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

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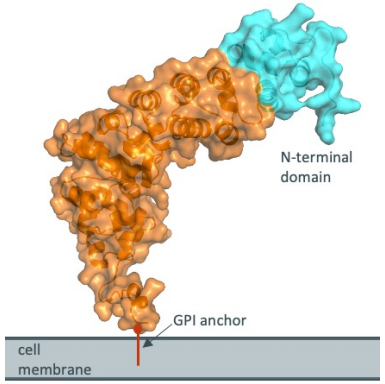
Mesothelin is a promising target in multiple indications

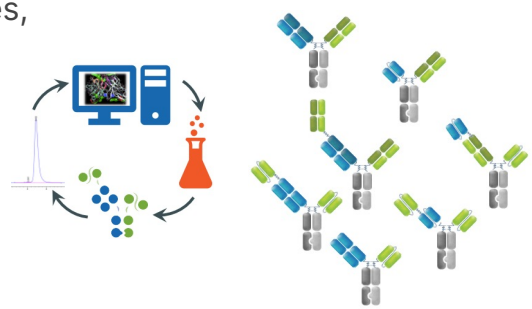
- Mesothelin (MSLN) is a GPI-linked membrane glycoprotein that is overexpressed in many cancer indications, including pancreatic, mesothelioma, and ovarian[1], for which there is a high unmet medical need
- While MSLN-targeting agents have shown early signs of clinical activity, there remains a need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



AlphaFold model of MSLN

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

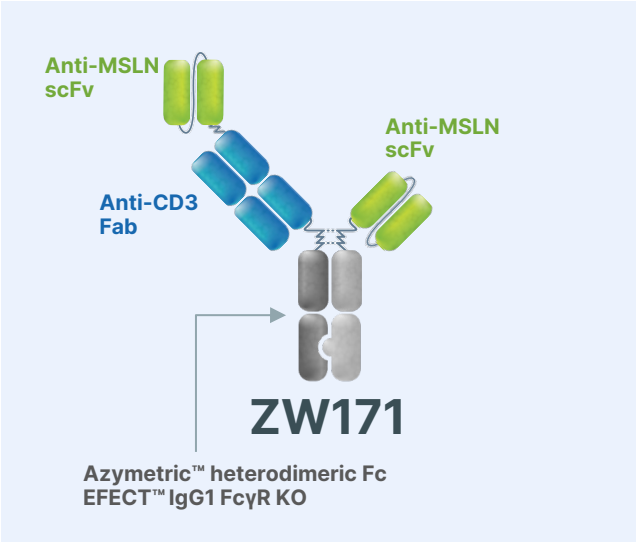
- Utilize our Azymetric™ and EFECT™ platforms and engineering strategies to generate a panel of MSLN-targeting TCEs with a variety of formats, geometries, and paratope affinities
- **Following extensive screening, a lead candidate with enhanced anti-tumor activity and safety, ZW171, was selected for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

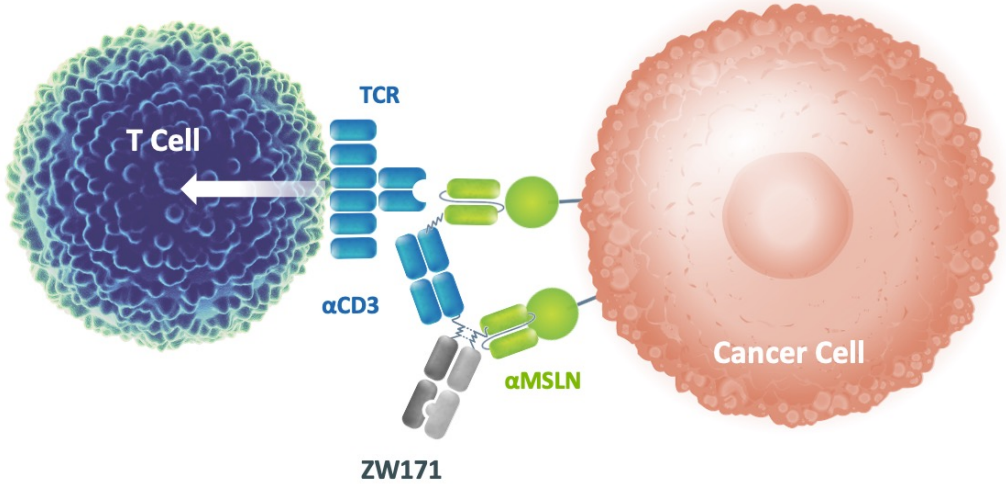


1. Morello, A., Sadelain, M., & Adusumilli, P.S. (2016). Mesothelin-Targeted CARs: Driving T Cells to Solid Tumors. *Cancer discovery,* 6(2), 133-46.
2. Faust, J. R., Hamill, D., Kolb, E. A., Gopalakrishnapillai, A., & Barwe, S. P. (2022). Mesothelin: An Immunotherapeutic Target beyond Solid Tumors.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

