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

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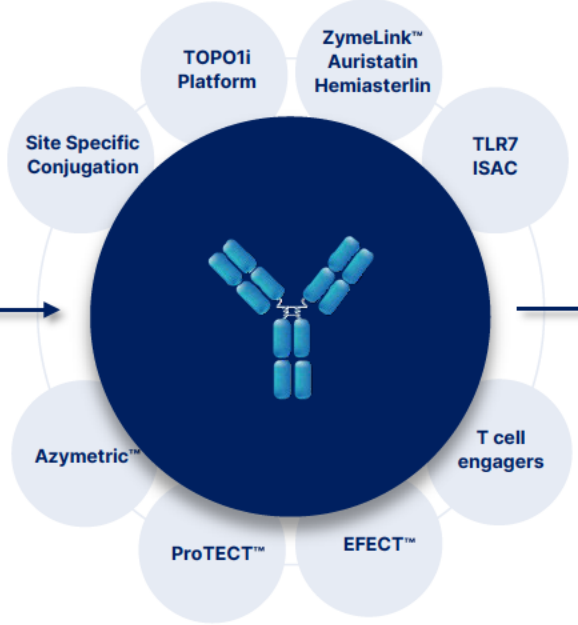
ADC and Multispecific Modalities Driving Zymeworks' Pipeline

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

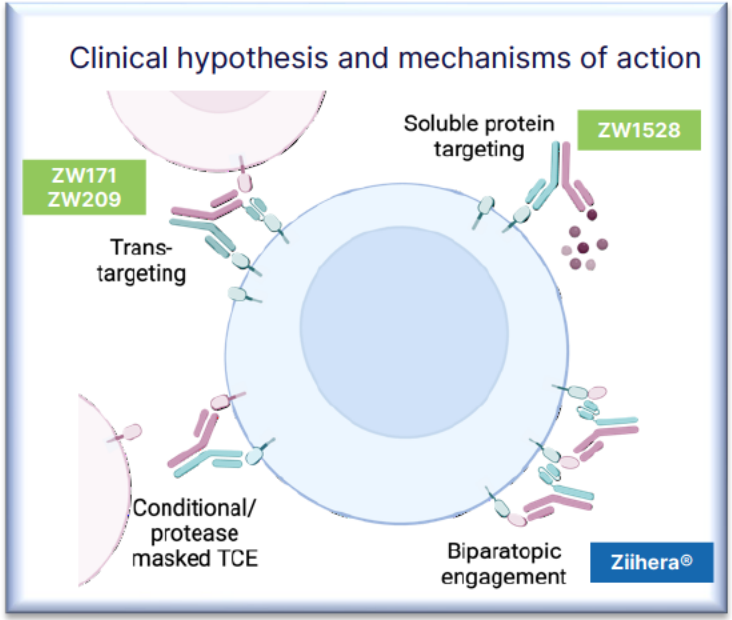
DAR: drug to antibody ratio; ISAC: immune stimulating antibody conjugate; MOA: mechanism of action

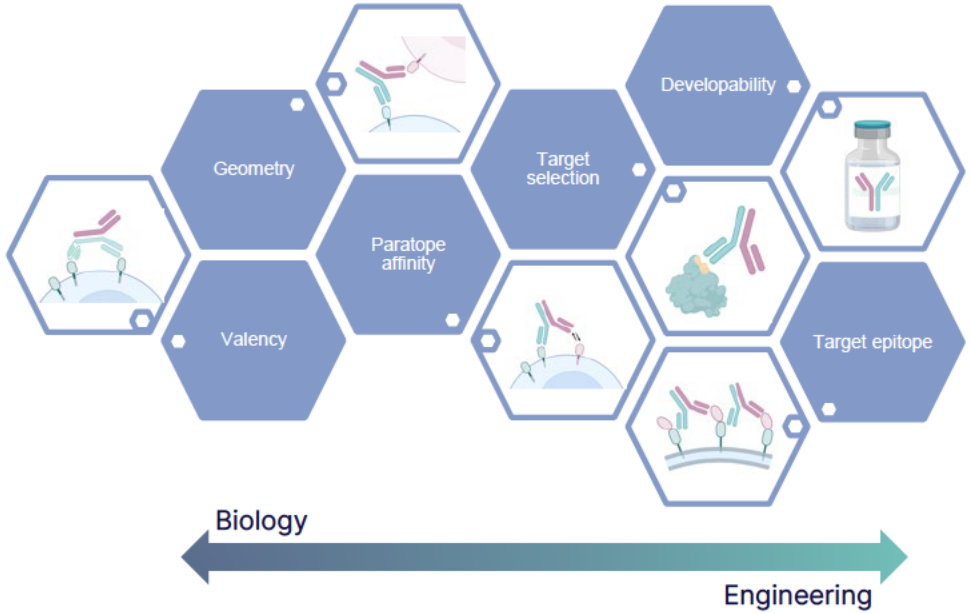
Differentiated Development of Multifunctional Therapeutics

Program	Technology	Target	Indication	Discovery	Preclinical	Phase 1	Phase 2	Phase 3
Solid Tumor Oncology: Antibody-Drug Conjugates (ADC)								
ZW191 Topo1i ADC | DAR 8 | Fc WT	ZD06519 Payload	FRα	Gynecological Thoracic			NCT06555744		
ZW220 Topo1i ADC |DAR 4 | Fc Mut	ZD06519 Payload	NaPi2b	Gynecological Thoracic					
ZW251 Topo1i ADC | DAR 4 | Fc WT	ZD06519 Payload	GPC3	Digestive System (HCC, PDAC)					
Solid Tumor Oncology: Multispecifics								
Zanidatamab Bispecific	Azymetric™	HER2	Multiple indications			HERIZON-BTC-302, HERIZON-GEA-01, EMPOWHER, +8 ongoing P1 and P2		
ZW171 Trivalent TCE | 2+1 Format	Azymetric™ Novel anti-CD3	MSLN x CD3	Gynecological Thoracic			NCT06523803		
ZW209 Trispecific TCE | Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	DLL3xCD3x CD28	Thoracic					
ZW239 Trispecific TCE | Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	CLDN18.2x CD3xCD28	Digestive System					
AIID								
ZW1528 Dual Cytokine Blocker	Azymetric™ Hetero-Fab | YTE	IL4RαxIL33						
ZW1572 Dual Cytokine Blocker	Azymetric™ Hetero-Fab | YTE	IL4RαxIL-31						

