Trispecific T Cell Engagers Incorporating Conditional CD28 Co-stimulation (TriTCE Co-stim) to Improve Treatment Responses in Oncology

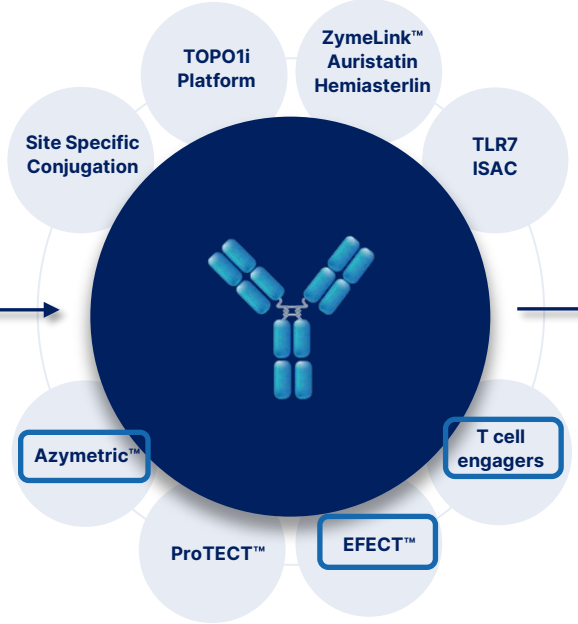
PEGS Europe Protein & Antibody Engineering Summit
November 5, 2024

Nina Weisser, PhD
Director, Multispecific Antibody Therapeutics

ADC and Multispecific Modalities Driving Zymeworks' Pipeline

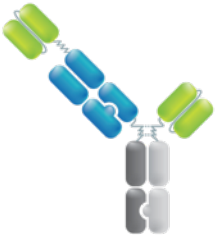
Select Difficult-to-Treat Cancers & Target

Design with Complementary Technology



Optionality with Two Foundational Fit-for-Purpose Modalities

Antibody-Drug Conjugates
Customization:
- Antibody properties
- Antibody format
- Payload
- DAR



Multispecifics
Customization:
- Multiple MOA in single molecule
- Synergistic biology
- Precision targeting through multivalency

DAR: drug to antibody ratio; ISAC: immune stimulating antibody conjugate; MOA: mechanism of action

Differentiated Development of Multispecific Antibody Therapeutics

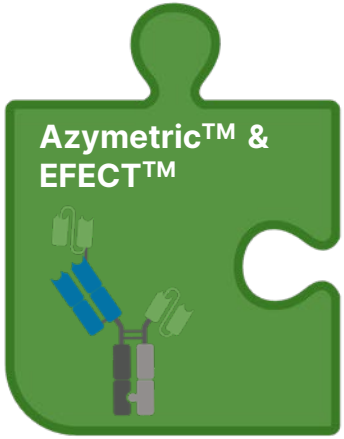
Versatile multispecific antibody therapeutics enhancing potency and precision with proven track record and robust clinical pipeline

Program	Potential Indication	Target(s)	Preclinical / Phase 1 / Phase 2 / Pivotal	Collaboration Partners
Zanidatamab Bispecific	BTC	HER2 x HER2	HERIZON-BTC-302	Jazz Pharmaceuticals, BeiGene
	GEA	HER2 x HER2	HERIZON-GEA-01	Jazz Pharmaceuticals, BeiGene
	BC	HER2 x HER2	EMPOWHER[1]	Jazz Pharmaceuticals, BeiGene
	BC and other solid tumors	HER2 x HER2	8+ ongoing Phase 1 and Phase 2 trials (view)	Jazz Pharmaceuticals, BeiGene
ZW171 Bispecific T cell Engager	OVCA, NSCLC and other MSLN-expressing cancers	MSLN x CD3 (2+1)	NCT06523803 — Phase 1 clinical trial Initiated	
TriTCE Co-Stimulatory Trispecific T cell engager	Under active evaluation	CLDN18.2 x CD3 x CD28 DLL3 x CD3 x CD28	IND candidate nomination studies	
TriTCE Checkpoint Inhibition Trispecific T cell engager	Under active evaluation	TAA x PD-L1 x CD3	Pilot toxicology studies	
Selected Partnered Programs				
JNJ-78278343 Bispecific	Castration-Resistant Prostate Cancer	CD3 x KLK2	Azymetric™ | EFECT™	Johnson & Johnson Innovation

CD28: cluster of differentiation 28; CLDN: claudin; DLL3: delta-like ligand 3; KLK2: kallikrein-related peptidase 2; PD-L1: programmed cell death ligand 1; TAA: tumor associated antigen.
1. Trial initiation expected in the second half of 2024.

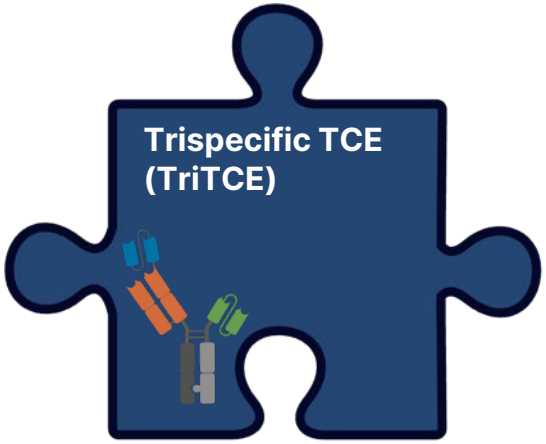
Plug and Play Platforms to Build Differentiated Therapeutic Cell Engagers

Tailored Designs



- **Azymetric™:** Foundational platform enabling generation of multiple bi- and multispecific IgG formats (**zanidatamab**, **ZW171**)
- **EFECT™:** FcγR modulation (**ZW171**)