ZW171 is a MSLN-targeting 2+1 format T cell engager

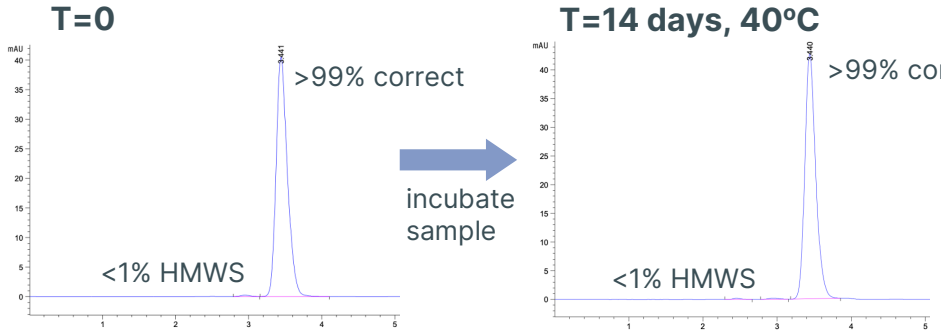
- Bispecific design built upon our Azymetric™ heterodimeric Fc platform technology
- Fc effector function knockout using EFECT™
- Bivalent binding to MSLN via dual scFv engagement
- Monovalent binding to CD3ε via Fab arm

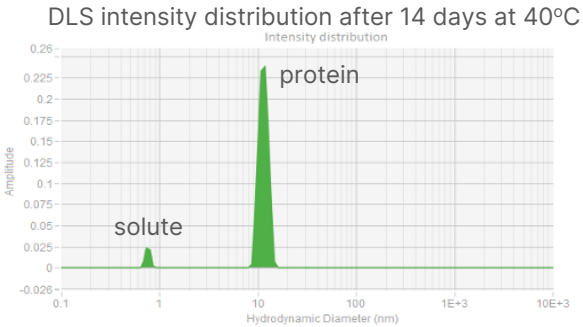




ZW171 is stable and displays good developability metrics

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:

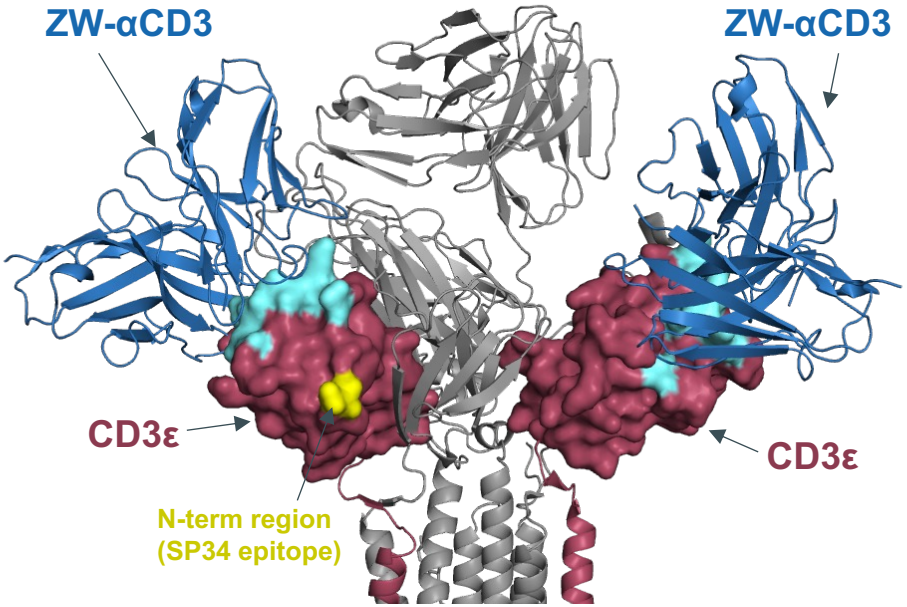
- ZW-αCD3 binds a distinct discontinuous epitope on CD3ε
- Cross-reactive with cyno CD3
- SP34, the αCD3 paratope broadly used by others, targets the N-terminal linear sequence of CD3ε