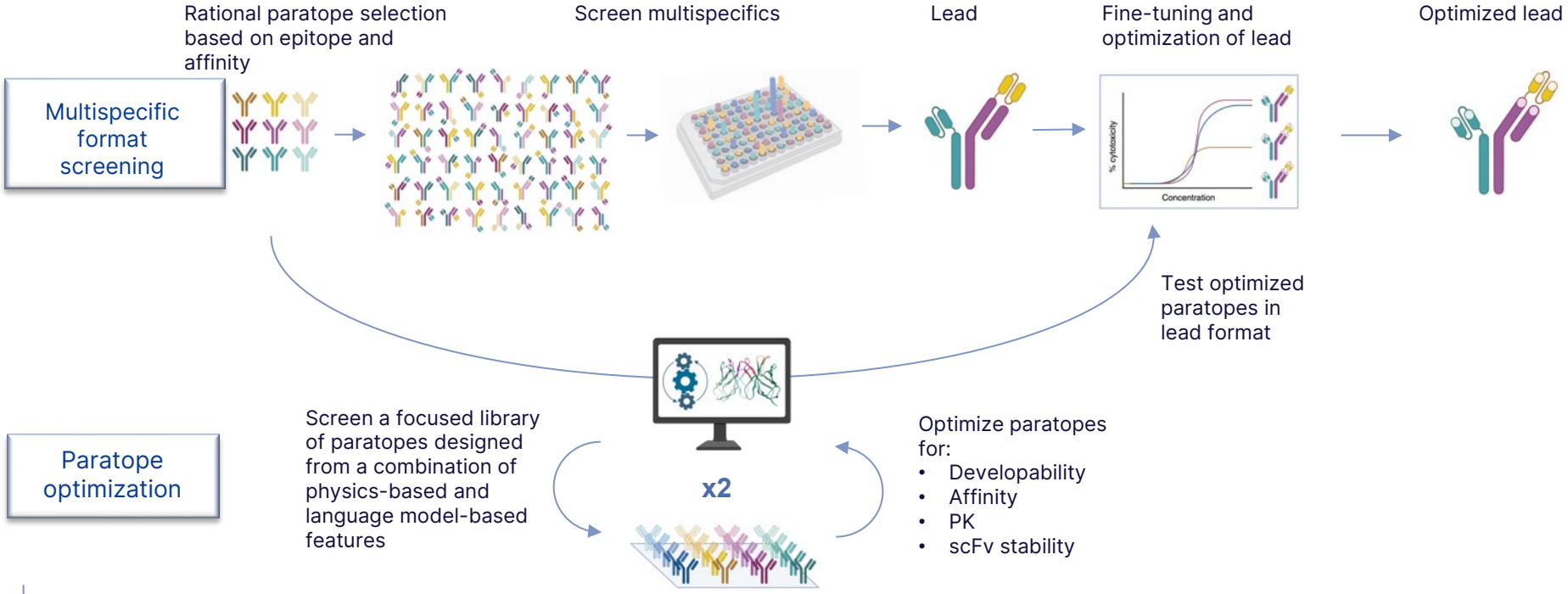
Multispecific Antibody Development Requires Optimization of Multiple Parameters Specific to the Desired Mechanism of Action

- Understanding the interplay of antibody geometry with optimal paratope affinity, valency, and target epitope is critical to identifying multispecific antibody therapeutics





Parallelization of Format Selection and Paratope Optimization Using HTP Screening Together with AI/ML Methods



Next Generation CD28 Co-Stimulatory Trispecific T cell Engager Platform

Designed to provide more durable responses in solid tumors and superior activity in ‘cold’ tumors

Therapeutic Rationale

- Next Gen TriTCE Co-stim can provide increased T cell fitness, activation, and proliferation via tumor-dependent T cell co-stimulation

Product Differentiation

- Novel approach of modular geometry and avidity screening of trispecifics to optimize T cell activation by Signal 1 and Signal 2
- TriTCE Co-stim show superior anti-tumor activity to bispecific benchmarks and exhibit no activation of T cells in absence of tumor cells

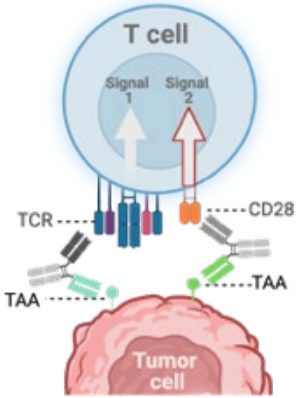
Next Milestones

- Expand utility to additional tumor targets

Current Bi- vs. Trispecific Co-Stimulatory T Cell Engager Approaches

Bispecific CD28 T cell Engagers (monotherapy or in combination)

Regeneron (MUC16xCD3 + MUC16xCD28, CD20xCD3 + CD22xCD28)
Janssen (PSMAxCD28 + KLK2xCD3)
Roche (CD20xCD3 + CD19xCD28)

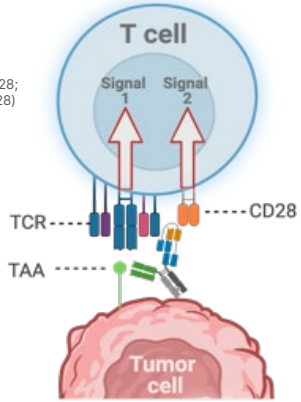


Limitations:

- Independent engagement of CD28-TAA, needs to be optimized to pair with CD3-TAA. **Difficult to optimize** by clinical dose adjustment.
- **Exposure** of two molecules at required dose levels potentially suboptimal

Trispecific CD28 T cell Engagers

Sanofi (HER2xCD3xCD28; CD38xCD3xCD28)



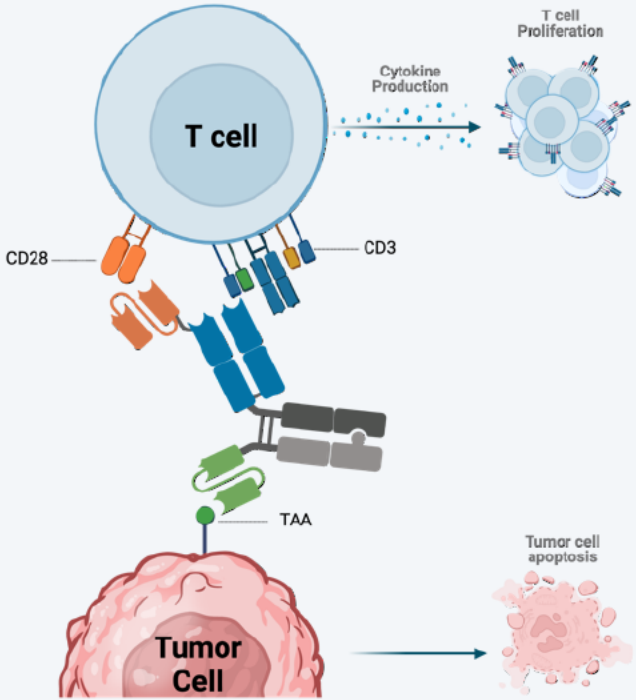
Limitations:

- First generation used high affinity CD3 and CD28 superagonist paratopes[1,2]
- Peripheral T cell binding, activation and TMDD[1,2]
- **Target-independent** activity and **T cell-T cell** activation

[1] Seung et al., Nature (2022); [2] Promsote et al., Nature Communications (2023)

TriTCE Co-Stim Designed to Optimize T cell Binding, Activation and Anti-Tumor Activity

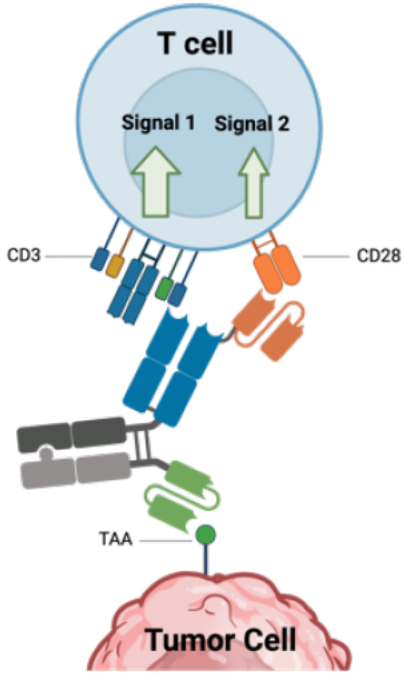
Conditional CD28 Co-Stimulation and Obligate *cis* T cell Binding



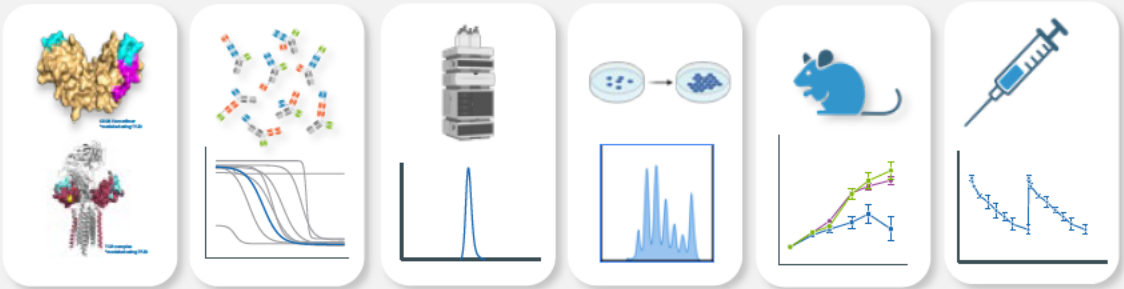
	Design Feature	Expected Benefit
1	**Balanced activation of CD3 and CD28**	Potential to provide more durable responses and activate T cell responses in 'cold' tumors with lower T cell infiltration
2	**Low affinity CD3 and CD28 binding**	Prevents overactivation of T cells and reduces risk of CRS and irAEs
3	**Obligate *cis* T cell binding**	No T cell-to-T cell bridging or T cell fratricide
4	**Conditional CD28 engagement**	Requires co-engagement of CD3
5	**Enhanced target-dependent activity**	Low T cell binding and no T cell activation in periphery or absence of tumor target

CRS: Cytokine release syndrome; irAEs: immune-related adverse events

TriTCE Co-Stim: A Next Generation Trispecific T Cell Engager Platform

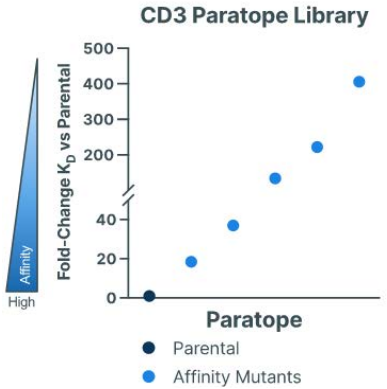


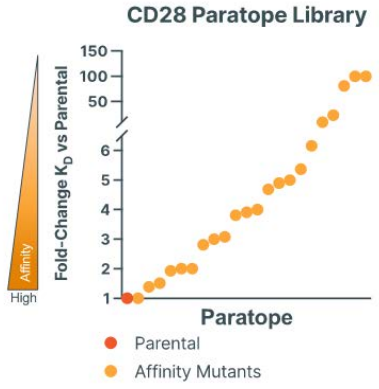
TriTCE Co-Stim Platform Workflow



TriTCE Co-Stim Lead Format Selection

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?	✓	✓	✓	✗	✓	✗	✗	✗	✗	✗
Potency (IC_{50}; pM)										
Yield (mg/L)										
Thermal Stability (T_m; °C)										