Enhanced Functionality

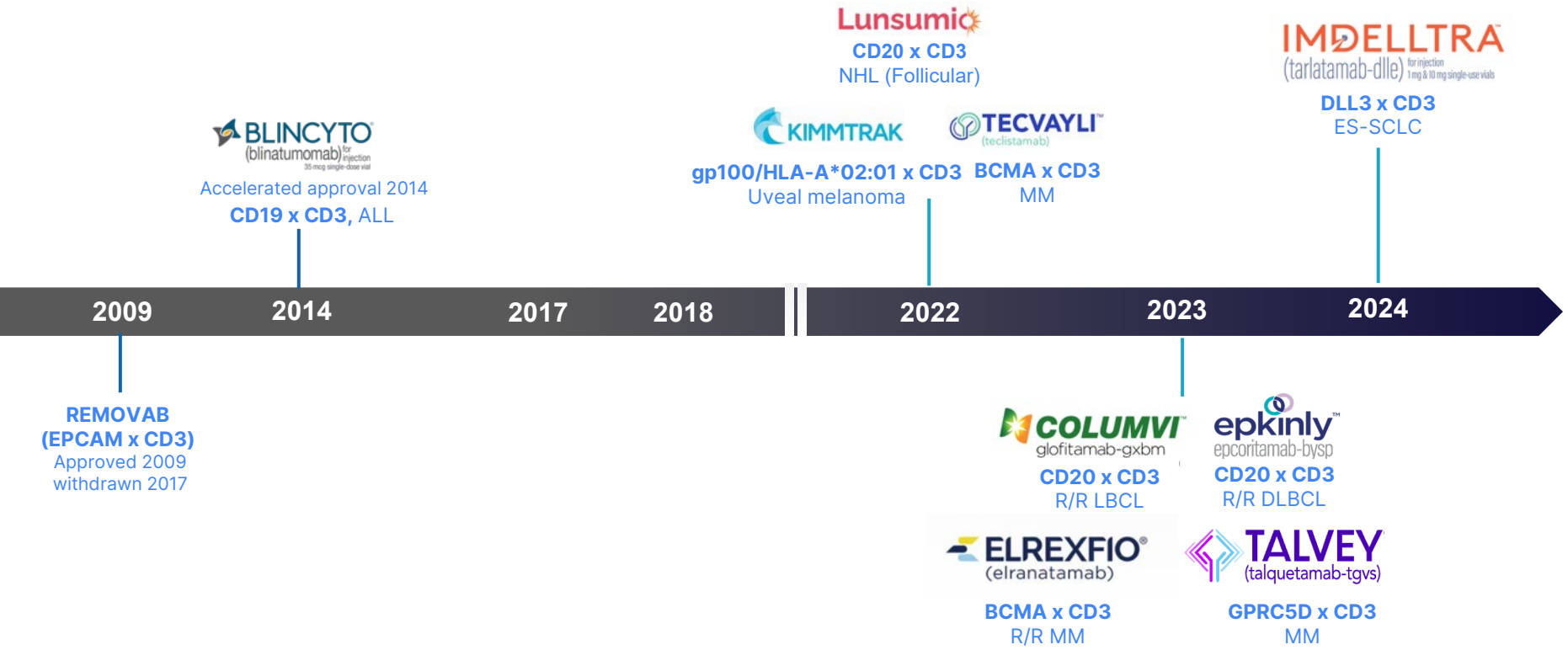


- **TriTCE Co-stim** with integrated CD28 costimulation
- **TriTCE CPI** with integrated checkpoint inhibition

Increased Specificity

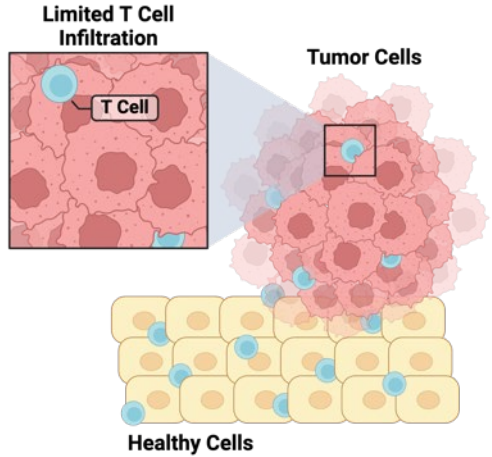
- Dual and Tri-TAA targeting
- Novel tumor-specific targets
- Conditional activation

Accelerated Pace of TCE Approvals in Liquid Tumors but Unmet Need Remains in Solid Tumors

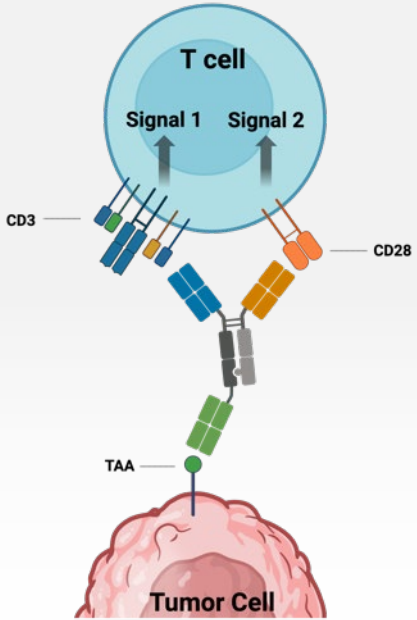


Zymeworks Trispecific Co-stimulatory T Cell Engagers: Overcoming Lack of Efficacy and Durability of Responses in Solid Tumors by Optimization of Signal 1 and 2

Low T cell infiltration and T cell anergy remain challenges in the treatment of solid tumors



Zymeworks Trispecific Co-stimulatory Program



Provides Signal 1 (CD3) and Signal 2 (CD28) in one molecule to **increase T cell activation and proliferation**

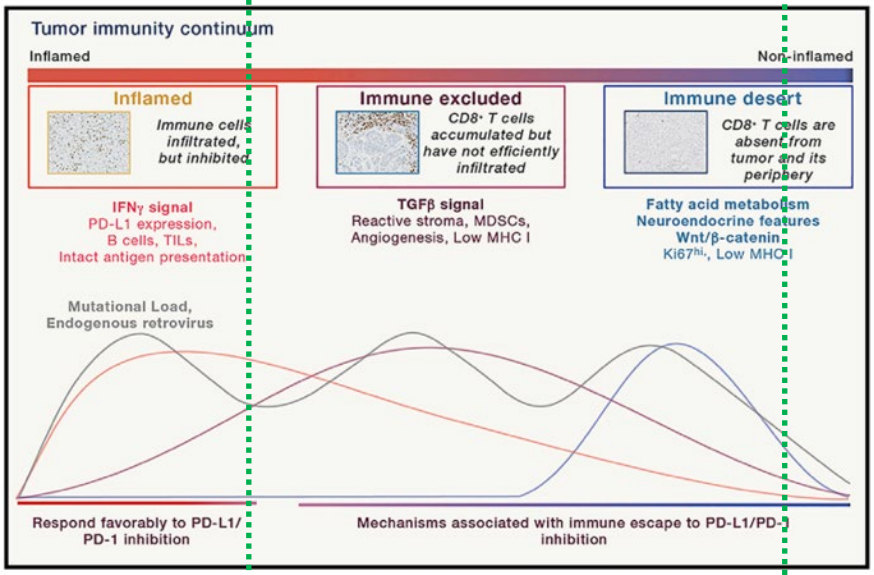
Engineered to balance signal 1 and 2 for optimized **target-dependent T cell activation** and expansion

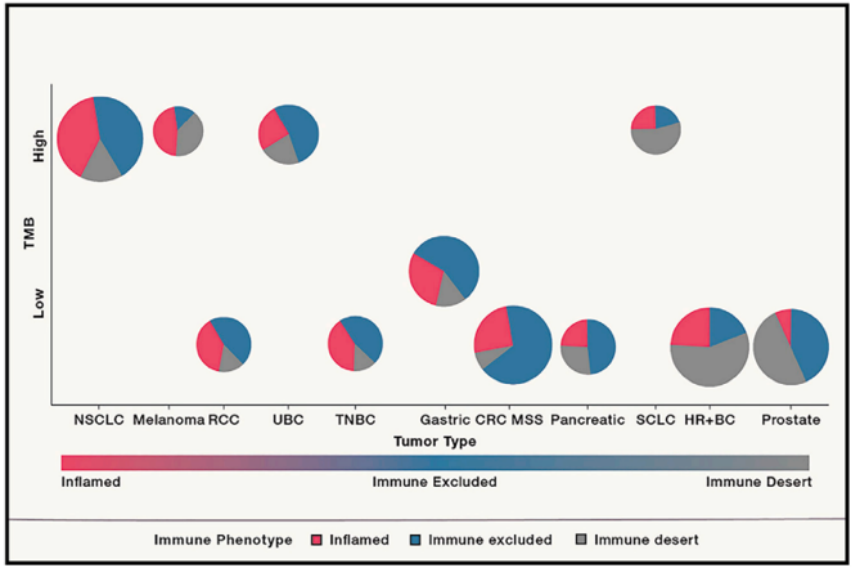
TriTCE Co-stim have the potential to provide **more durable responses** and reinvigorate T cell responses in 'cold' tumors with lower T cell infiltration

Arvedson T et al Ann Rev Cancer Biol 2022

TriTCE Co-stim Designed to Improve Responses in Low T cell Environments

Goal for ZYME TriTCE Co-Stim





Most Tumors Exist on a Continuum of Inflamed, Excluded and Desert Immune Phenotypes

