Engineering and Preclinical Development of ZW171: A 2+1 Format Anti-MSLN T Cell Engager

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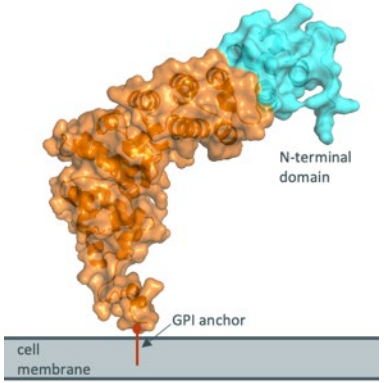
Chayne Piscitelli
Senior Scientist, Protein Engineering

Mesothelin is a Promising Target in Multiple Indications

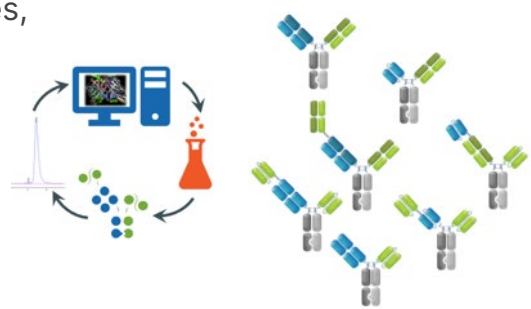
- Mesothelin (MSLN) is a GPI-linked membrane glycoprotein that is overexpressed in many cancer indications, including pancreatic, mesothelioma, and ovarian[1], for which there is a high unmet medical need
- While MSLN-targeting agents have shown early signs of clinical activity, there remains a need for therapies with improved safety and efficacy[2]



AlphaFold model of MSLN

Zymeworks approach: A multi-valent bispecific MSLN-targeted TCE

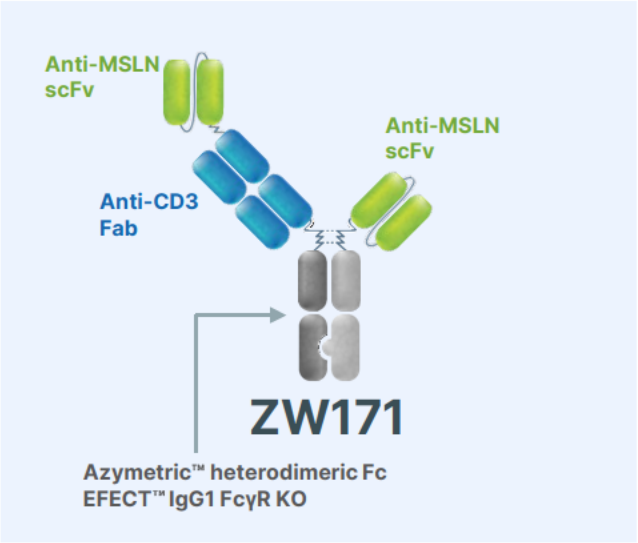
- Utilize our Azymetric™ and EFECT™ platforms and engineering strategies to generate a panel of MSLN-targeting TCEs with a variety of formats, geometries, and paratope affinities
- **Following extensive screening, a lead candidate with enhanced anti-tumor activity and safety, ZW171, was selected for development**

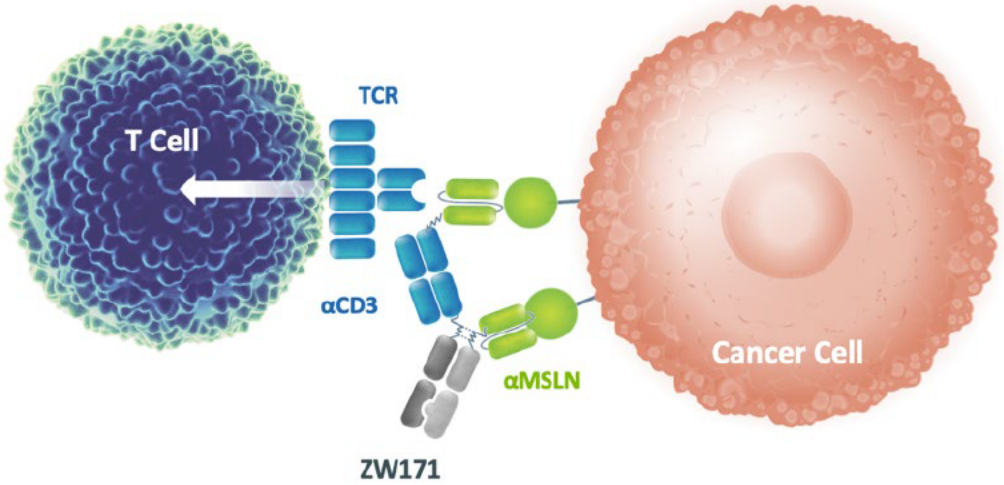


1. Morello, A., Sadelain, M., & Adusumilli, P.S. (2016). Mesothelin-Targeted CARs: Driving T Cells to Solid Tumors. *Cancer discovery,* 6(2), 133-46.
2. Faust, J. R., Hamill, D., Kolb, E. A., Gopalakrishnapillai, A., & Barwe, S. P. (2022). Mesothelin: An Immunotherapeutic Target beyond Solid Tumors. Cancers, 14(6), 1550.