Affinity:

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

Developability:

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

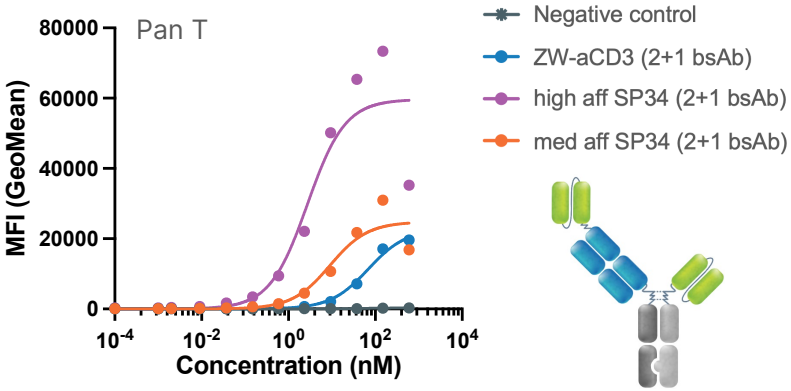


ZW-αCD3:CD3ε complex crystal structure superposed onto TCR structure (PDB id 7FJD)

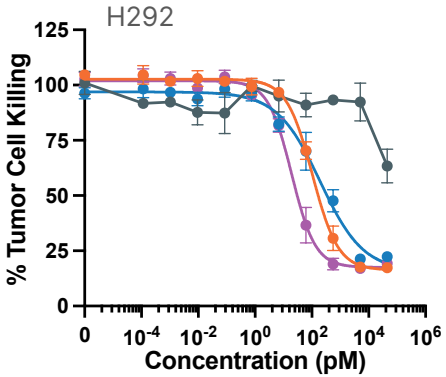
ZW CD3 engager has low affinity, potent cytotoxicity and low cytokine release