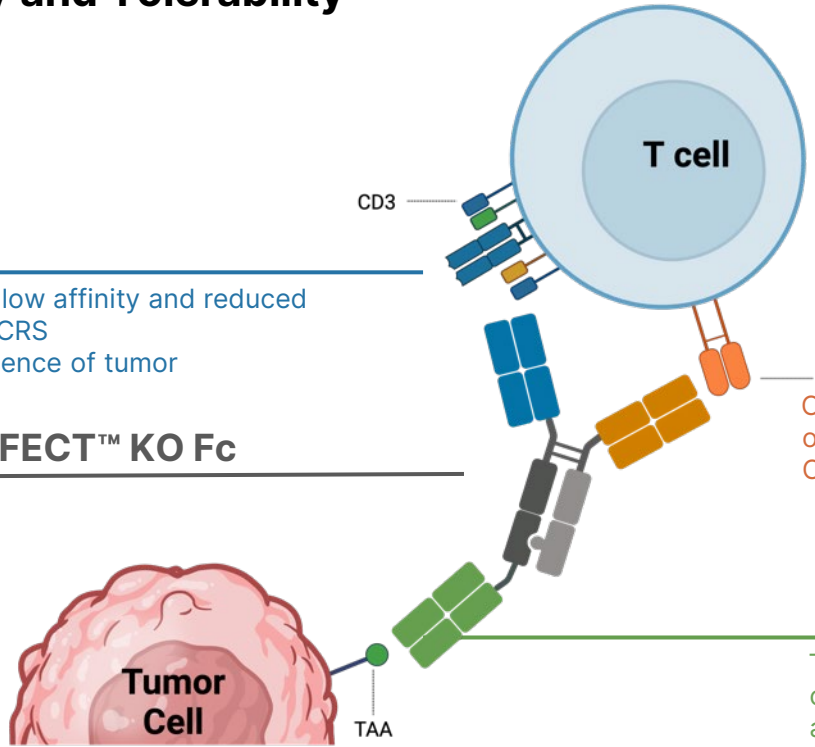
Hedge and Chen 2020 Immunity 52

TriTCE Co-stim Engineered for Enhanced T Cell Functionality, Anti-Tumor Activity and Tolerability

αCD3 Paratope

Novel CD3 paratope with low affinity and reduced T cell binding to mitigate CRS
No T cell activation in absence of tumor

Azymetric™ and EFECT™ KO Fc



αCD28 Paratope

Conditional CD28 binding, requiring co-engagement of CD3, and exhibiting obligate cis-binding with CD3, reducing the risk of T cell-mediated fratricide

αTAA Paratope

Tumor–targeting paratope drives target-dependent T cell responses, mitigating the risk of peripheral T cell activation

T cell engager antibody design is critical to elicit **optimal T cell synapse formation** and to the **widened therapeutic index**

CRS: cytokine release syndrome; KO Fc: knocked out fragment crystallizable region of antibody (Fc).

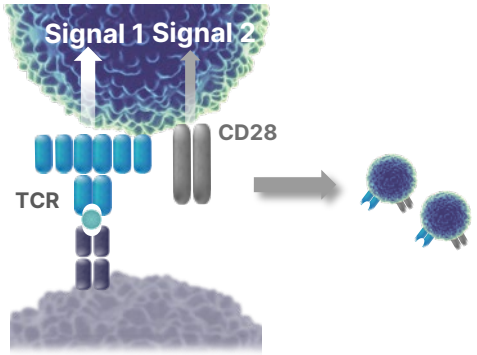
CD28 Selected Based on Biology and Ability to Engineer Conditional Co-stimulatory Molecule

	CD2	41BB	CD28
Expression	Expressed on virtually all T cells, thymocytes, NK cells, dendritic cells	Inducible CD8+ T cells and helper CD4+ T cells, NK cells, B cells, monocytes, and DCs	Constitutive, inducible Naïve and activated CD4+ and CD8+ T cells, plasma cells
Activation Requirements	Monovalent, moderate/low binding affinity	Multimerization, moderate/low binding affinity	Monovalent, low binding affinity
Activation Output	Enhances T-cell activation, T- or NK-mediated cytolysis, apoptosis in activated peripheral T-cells Limited IL-2 induction precluding local (intratumoral) expansion of T cells via paracrine/autocrine IL-2	Enhances T cell proliferation, survival and cytokine secretion, inducing IL-2 production Enhances anti-tumor activity	Enhances T cell proliferation, survival and cytokine secretion, priming naïve T cells, inducing IL-2 production Enhances anti-tumor activity Ability to expand and maintain Tpex and prevent Tex[2]
Ability to Signal in Absence of Signal 1?	Yes[1]	No	**No (monovalent engagement)** Yes (superagonist, bivalent TGN1412)
Ability to Engineer Ab with Co-stimulation Conditional on Signal 1?	Unlikely (signal-1 independent signaling)	Yes	**Yes**

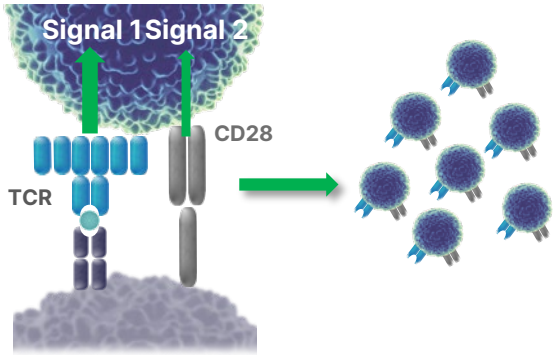
1. De Sousa et al. 2024 iScience 27, 109267
2. Humblin et al., 2023, Sci. Immunol. 8, 25

Balance of T Cell Activation by Signal 1 and Signal 2 Critical to Achieve Optimal T Cell Activation And Prevent Severe Adverse Events

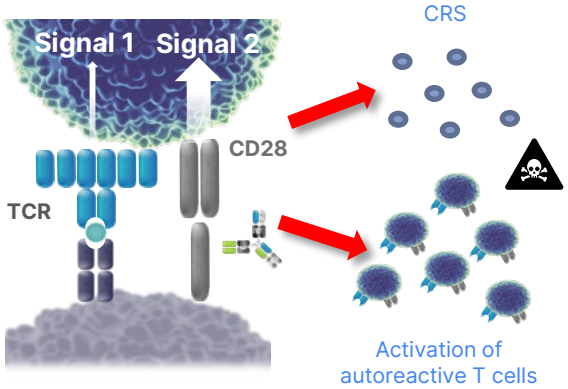
No signal 2
T cell anergy and limited T cell proliferation



Optimal Signal 1 and Signal 2
T cell activation and proliferation



Strong T cell (CD28) Activation
Induction of cytokine release syndrome (CRS) and/or immune-related adverse events (irAEs)



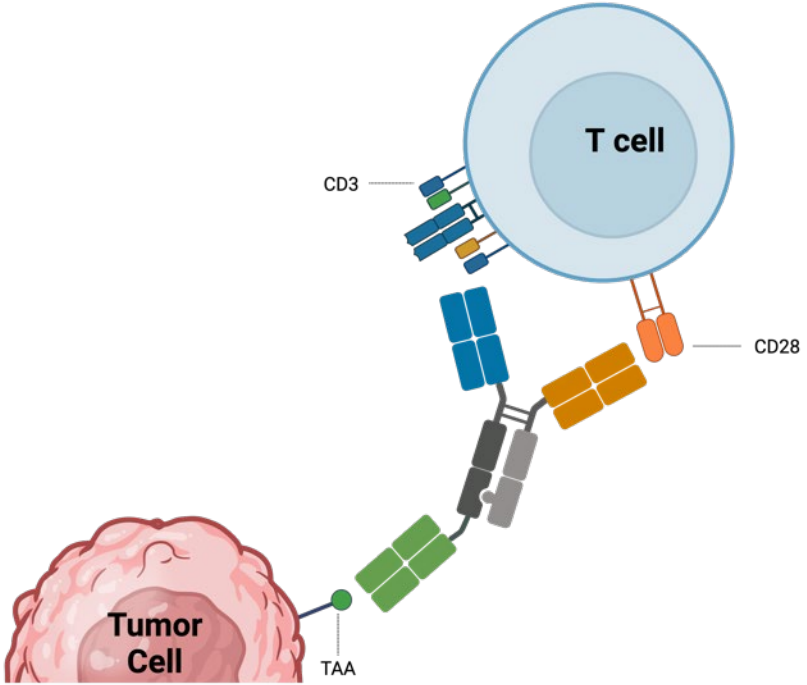
T cell anergy, reduced T cell activation and proliferation