ZW171 is a MSLN-targeting 2+1 Format T Cell Engager

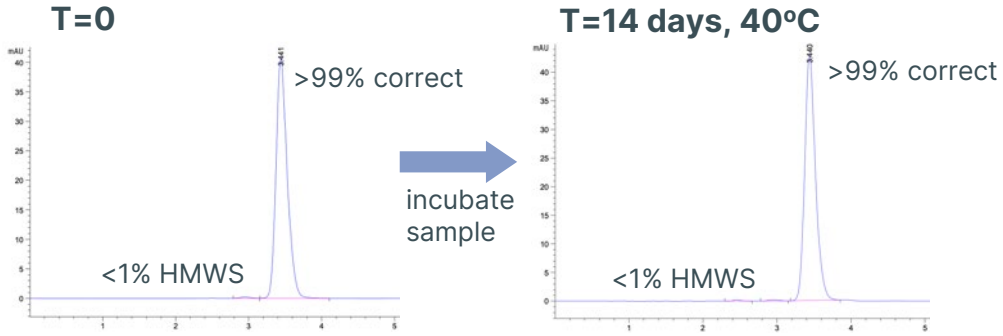
- Bispecific design built upon our Azymetric™ heterodimeric Fc platform technology
- Fc effector function knockout using EFECT™
- Bivalent binding to MSLN via dual scFv engagement
- Monovalent binding to CD3ε via Fab arm

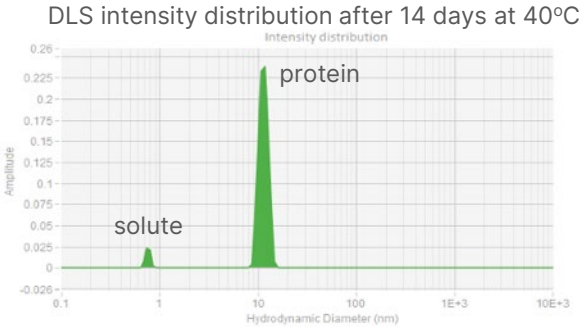




ZW171 is Stable and Displays Good Developability Metrics

ZW171 maintains monodispersity after 14 days at 40°C





In vitro developability analytics are within normal ranges

Sample	cIEF	AC-SINS	Polyspecificity binding ELISA				
	pI	Δλ (nm)	1	2	3	4	5
ZW171	8.97	3.00	3.21	2.78	1.50	2.26	1.93
fast clearance control Ab	9.34	28.7	18.0	27.4	29.2	24.8	28.9

Good plasma stability (mouse) with no evidence of scFv clipping after 2 weeks at 37°C

ZW αCD3 Paratope is Differentiated from SP34-based Engagers

Epitope:

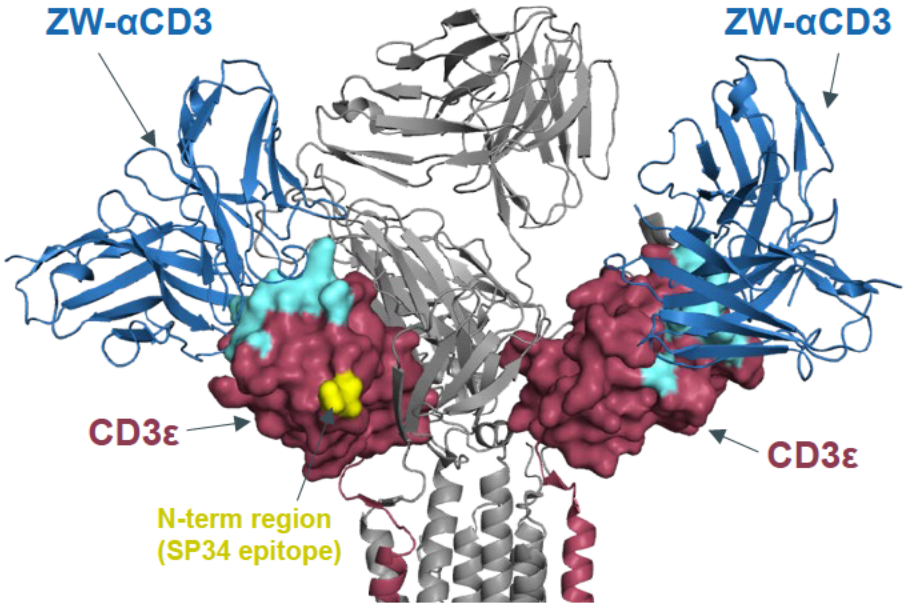
- ZW-αCD3 binds a distinct discontinuous epitope on CD3ε
- Cross-reactive with cyno CD3
- SP34, the αCD3 paratope broadly used by others, targets the N-terminal linear sequence of CD3ε

Affinity:

- Tuned for low affinity to improve tolerability and minimize TMDD effects

Developability:

- Good thermal stability (Fab >70ºC)
- No deamidation/iso-Asp liabilities

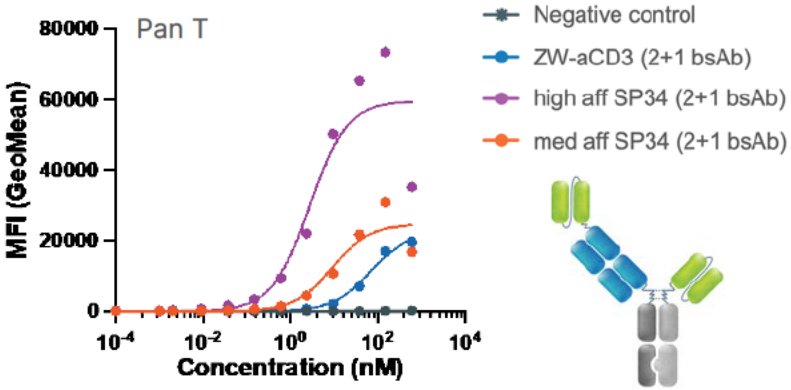


ZW-αCD3:CD3ε complex crystal structure superposed onto TCR structure (PDB id 7FJD)

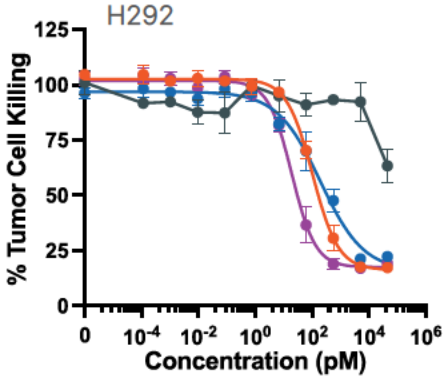
ZW CD3 Engager has Low Affinity, Potent Cytotoxicity and Low Cytokine Release

- Comparing ZW-αCD3 activity with high affinity and med affinity SP34-based constructs
- Format: 2+1 MSLNxCD3 (lower affinity MSLN paratopes)