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

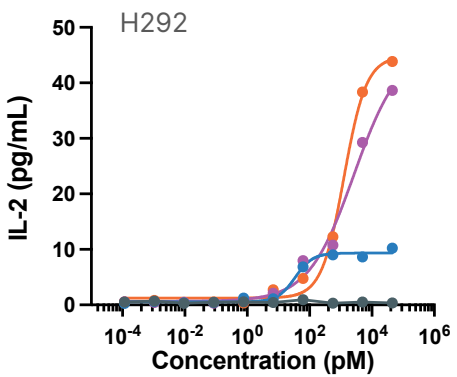
CD3 on-cell binding



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

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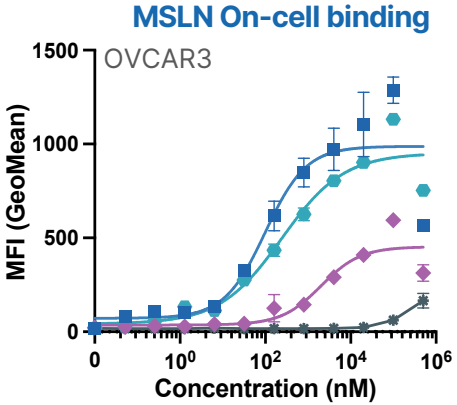
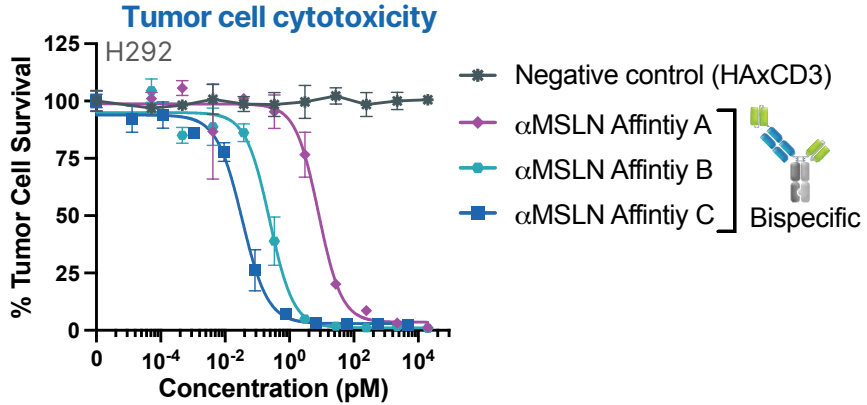
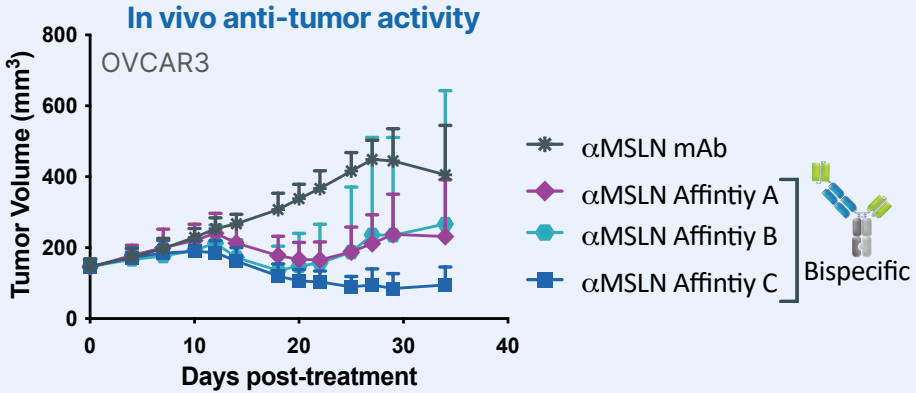
Tuning MSLN affinity

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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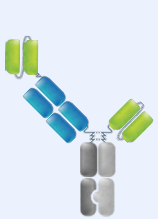
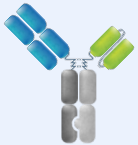
Format and valency have a high impact on activity

Three formats compared with same MSLN and CD3 paratopes



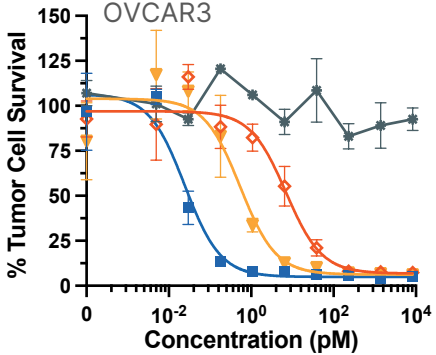
2+1 dual scFv

2+1 triple Fab

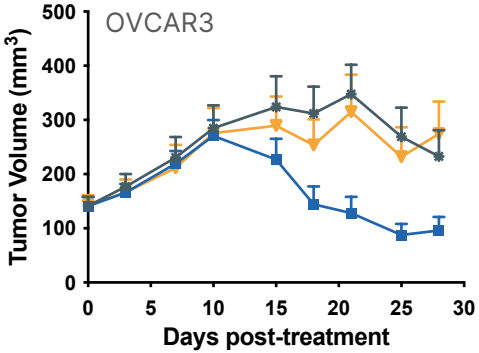


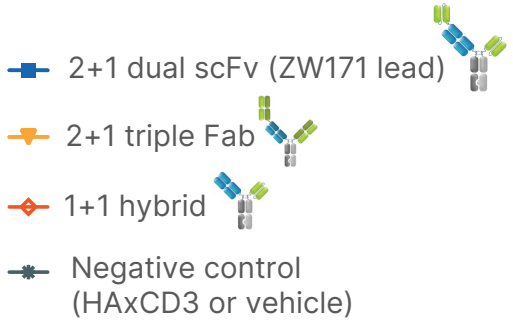
1+1 hybrid

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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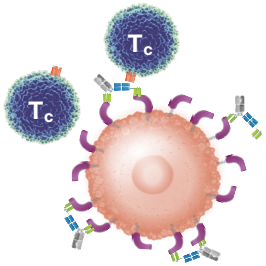
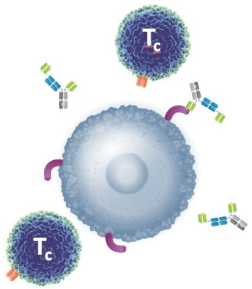
ZW171 activity is MSLN-dependent and shows low activity on MSLN-low cell lines