Optimal signal strength for T cell activation

Toxicities associated with T cell overactivation

TriTCE Co-stim Key Design Criteria: Optimized T cell Binding & Activation

Conditional CD28 Co-stimulation and Obligate *cis* T cell Binding



✓ **Balanced activation** of CD3 and CD28

✓ Low affinity CD3 and CD28 binding

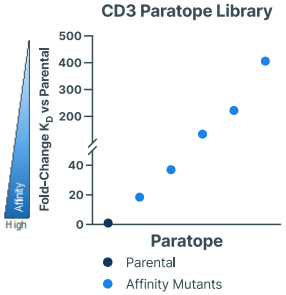
✓ **Conditional CD28 engagement**, requires co-engagement of CD3

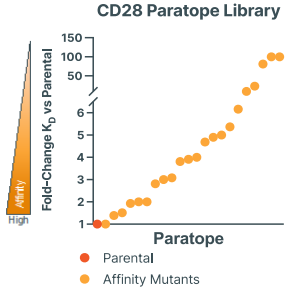
✓ **Obligate cis T cell binding**, no T-T bridging or T cell fratricide

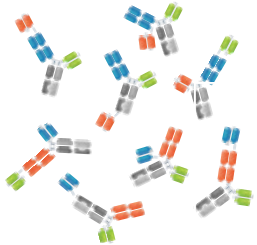
✓ Enhanced **target-dependent** activity

✓ Plug and play platform, transferable to other tumor targets

Lead TriTCE Co-stim Selected Following Extensive Format Screening for Potent, Target-Dependent T cell Activation

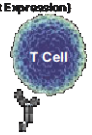
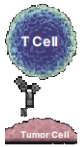






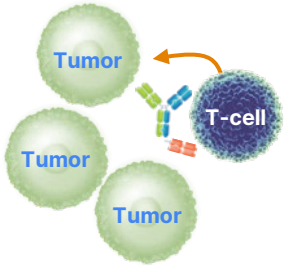
Antibody Format	1	2	3	4	5	6	7	8	9	10
Target-Dependent?	✓	✓	✓	x	✓	x	x	x	x	x
Potency (IC_{50}; pM)										
Yield (mg/L)										
Thermal Stability (T_m; °C)										

10^{0} 10^{-1} 10^{-2}
10 20 30
70 80 90

	Desired	Not Desired
	Lead TriTCE	Target-Independent TriTCE Co-Stim (Format 10)
Periphery (No Target Expression) 	✓ No T Cell Activation ✓ No Loss of T cell Viability	x T cell Activation x Loss of T cell Viability
Tumor (Target Expression) 	✓ Cytotoxicity of Target Cells ✓ T Cell Activation	✓ Cytotoxicity of Target Cells ✓ T Cell Activation

Trispecifics Exhibit Improved Potency & Maximum Cytotoxicity Over Bispecifics with Long Term Co-culture at Low T cell to Tumor Cell Ratios

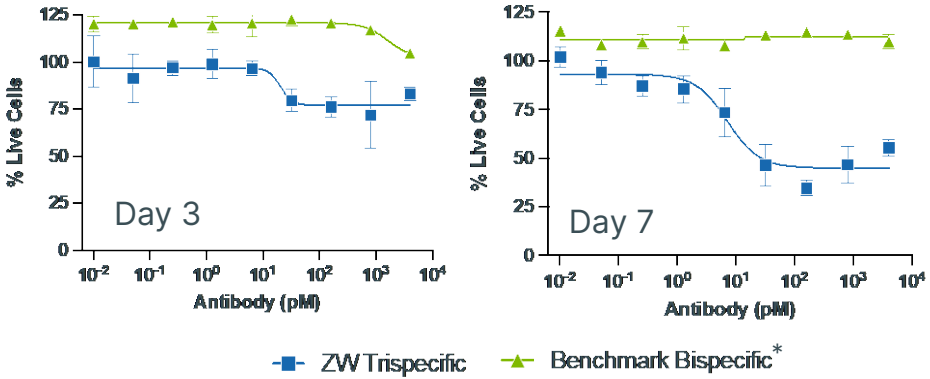
Increased T cell Proliferation and Anti-tumor Activity in Long Term Low E:T Co-cultures



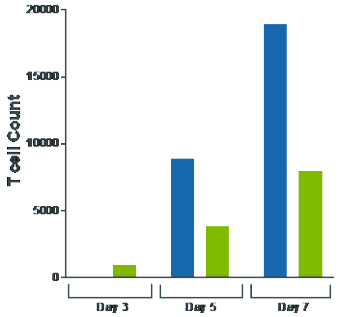
Low E:T Assay System

Cytotoxicity and T cell proliferation

T Cell-Dependent Cytotoxicity



T Cell Proliferation



Developed **long term co-cultures** at **low T cell to tumor cell (E:T) ratios** to better represent conditions in solid tumors

Activity in long term low E:T cultures differentiates trispecifics vs. bispecific benchmarks

* Benchmark Bispecific targets same TAA as trispecific

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

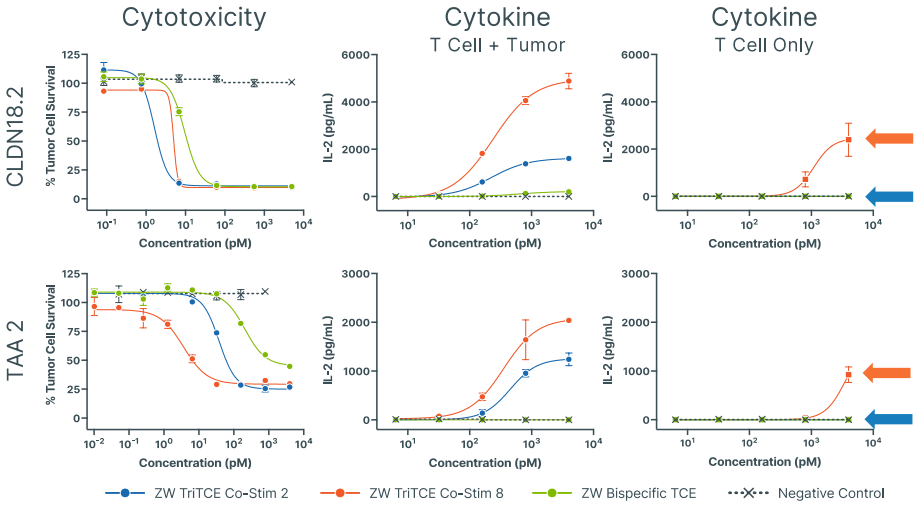
Lead TriTCE Co-stim Format Identified Through Extensive Screening and is Transferable Across Targets

Antibody Format	1	2	3	4	5	6	7	8	9	10
Target-Dependent?	✓	✓	✓	x	✓	x	x	x	x	x
Potency (IC_{50}; pM)										

