomes.**