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

CD3 and CD28 paratope library and format screening identifies lead constructs with good target-dependency, potency, and stability

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

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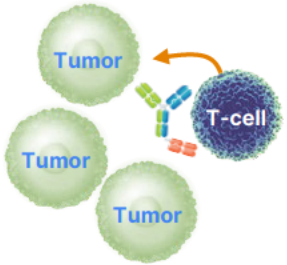
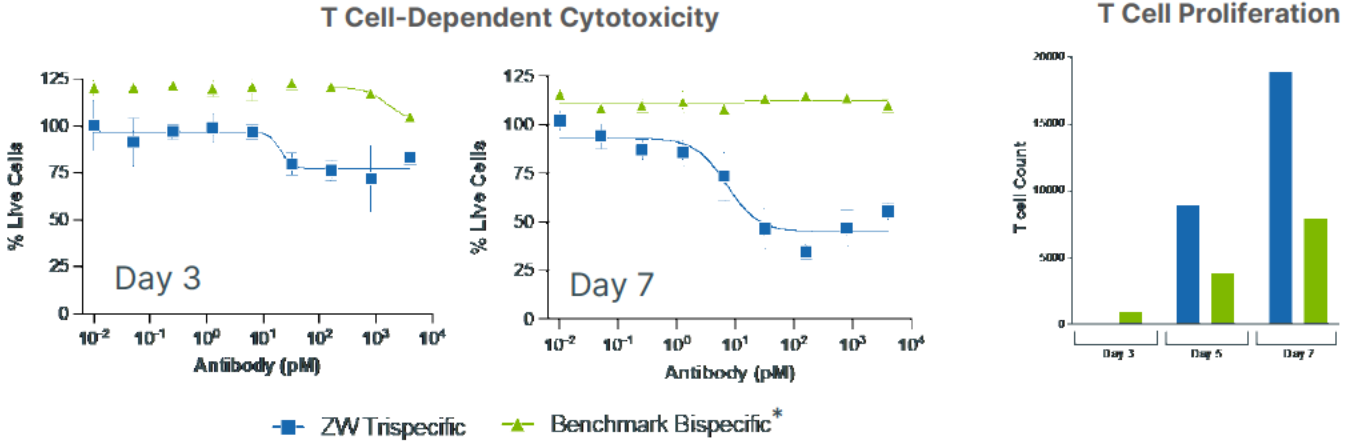
Increased T cell Proliferation and Anti-Tumor Activity in Long Term Low E:T Co-Cultures



Low E:T Assay System

Cytotoxicity and T cell proliferation



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

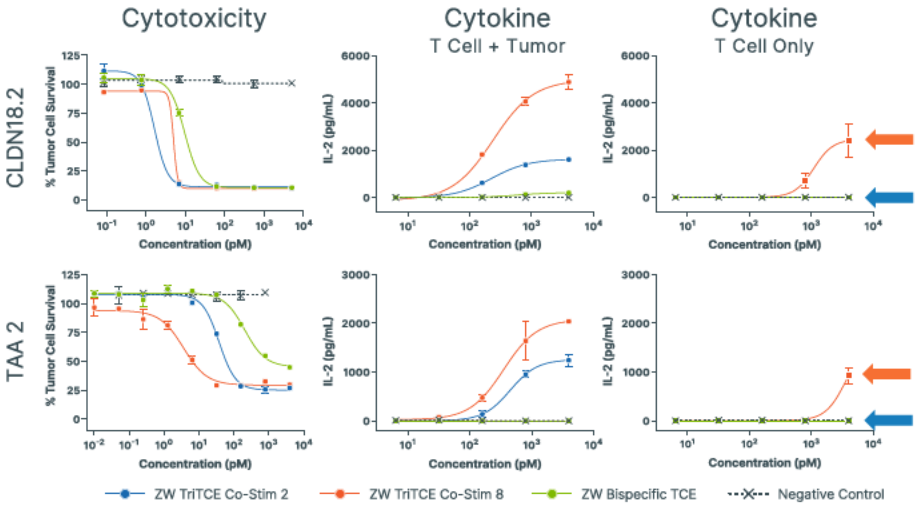
Lead TriTCE Co-Stim Format Identified Through Extensive Screening and is Transferable Across Targets

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



CLDN 18.2 TriTCE Co-Stim Enhances T Cell Responses and Anti-Tumor Activity Versus Benchmark Bispecific TCEs