10^{0} 10^{-1} 10^{-2}

TAA-Dependent

TAA-Independent



TriTCE Co-stim Platform and Workflow Established

Platform established and evaluated with multiple targets including CLDN18.2 and DLL3

CLDN18.2 TriTCE Co-stim

Therapeutic Program for the Treatment of CLDN18.2-Expressing Solid Tumors

Design
Trispecific TCE with optimized TAA, CD3, CD28 binding affinity and geometry using Azymetric™ and EFECT™ platforms

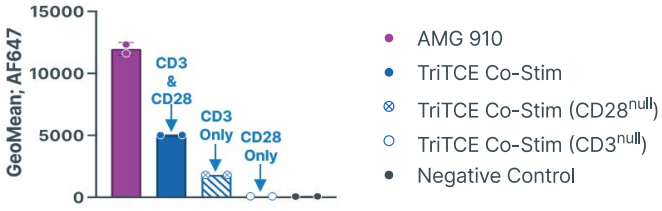
Mechanism
Targets CLDN18.2-expressing tumor cells and CD3 and CD28 on T cells, TAA-dependent T cell mediated cytotoxicity prevents activation of effector T cells in the absence of TAA

Profile
Differentiated long term cytotoxicity at low effector to T cell ratios, increased T cell proliferation, survival, and anti-tumor activity with reduced cytokine release. Safe in in vitro and in vivo CRS models

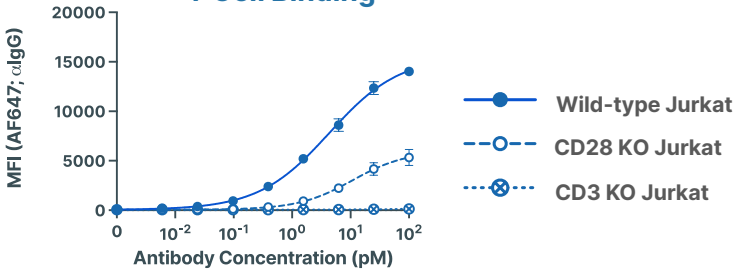
CLDN18.2 TriTCE Co-stim Exhibits Conditional CD28 Binding and Obligate Cis T Cell Engagement

Conditional Binding of CD28, Requiring Co-engagement of CD3

T Cell Binding

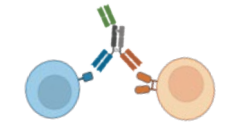


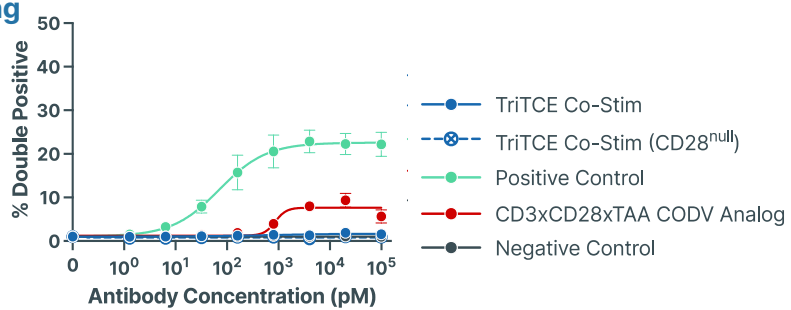
T Cell Binding



Obligate *cis* Binding of CD3 and CD28 on T Cells

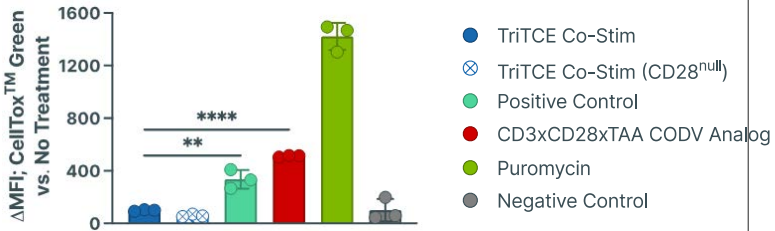
T Cell: T cell Bridging





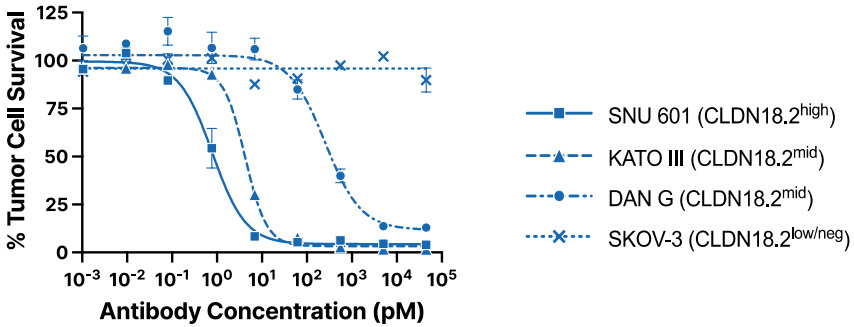
No Reduction of T Cell Viability

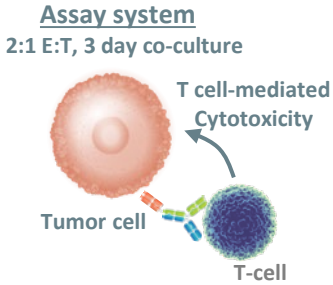
T Cell Monoculture Viability



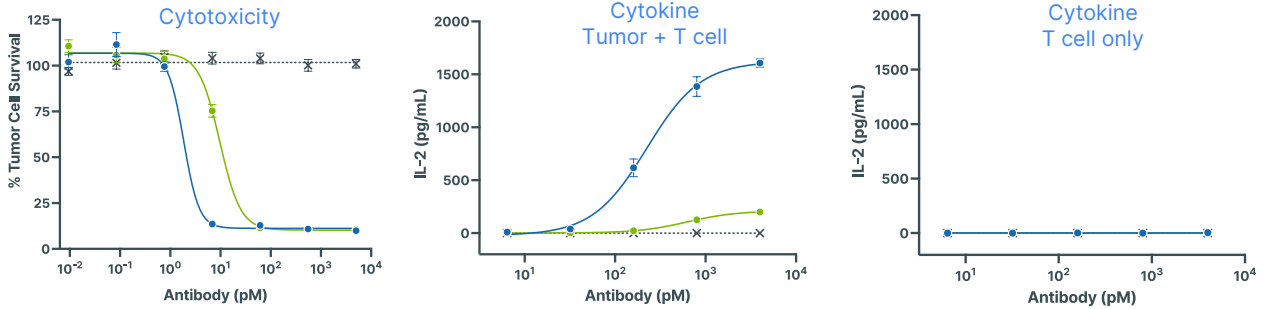
CLDN18.2 TriTCE Co-stim Mediates Conditional CD28 Co-stimulation Dependent on CD3 Engagement and TAA Expression

Target-dependent Cytotoxicity





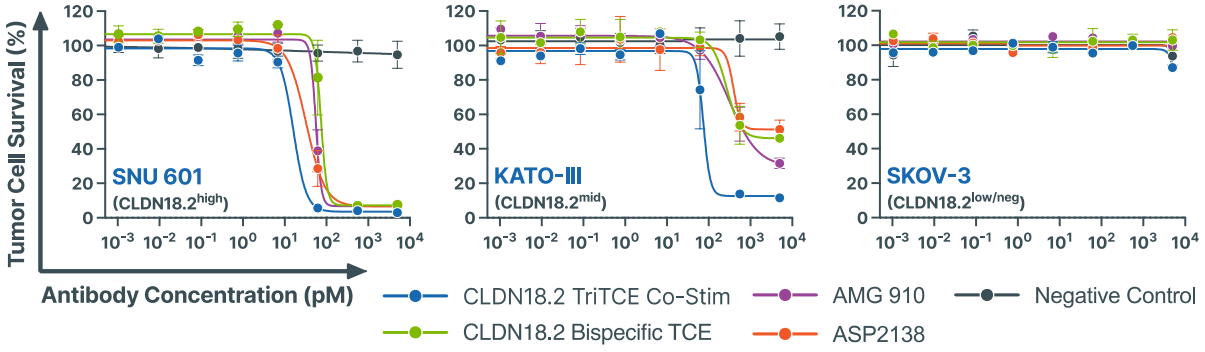
Enhanced Target-Dependent Cytotoxicity and CD28 Co-stimulation Relative to Bispecific