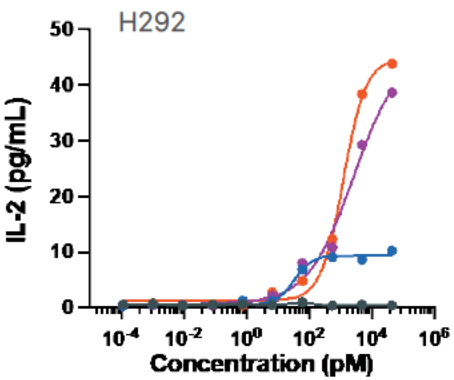
CD3 on-cell binding



Tumor cell cytotoxicity



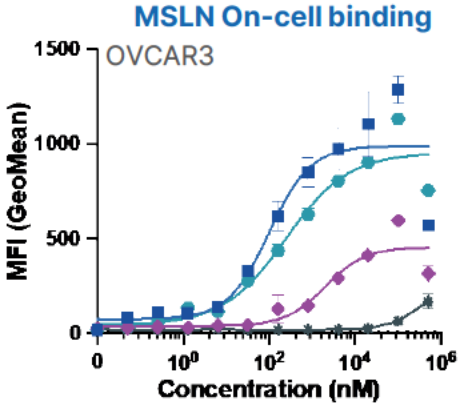
Cytokine release

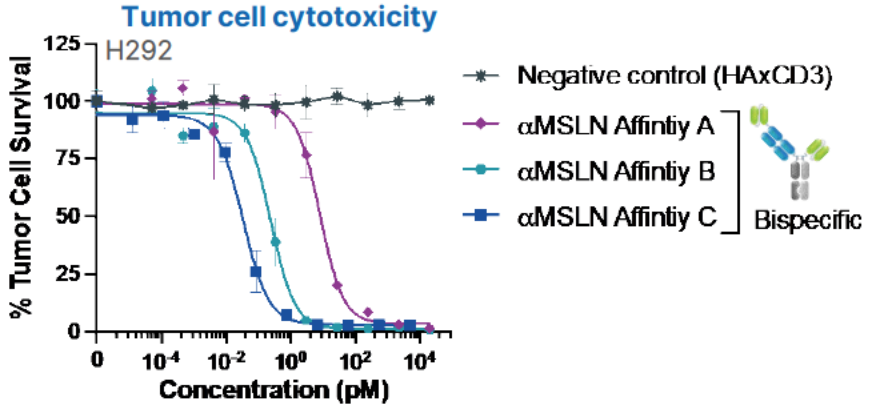


2+1 format with low affinity ZW-αCD3 shows comparable cytotoxic potency to higher affinity SP34 constructs but significantly less cytokine release

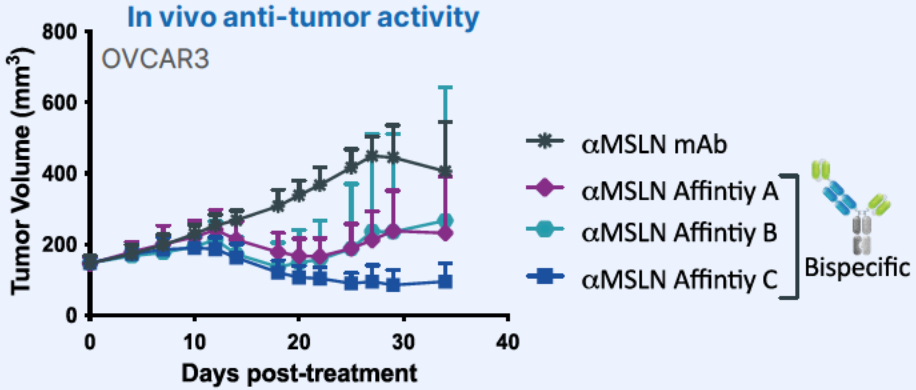
Tuning MSLN affinity

Three MSLN affinities were tested in the lead 2+1 dual scFv format





MSLN affinity level correlates with high activity both in vitro and in vivo



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Format and Valency has a High Impact on Activity

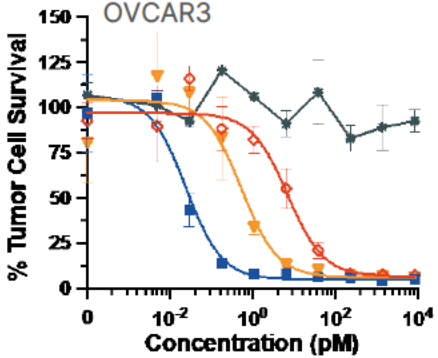
Three formats compared with same MSLN and CD3 paratopes

2+1 dual scFv

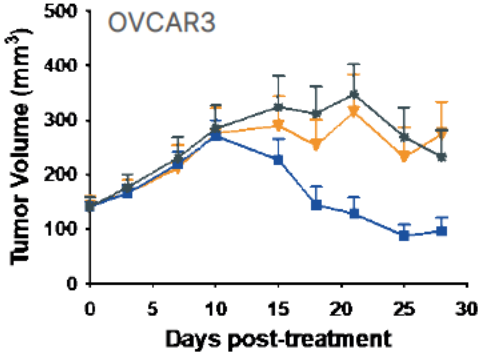
2+1 triple Fab

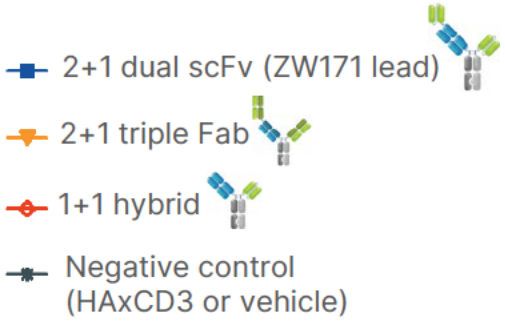
1+1 hybrid

Tumor cell killing



In vivo anti-tumor activity





2+1 dual scFv shows significantly higher activity in vitro and in vivo

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ZW171 Activity is MSLN-dependent and Shows Low Activity on MSLN-low Cell Lines