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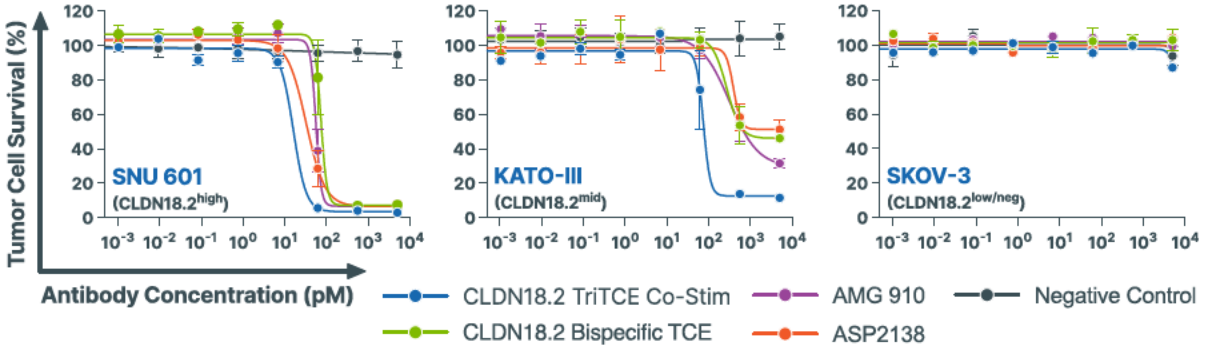
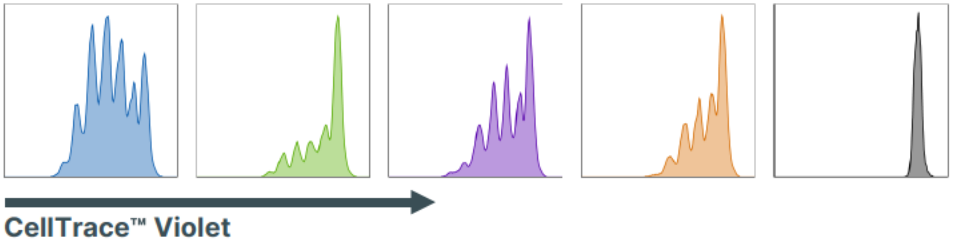
Enhanced Cytotoxicity at Low E:T

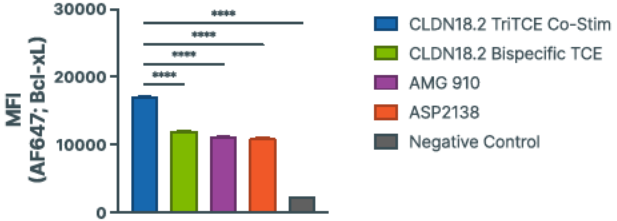


Improved T cell Proliferation and Survival

Proliferation



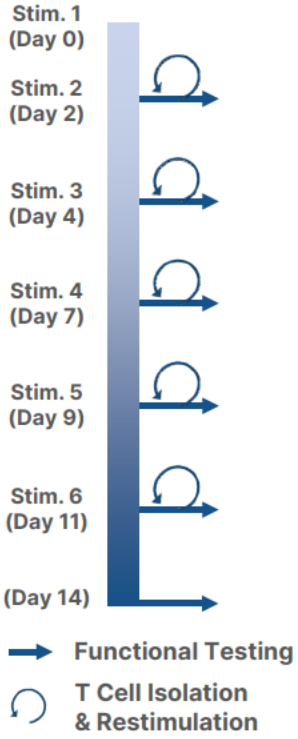
Survival

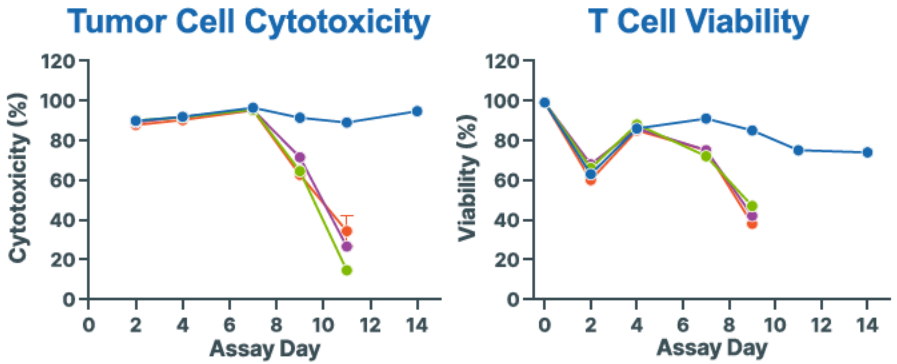


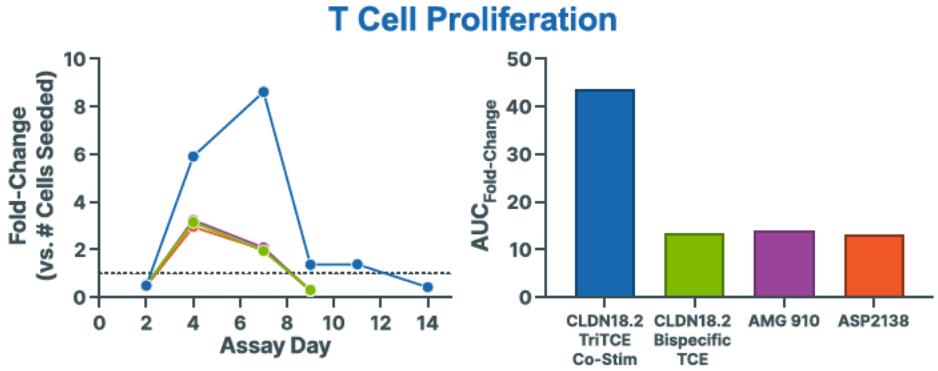
Newhook L, et al. Presented at: AACR Annual Meeting. 2024 (abstr # 6719)

CLDN 18.2 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 Stimulation







CD28 co-stimulation mediates sustained T cell activity *in vitro* relative to bispecific TCE, which may translate to more durable antitumor responses

TCE: T cell engager
Newhook L, et al. Presented at: AACR Annual Meeting. 2024 (abstr # 6719)

CLDN 18.2 TriTCE Co-Stim Mediates Enhanced Anti-Tumor Activity and Increases Activated Intratumoral T cells *in vivo* Compared to Benchmark Bispecific TCEs