CLDN18.2 TriTCE Co-stim Enhances T cell Responses and Anti-tumor Activity Versus Benchmark Bispecific TCEs

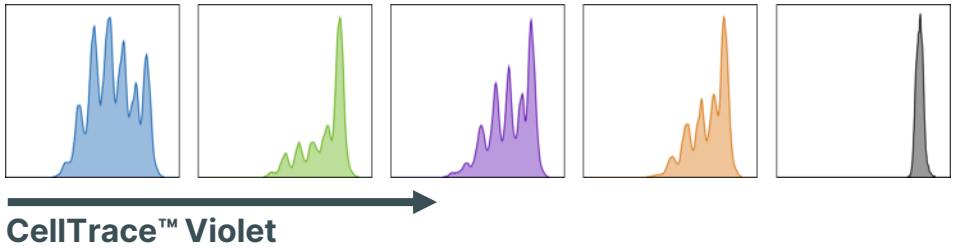
zymeworks

Enhanced Cytotoxicity at Low E:T

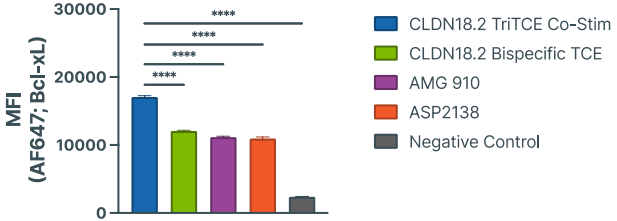


Improved T cell Proliferation and Survival

Proliferation

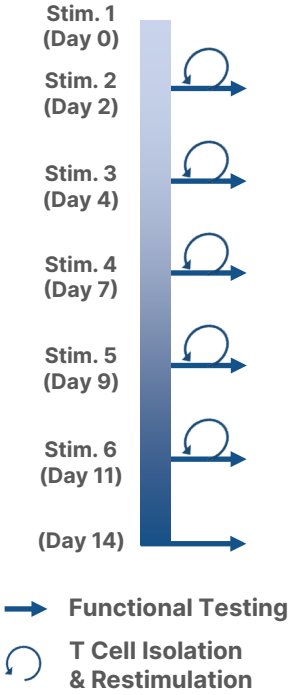


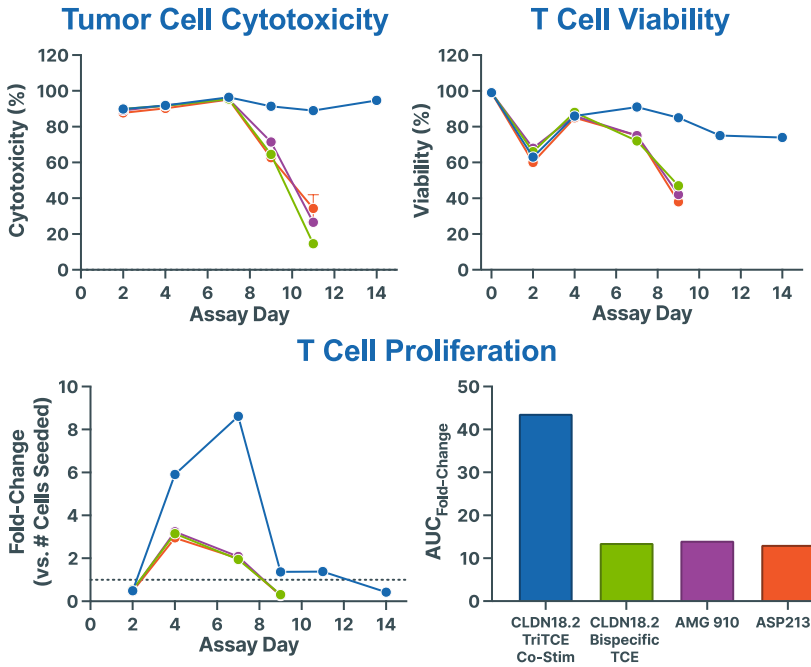
Survival



CLDN18.2 TriTCE Co-stim Displays Sustained T Cell Fitness and Anti-tumor Activity in a Serial Repeat Challenge Assay

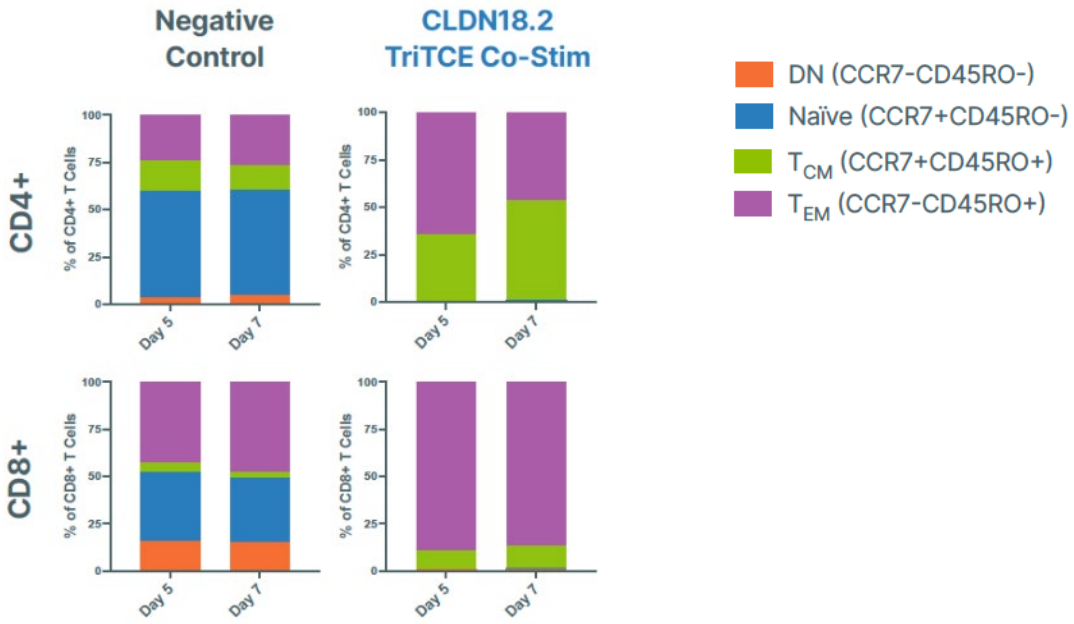
Sustained Tumor Cell Cytotoxicity, T cell Viability and T Cell proliferation Over Repeated T cell Simulation





CLDN18.2 TriTCE Co-stim Treatment Results in Activation of Naive and Expansion of T_{CM} and T_{EM} Memory Cell Subsets