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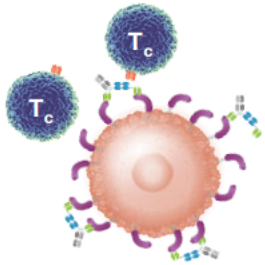
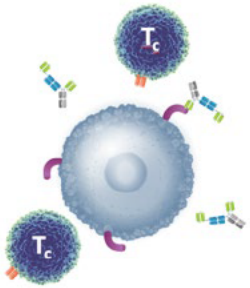
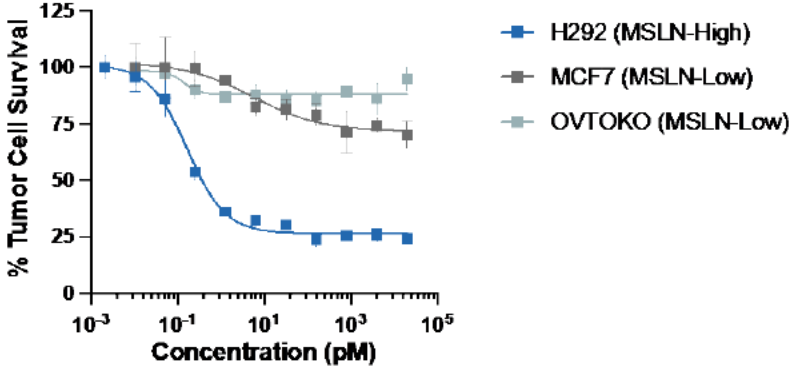
Cancer cell (MSLN-high)



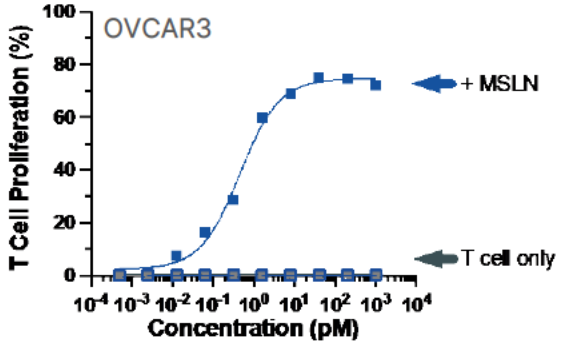
Healthy cell (MSLN-low)



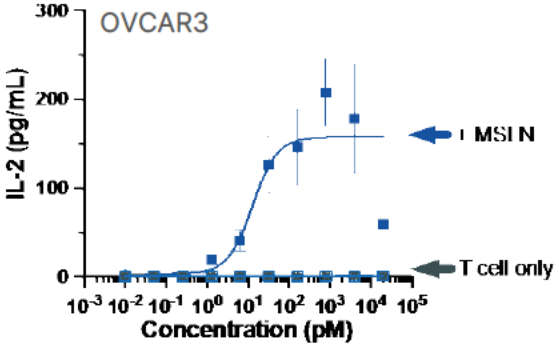
Tumor cell cytotoxicity



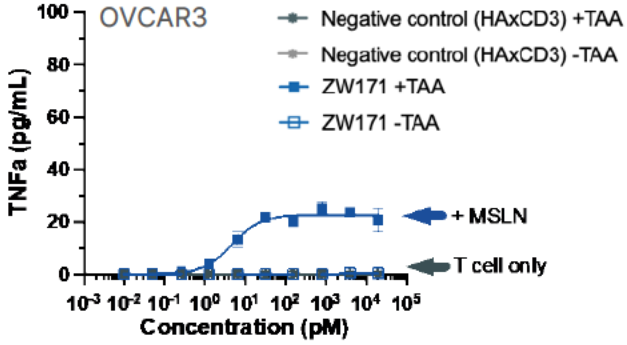
T cell proliferation



IL-2



TNF-α



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ZW171 Mediates Greater Anti-tumor Activity Compared to Benchmark in MSLN-expressing Tumor Models

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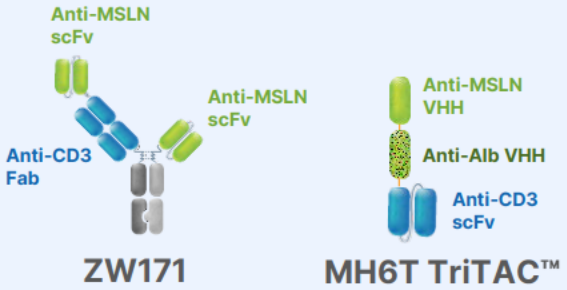
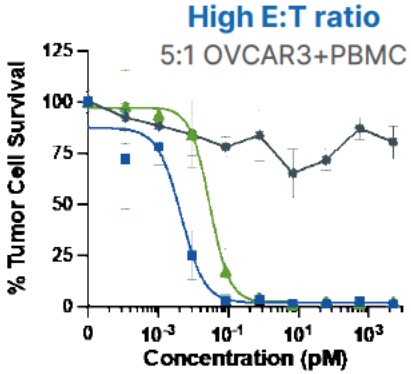
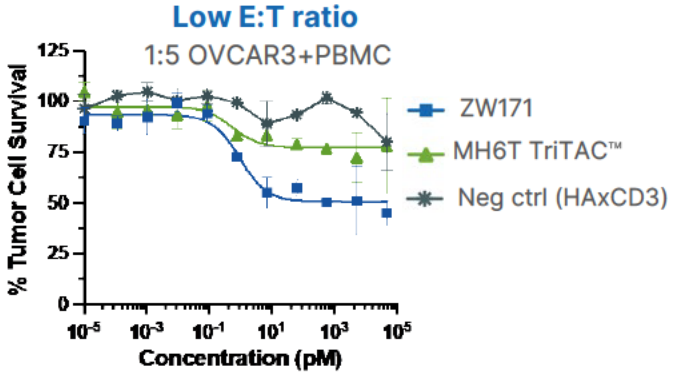
Benchmarked activity of ZW171 against Harpoon's MH6T TriTAC™