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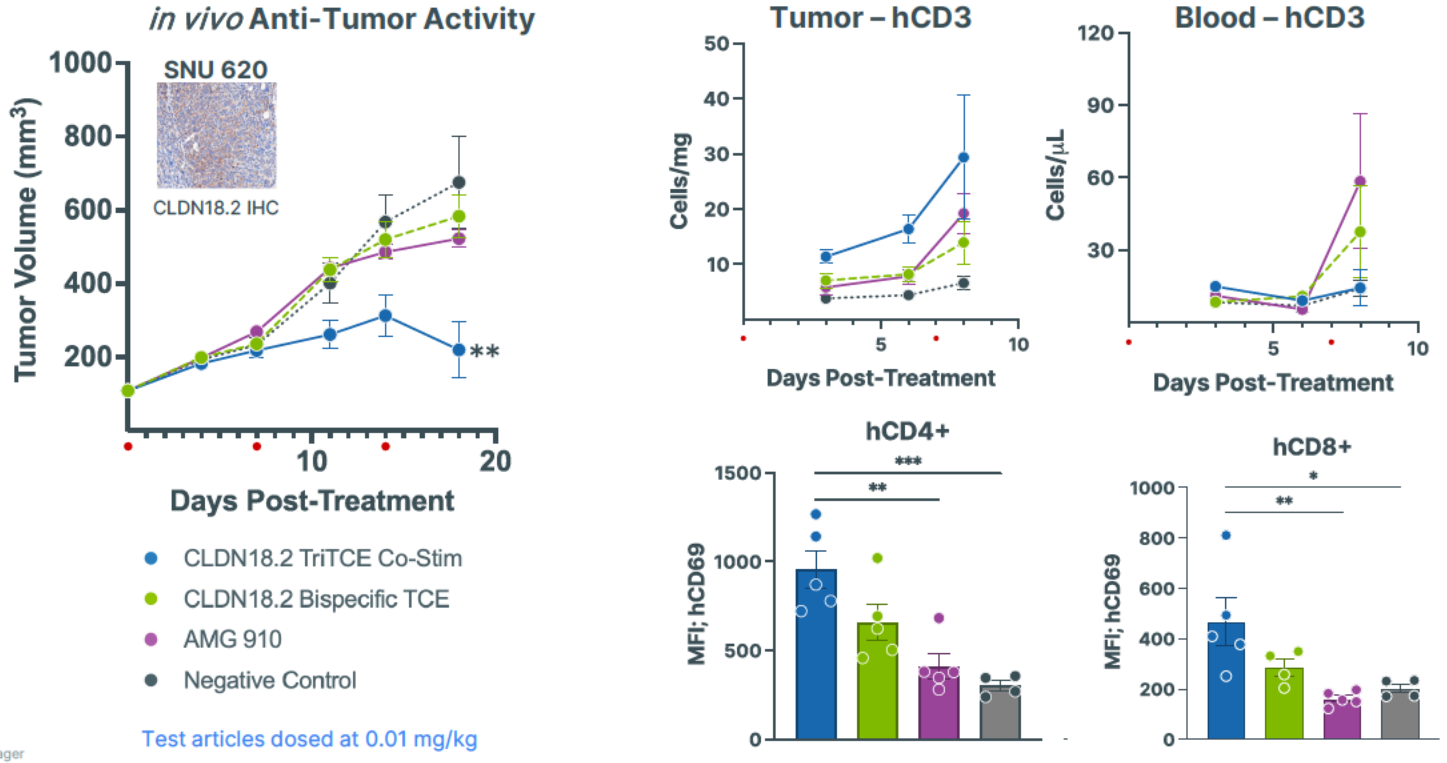
Greater Anti-Tumor Activity and Increased Activated T cell Infiltration in Tumor But Not in Blood



Test articles dosed at 0.01 mg/kg

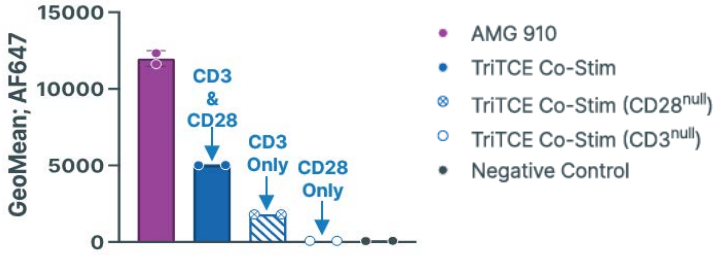
TCE: T cell engager

Newhook L, et al. Presented at: SITC Annual Meeting. 2023 (abstr # 1372)

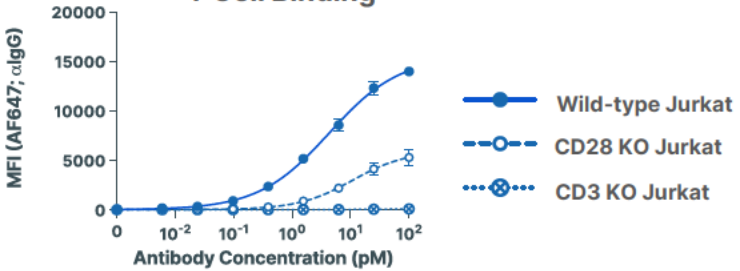
CLDN18.2 TriTCE Co-Stim Exhibits Conditional CD28 Binding and Obligate Cis T Cell Engagement