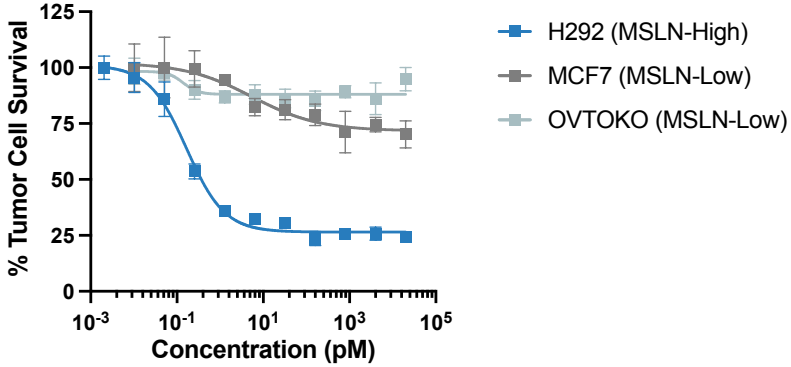
Cancer cell (MSLN-high)



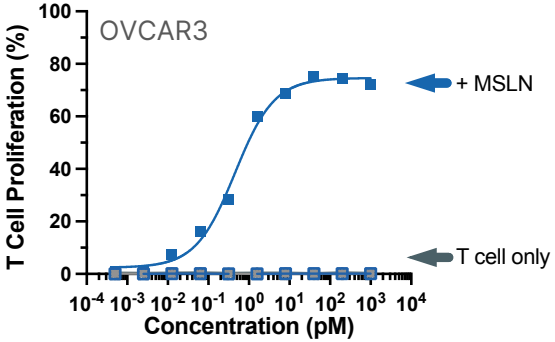
Healthy cell (MSLN-low)



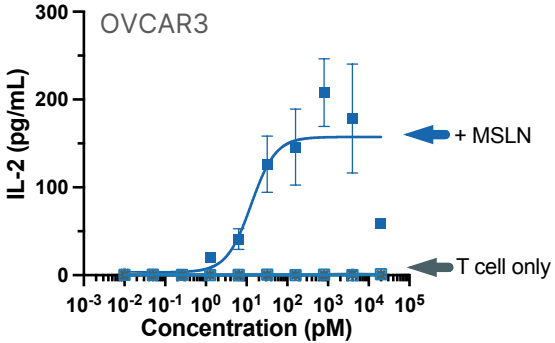
Tumor cell cytotoxicity



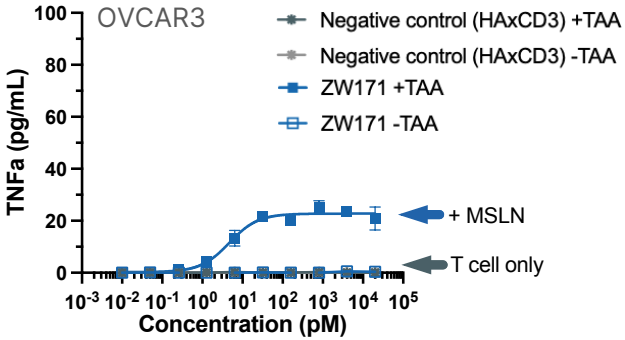
T cell proliferation



IL-2



TNF-α



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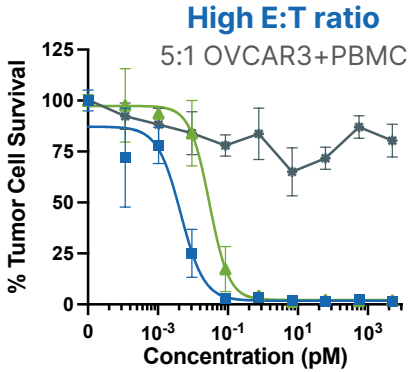
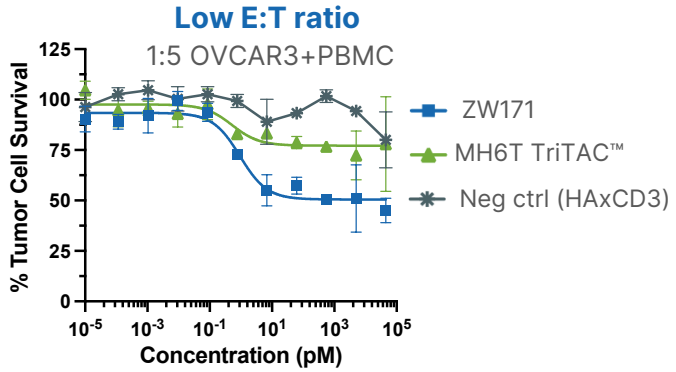
ZW171 mediates greater anti-tumor activity compared to benchmark in MSLN-expressing tumor models