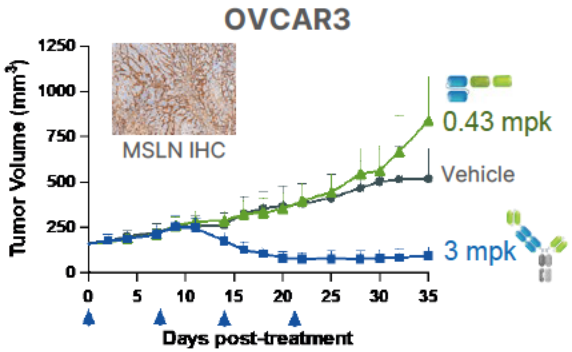


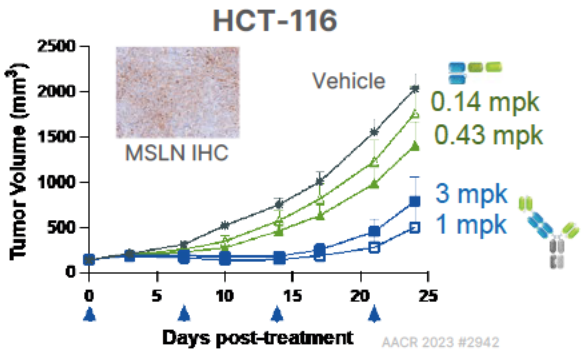




In vivo studies

- **OVCAR3**: Tumor fragments engrafted s.c. in NOG mice, followed by PBMC engraftment
- **HCT-116**: NPG mice engrafted with HCT-116 and PBMC i.p.
- Dosing of MH6T TriTAC™ and ZW171 tailored for **matched exposure** (verified by PK)
- ZW171 dosed QWx4, MH6T TriTAC™ dosed ODx18





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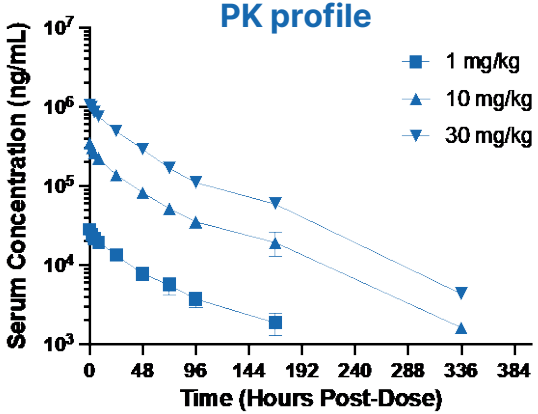
ZW171 is Well-tolerated in Cynomolgus Monkeys

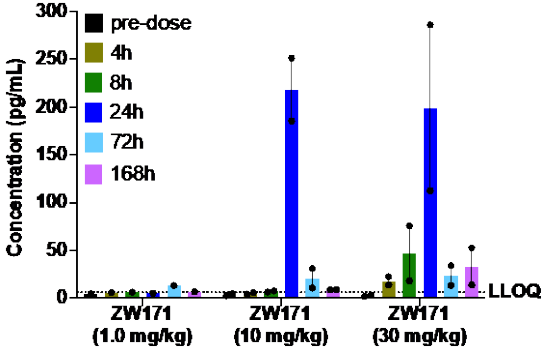
Cynomolgus monkeys administered single dose of ZW171 at 1, 10, 30 mg/kg i.v.

- Transient increase in IL-6, MCP-1, and GM-CSF at higher doses
- Dose-dependent elevation of Fibrinogen
- Mild hyperplasia/hypertrophy in mesothelium

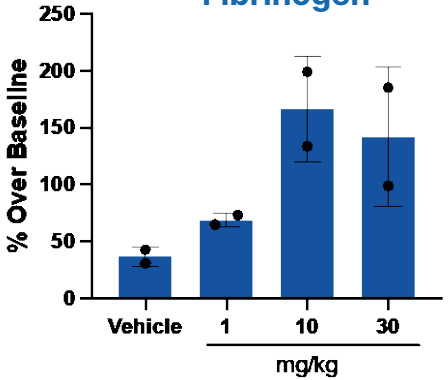
PK profile



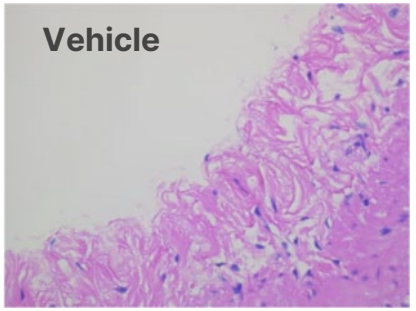
Serum IL-6

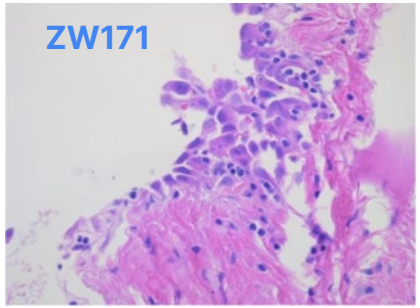


Fibrinogen



IHC of stomach mesothelium





Toxicology findings were mild and associated with the known mechanism of action for ZW171

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Summary

- ZW171 was selected as a lead candidate through iterative engineering and screening of paratope affinities and formats
- Dual scFv 2+1 format showed superior activity compared to triple Fab and 1+1 hybrid bispecifics
- ZW171 is a stable protein with good therapeutic developability and manufacturability characteristics
- The 2+1 TCE format of ZW171 facilitates avidity-driven tumor cell binding and stimulates MSLN-dependent T cell activation, limiting on-target off-tumor toxicities
- ZW171 exhibits potent tumor growth inhibition in MSLN expressing tumor models
- ZW171 compares favorably in vitro and in vivo when compared to currently available clinical benchmarks
- ZW171 is well tolerated in cynomolgus monkeys up to maximum dose tested of 30 mg/kg