Conditional Binding of CD28, Requiring Co-Engagement of CD3

T Cell Binding

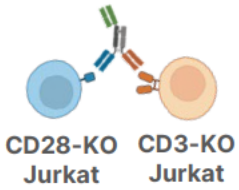


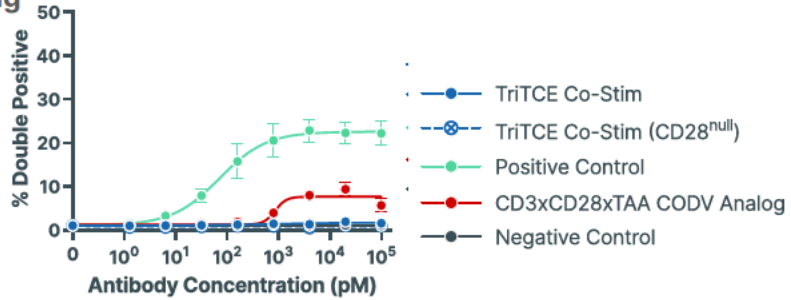
T Cell Binding



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

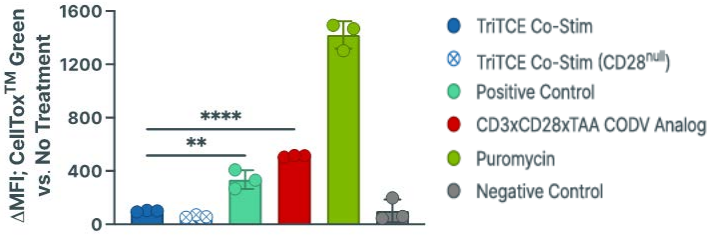
T Cell: T cell Bridging





No Reduction of T Cell Viability

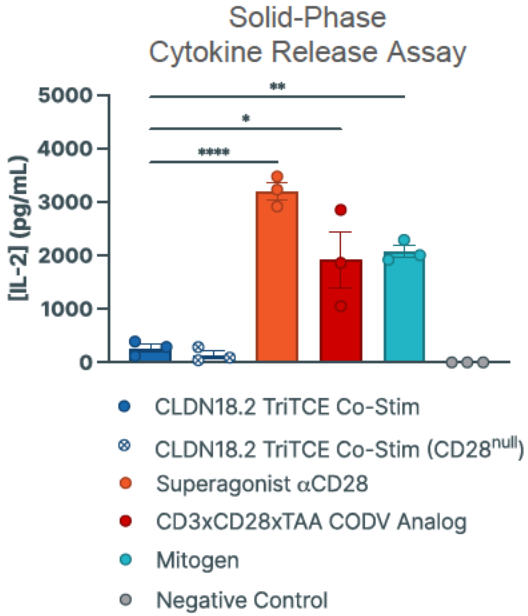
T Cell Monoculture Viability



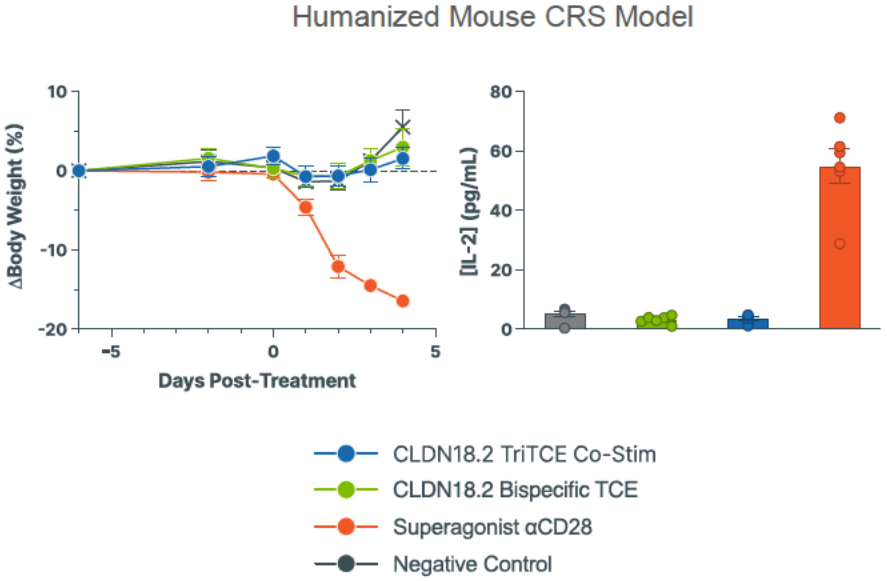
CLDN 18.2 TriTCE Co-Stim Has a Favorable Safety Profile *In Vitro* and in a Mouse CRS *In Vivo* Model

No cytokine release observed using *in vitro* or *in vivo* models of CRS

No Cytokine Release *in vitro* with Human PBMC Only



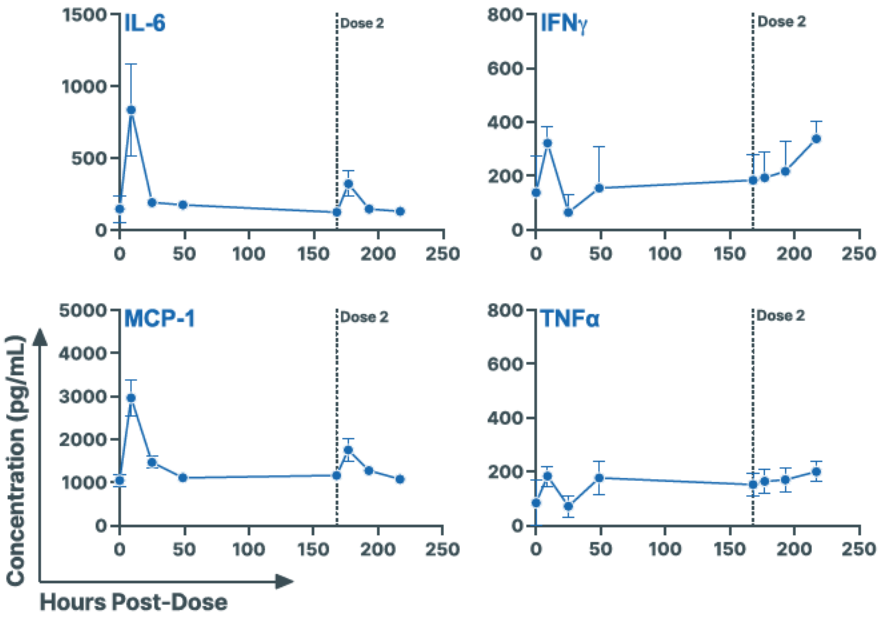
No Body Weight Loss or Systemic Cytokine Release *in vivo*