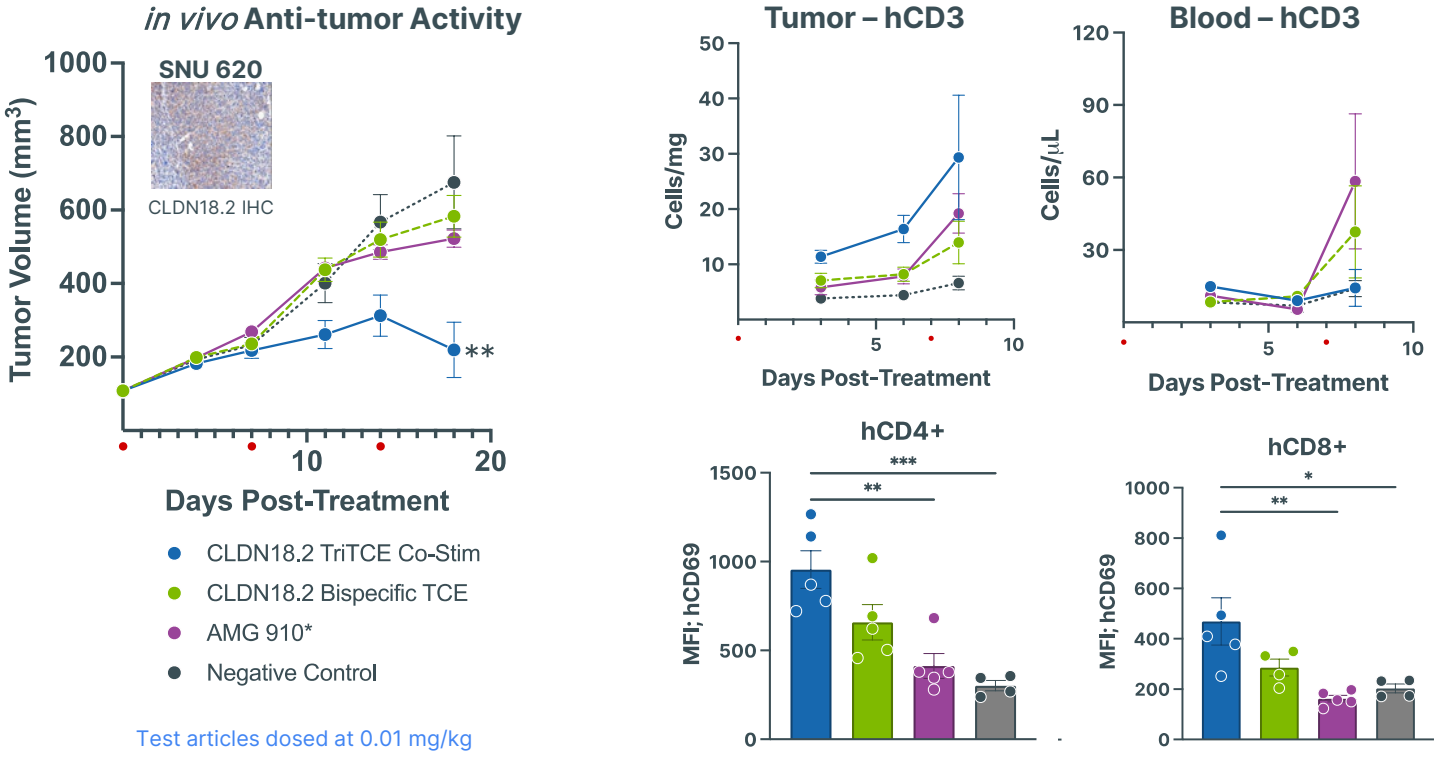
T_{CM} and T_{EM} Expansion of CD4+ and CD8+ T cell at E:T of 1:1



Test articles incubated with PBMCs co-cultured with CLDN18.2-expressing SNU 601 target cells and assessed for expansion of memory subsets. Memory subsets of CD4+ or CD8+ T cells were analyzed by flow cytometry after 5 and 7 days of co-culture at an E:T of 1:1.

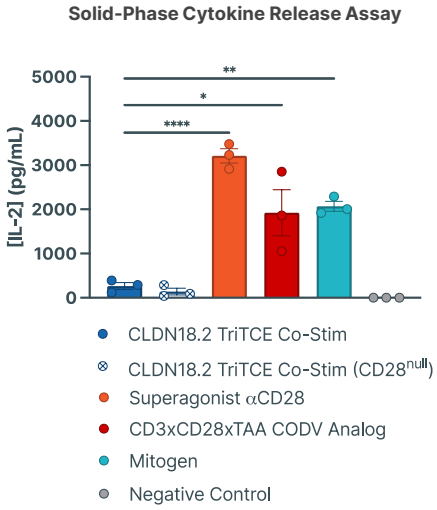
CLDN18.2 TriTCE Co-stim Mediates Enhanced Anti-tumor Activity and Increases Activated Intratumoral T cells *in vivo* Compared to Benchmark Bispecific TCEs

Greater Anti-tumor Activity and Increased Activated T cell Infiltration in Tumor But Not in Blood

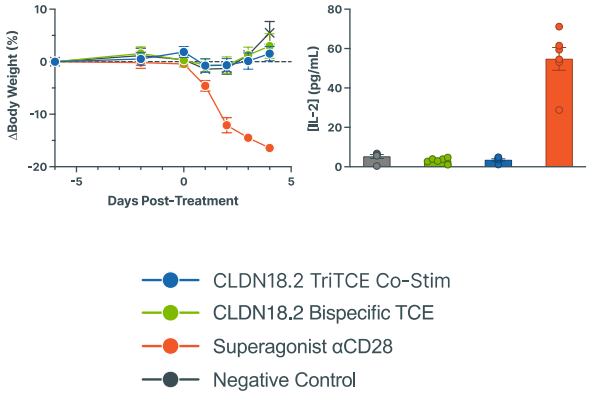


CLDN18.2 TriTCE Co-stim has a Favorable Safety Profile

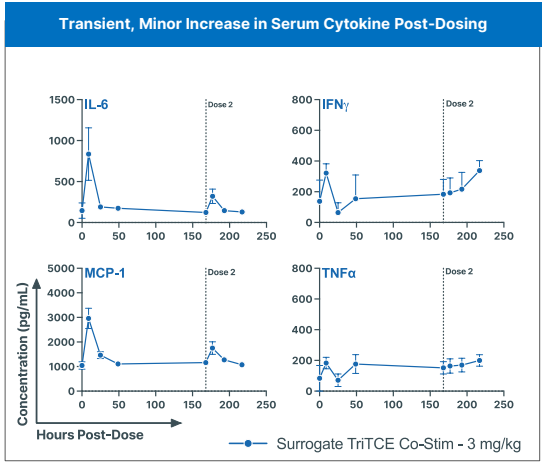
No Cytokine Activation with PBMCs Alone



No Systemic Cytokine Release in Humanized Mouse Model



Well Tolerated in NHP

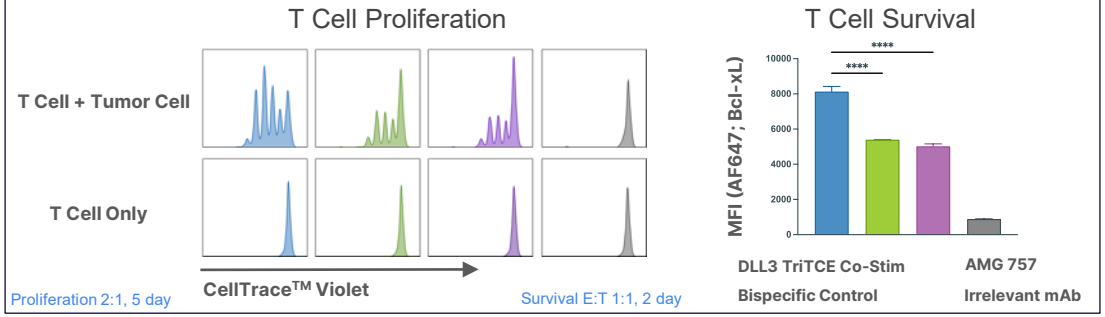


No histopathological changes observed in the stomach, where CLDN18.2 is expressed (Türeci et al., 2011)