Collectively, these data provide a strong therapeutic rationale to support the development of ZW171 for the treatment of MLSN-expressing tumors

- GMP process established, and GLP toxicology study scheduled
- On track for IND filing in early 2024

Next generation: TriTCE co-stim and TriTCE CPI

TriTCE Co-stim: Next Generation Co-stimulatory Trispecific T Cell Engagers for the Treatment of Solid Tumors

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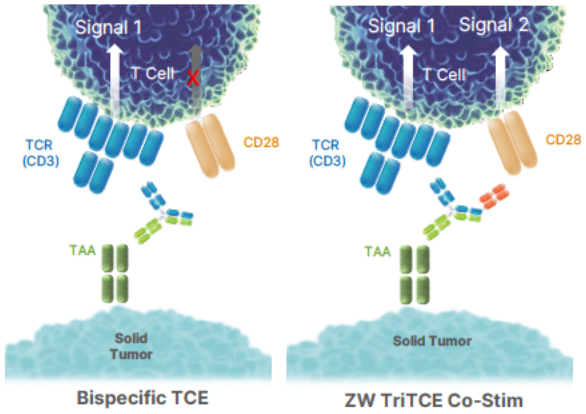
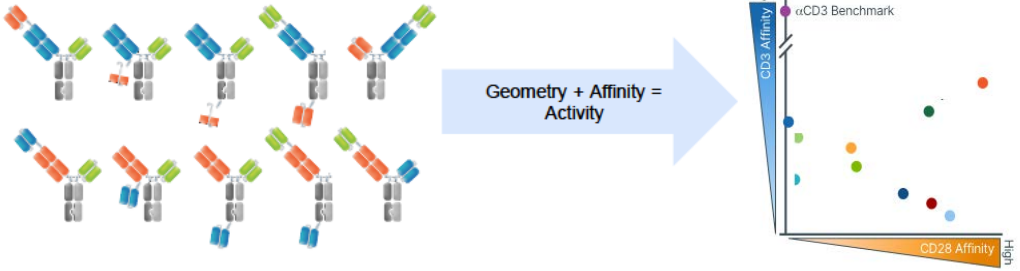
TriTCE co-stim may provide increased T cell fitness, activation and proliferation via tumor-dependent T cell co-stimulation



- TAA-driven TCR + CD28 co-stimulation
- Enhanced T cell activation, metabolism, and fitness
- TME-localized cytokine production and sustained proliferation

Structure+Affinity screening enables identification of optimal TriTCE format for robust 'Signal 1' + 'Signal 2' T cell activation and synapse formation

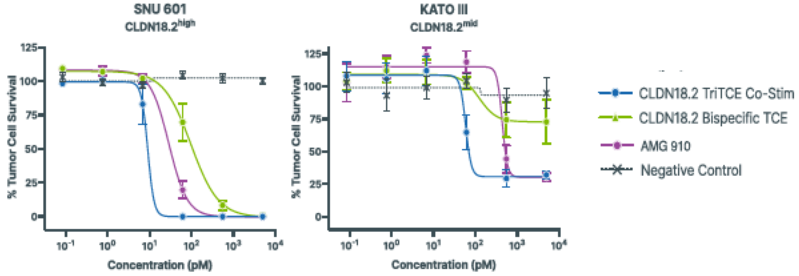


Antibody Format	1	2	3	4	5	6	7	8	9	10
IC_{50} (pM)										
TAA-Dependent?	✓	✓	✓	X	✓	✓	X	X	X	X

- TriTCE are screened for cytotoxic potency (IC50; pM) and TAA-dependent T cell agonism
- Formats that activate T cells in the absence of TAA and those that show inferiority to bispecific TCE are eliminated from consideration

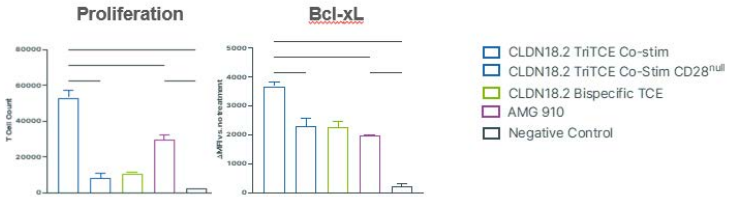
CLDN18.2 TriTCE Co-stim Therapeutic Program

Enhanced cytotoxicity at low effector-to-target ratios



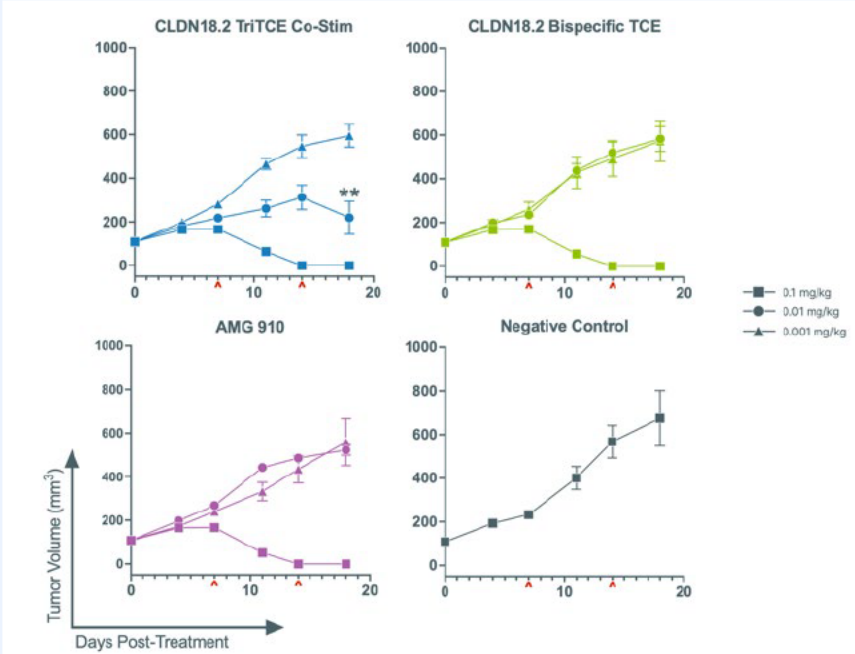
TriTCE Co-Stim may show superior activity in 'cold', poorly infiltrated tumors