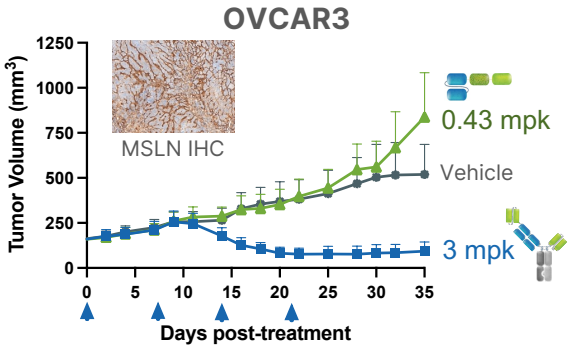
Benchmarked activity of ZW171 against Harpoon's MH6T TriTAC™

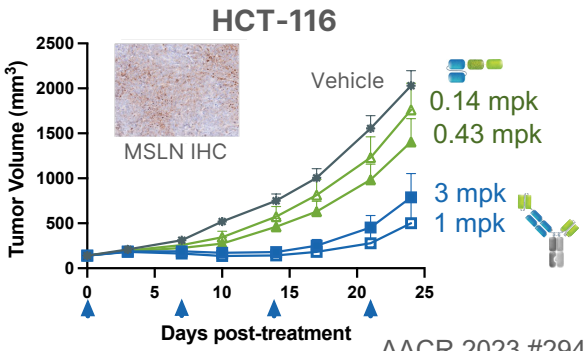




In vivo studies

- **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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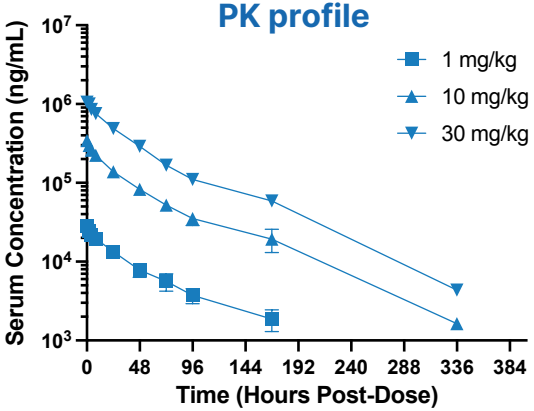
ZW171 is well-tolerated in cynomolgus monkeys

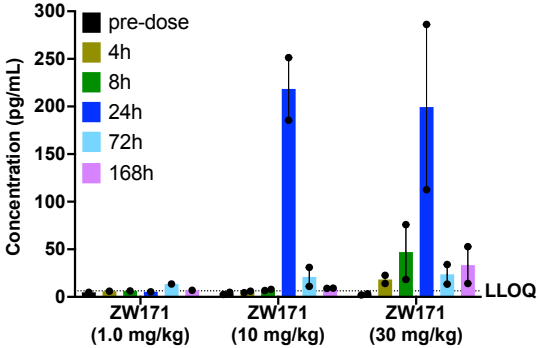
Cynomolgus monkeys administered single dose of ZW171 at 1, 10, 30 mg/kg i.v.