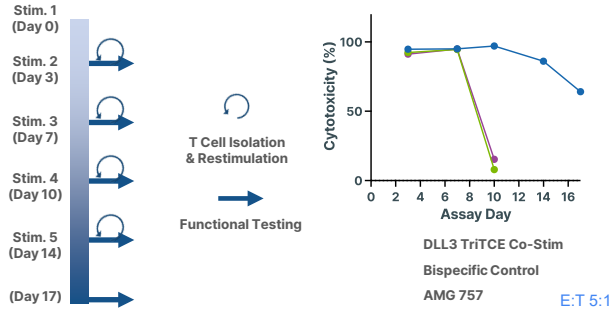
TriTCE Co-stim Applicable to Additional Targets

DLL3 TriTCE Co-stim: CD3 x CD28 x DLL3

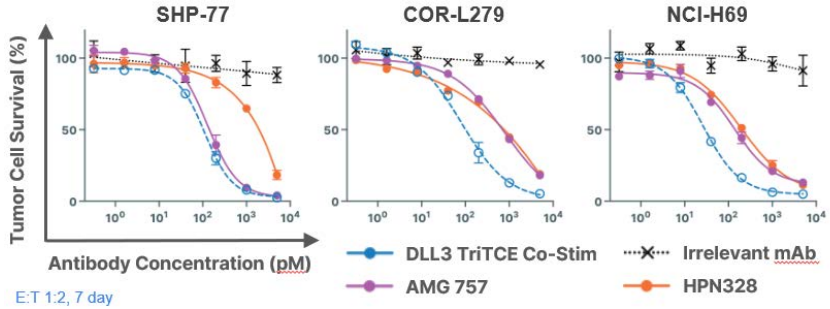
Improved T Cell Proliferation and Survival



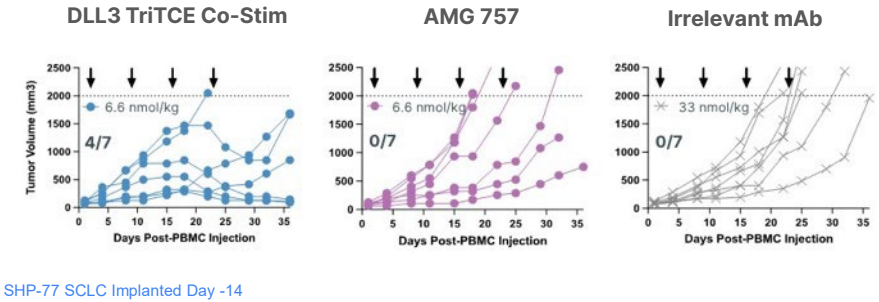
Sustained Cytotoxicity



Increased Cytotoxicity vs. Benchmarks at Low E:T

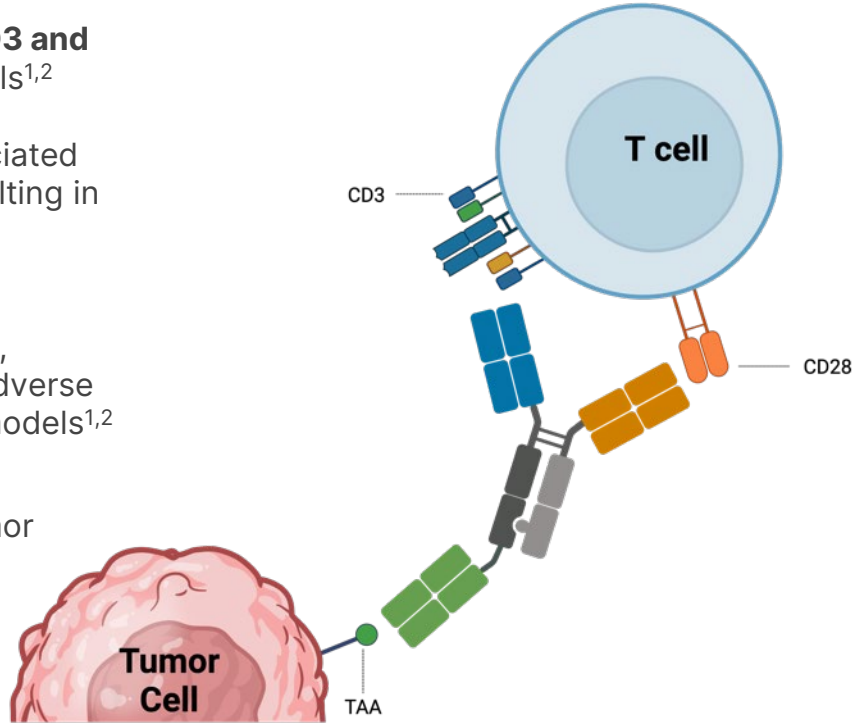


Anti-tumor Activity in Established Tumor Xenograft Model



TriTCE Co-stim Summary

- ✓ Zymeworks TriTCE Co-stim provides **balanced CD3 and CD28 activation** to prevent overactivation of T cells[1,2]
- ✓ **Enhanced tumor target-dependent activity** associated with sustained T cell viability and cytotoxicity resulting in improved anti-tumor activity in preclinical models compared to bispecific TCEs[1-5]
- ✓ **No CD28 binding in absence of CD3 engagement**, lowering risk of CD28-mediated immune related adverse events (irAEs), well tolerated in both in vivo CRS models[1,2] and in non-human primates[3]
- ✓ Platform established and transferable to other tumor targets



1. Newhook et al., Cancer Res. (2023); 2. Newhook et al., JITC (2023); 3. Newhook L et al., Abstract #6719 presented at AACR Annual Meeting 2024; 4. Repenning P et al., Abstract #6716 Presented at AACR Annual Meeting 2024. 5. Newhook et al., SITC (2023); 6. Skokos et al., Sci. Transl. Med. (2020); 7. Dragovich et al., Cancer Research (2023); 8. Stein et al., Journal Clinical Oncology (2023); 9. Martins et al., Nature Reviews Clin Oncol (2019); 10. Eastwood et al., BJP (2010); 11. Roemer et al., Blood (2011); 12. Hui et al., Science (2017); 13. Humphrey et al. (2011) J Natl Cancer Inst. 14. Seung et al., Nature (2022); 15. Promsote et al., Nature Communications (2023).

Acknowledgements...A Global Team Effort

https://www.zymeworks.com/publications/

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
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

DLL3 TriTCE Co-stim: A next generation Trispecific T cell engager with integrated CD28 co-stimulation for the treatment of DLL3-expressing cancers
Peter Repenning, Desmond Lau, Diana Canals Hernaez, Alec Robinson, Diego Perez Escanda, Mariana Rocha, Aditi Deshmukh, Begonia Silva Moreno, John Zhang, Polly Shao, Nichole Escalante, Lisa Newhook, Purva Bhojane, Chayne L. Piscitelli, Nicole Afacan, Paul A. Moore, Thomas Spreter von Kreudenstein, Nina E. Weisser

Next-generation co-stimulatory trispecific T cell engagers (TriTCEs) for the treatment of solid tumors
Lisa Newhook, Purva Bhojane, Peter Repenning, Diego Perez, Nichole Escalante, Patricia Zwierzchowski, Alec Robinson, Lauren Clifford, Harsh Pratap, David Douda, Chayne Piscitelli, Nicole Afacan, Thomas Spreter von Kreudenstein, Nina E. Weisser

Zymeworks' Multispecific Antibody Therapeutics Team