Improved TAA-dependent T cell proliferation and survival



TriTCE co-stim may provide more durable responses in solid tumors

CLDN18.2 TriTCE co-stim exhibits superior *in vivo* anti-tumor activity in a PBMC-engrafted xenograft model



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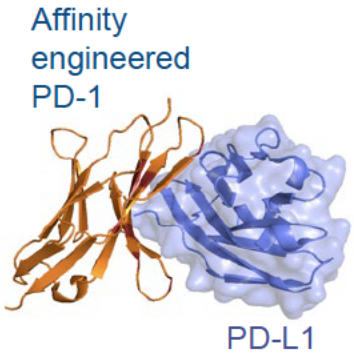
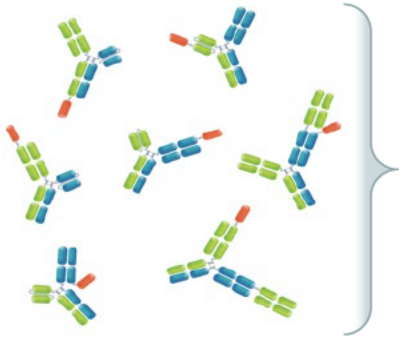
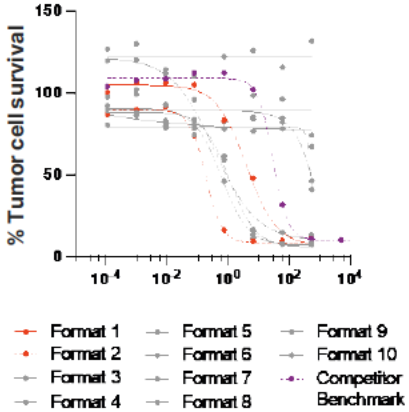
TriTCE-CPI: Trispecific T Cell Engagers with Checkpoint Inhibition for the Treatment of Solid Tumors



TriTCE-CPI format screening





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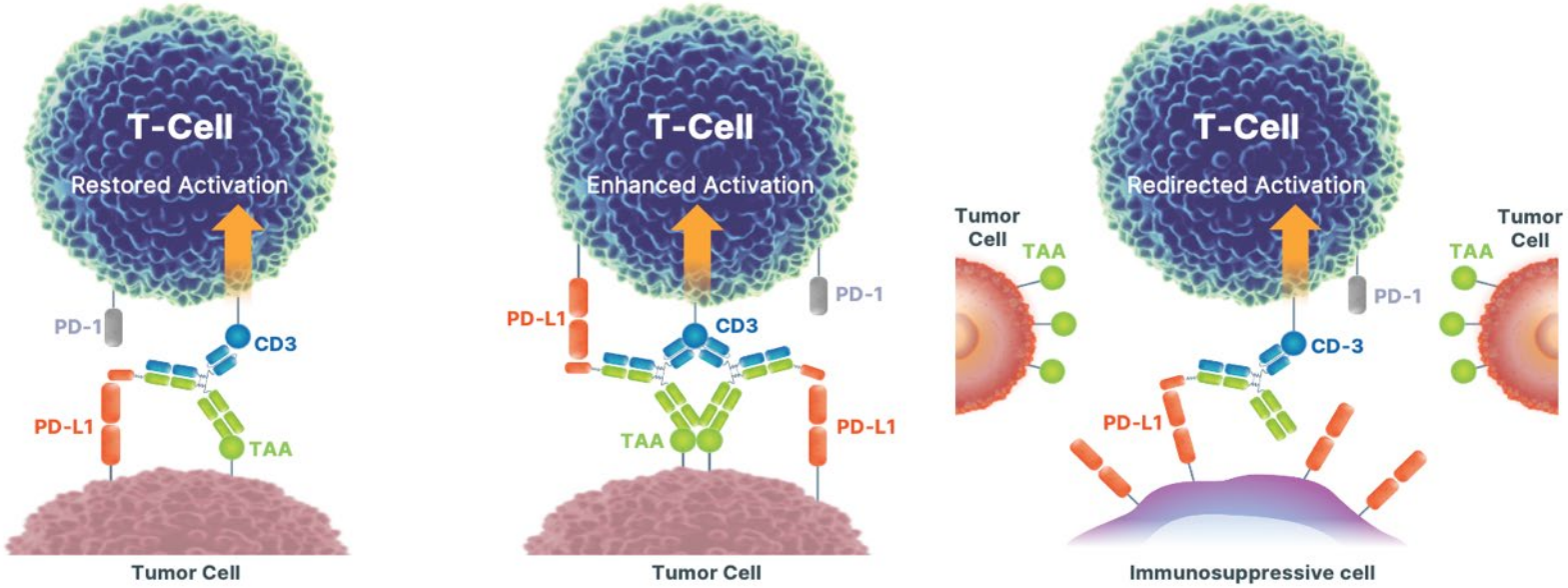
TriTCE-CPI: Trispecific T Cell Engagers with Checkpoint Inhibition for the Treatment of Solid Tumors



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TriTCE-CPI: Trispecific T Cell Engagers with Checkpoint Inhibition for the Treatment of Solid Tumors

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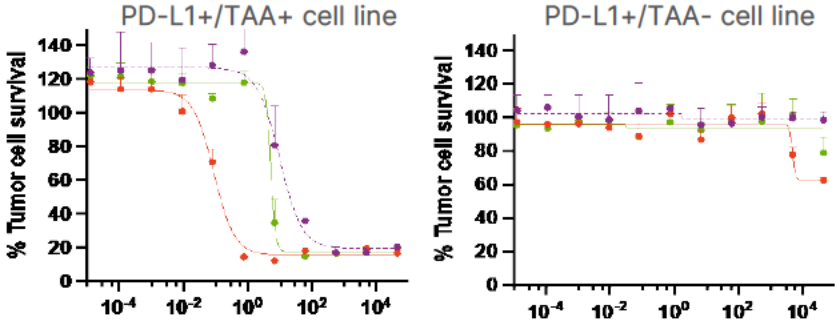
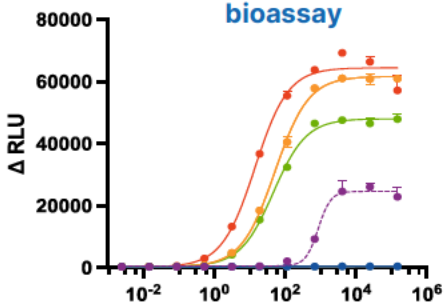
T cell-dependent cellular cytotoxicity