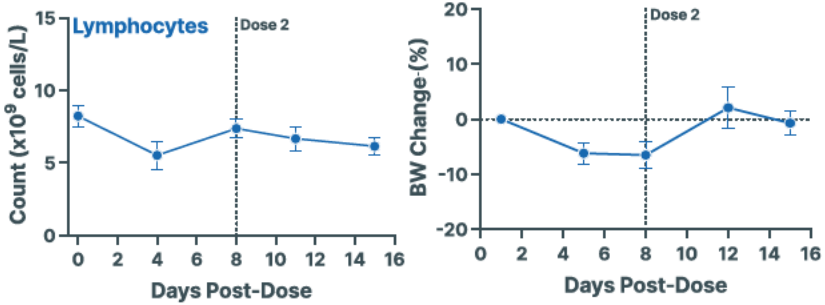
CRS: Cytokine release syndrome
Newhook L, et al. Presented at: SITC Annual Meeting. 2023 (abstr # 1372), Newhook L, et al. Presented at: AACR Annual Meeting. 2024 (abstr # 6719)

CLDN 18.2 TriTCE Co-Stim is Well-Tolerated in Cynomolgus Monkeys

Transient, Minor Increase in Serum Cytokine Post-Dosing



Transient, Minor Decrease in Lymphocyte Count and Body Weight Post-Dosing



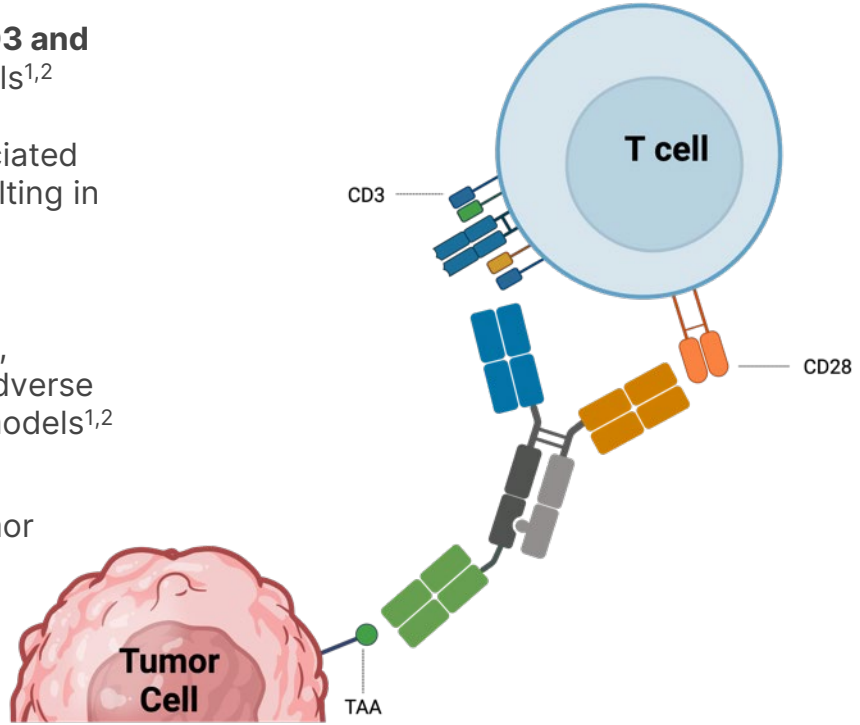
Surrogate TriTCE Co-Stim*- 3 mg/kg

- 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 (Türeci et al., 2011)
  - Other histopathological changes were secondary to decreased food consumption and body weight loss

*Surrogate TriTCE Co-Stim exhibited ~10-fold increased cytotoxic potency vs. lead TriTCE Co-Stim and ~15-fold reduced cytotoxic potency vs. AMG 910 in cynomolgus T cell-dependent cytotoxicity assays *in vitro*. AMG 910 dosed up to 0.03 mg/kg in a one-month, repeat dose NHP toxicology study (Bialis et al, 2020).
Newhook L, et al. Presented at: AACR Annual Meeting. 2024 (abstr # 6719)

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

Multispecific Antibody Therapeutic Department

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Nina Weisser
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Charles Chen
Paul Moore

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Health and Human Therapeutics department

Fresh Updates on Multispecifics Programs at AACR 2025!

Abstract #7318
ZW209, a DLL3 targeted trispecific T cell engager with integrated CD28 co-stimulation, demonstrates safety and potent preclinical efficacy in models of small cell lung cancer
Desmond Lau, Peter Repenning, Diana Canals Hernaez, Nichole Escalante, Alec Robinson, Diego Perez Escanda, John Zhang, Hamed Shirvani, Catherine Wu, Kurt Stahl, Marylou Vallejo, Aditi Deshmukh, Aaron Tieu, Mariana Rocha, Begonia Silva Moreno, Lisa Newhook, Purva Bhojane, Paul A. Moore, Nina E. Weisser, Thomas Spreter von Kreudenstein

Abstract #3503
ZW171, a differentiated 2+1 T cell engaging bispecific antibody with antitumor activity in a range of mesothelin expressing cancers
Nicole Afacan, Patricia Zwierzchowski, Wingkie Wong, Maya C. Poffenberger, Chayne Piscitelli, Catherine Wu, Richard Kunze, Caitlin Low, Sifa Arrafi, Mina Khoddami, Alexia Piercey, Maya Poffenberger, Thomas Spreter von Kreudenstein, Nina E. Weisser, Paul Moore

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