PD-1/PD-L1 checkpoint bioassay



- PD-1 TriTCE-CPI
- Bispecific TCE
- Competitor Benchmark
- Bispecific TCE + α-PD-L1 mAb
- α-PD-L1 mAb
- Irrelevant antibody

TriTCE-CPI constructs are high potency **TAA-dependent** T cell engagers with robust checkpoint blockade activity

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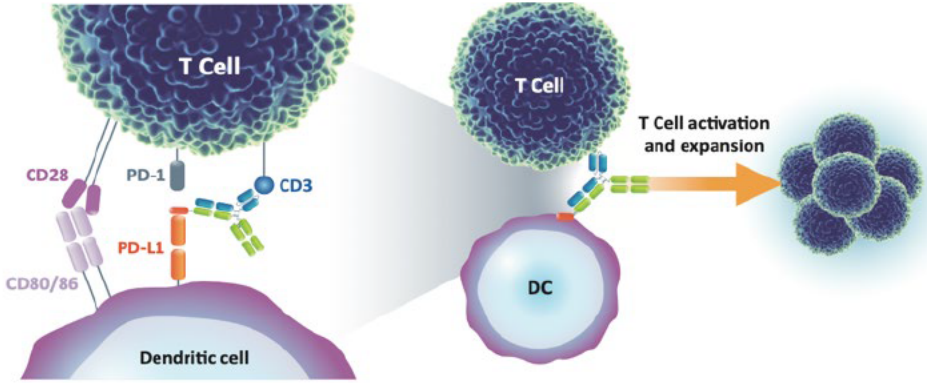
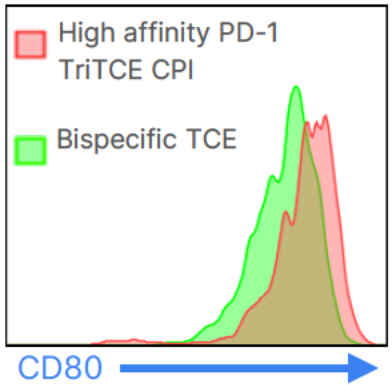
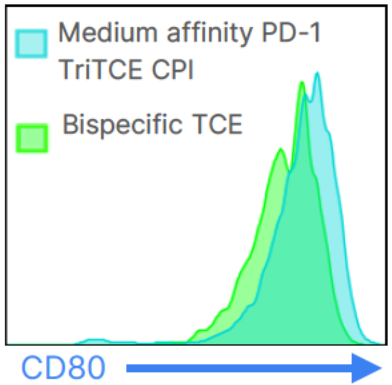
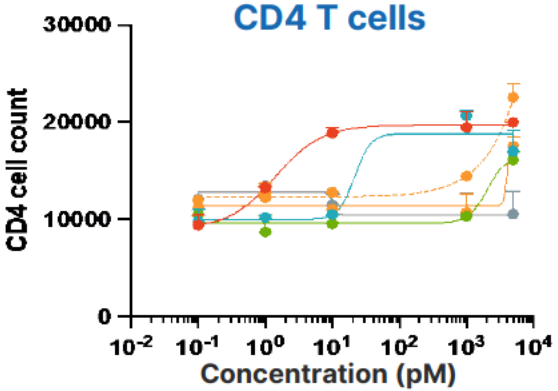
TriTCE CPI Formats can be Tuned to Optimize Dendritic Cell (DC)-dependent T Cell Activation

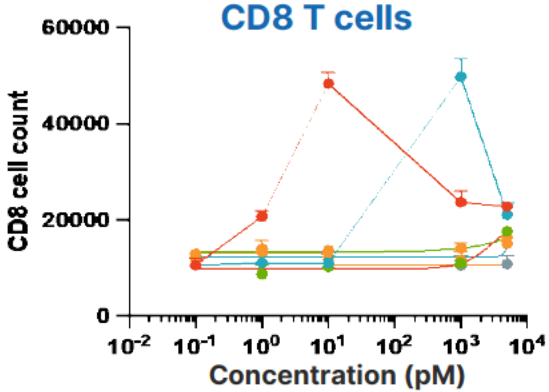


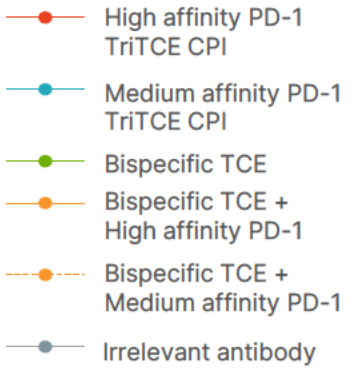
DC - T cell co-culture CD80 expression











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Purva Bhojane

Lisa Newhook

Peter Repenning

Diego Perez Escanda

Nichole Escalante

Patricia Zwierschowski

Alec Robinson

Lauren Clifford

Harsh Pratap

David Douda

Alexandra Livernois

TriTCE CPI

Meghan Verstraete

Maya Poffenberger

Anna von Rossum

Matteo Zago

Veronica Luu

Siran Cao

Sifa Arrafi

Patricia Zwierzchowski

Janessa Li

Harsh Pratap

Brenda Ma

Alexandra Livernois

Multispecific Antibody Therapeutics (MSAT) Leadership

Nina Weisser

Paul Moore

Thomas Spreter von Kreudenstein

National Research Council (NRC) Canada

Health and Human Therapeutics department