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

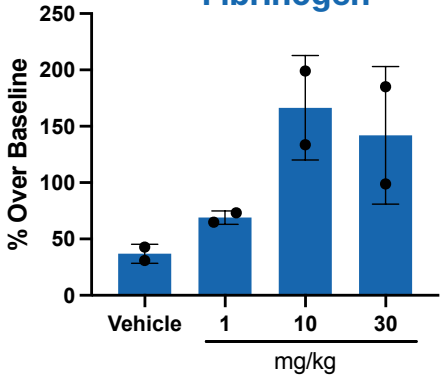
PK profile



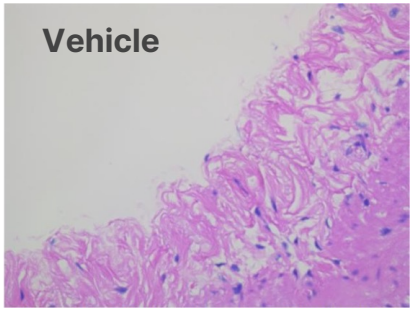
Serum IL-6

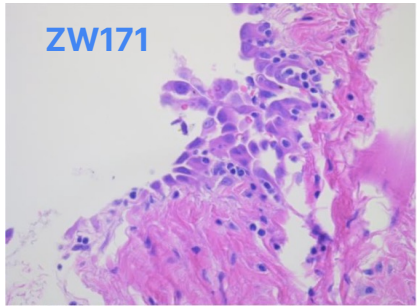


Fibrinogen



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

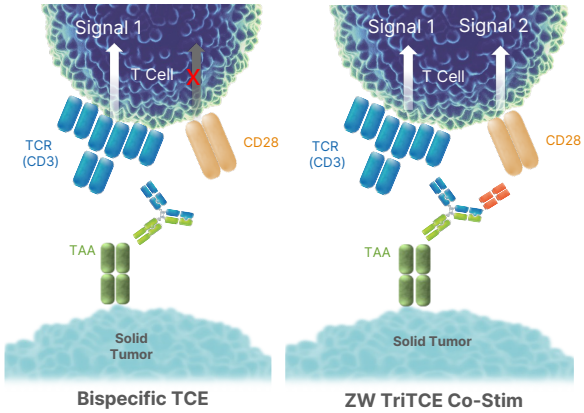
Collectively, these data provide a strong therapeutic rationale to support the development of ZW171 for the treatment of MLSN-expressing tumors

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

Next generation: TriTCE co-stim and TriTCE CPI

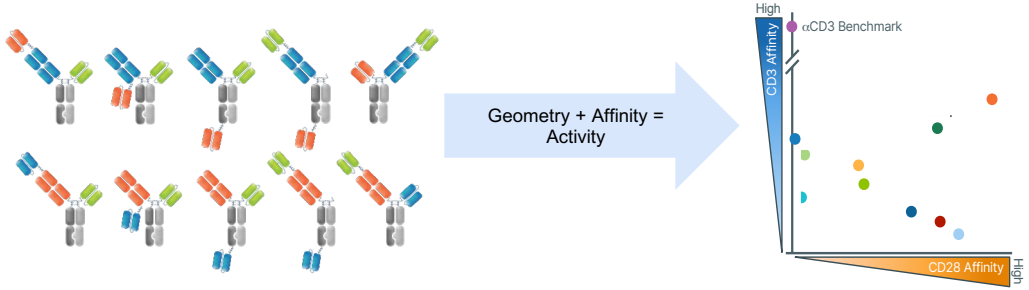
TriTCE Co-stim: Next generation co-stimulatory trispecific T cell engagers for the treatment of solid tumors

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



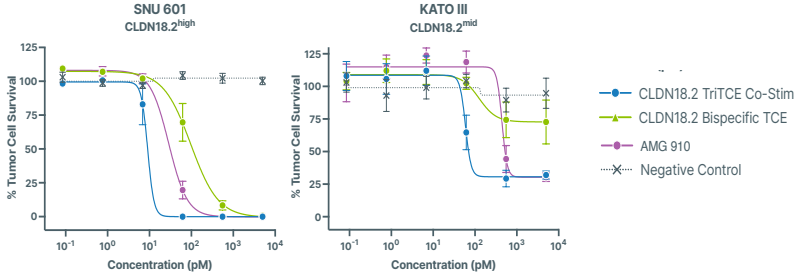
Antibody Format	1	2	3	4	5	6	7	8	9	10
IC_{50} (pM)										
TAA-Dependent?	✓	✓	✓	X	✓	✓	X	X	X	X

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

- 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

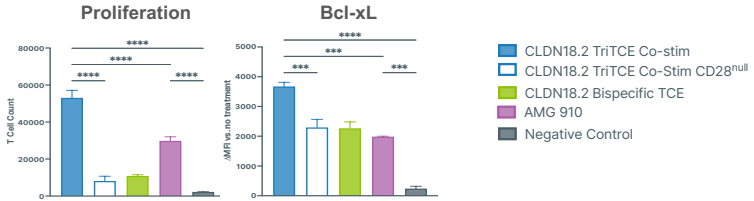
CLDN18.2 TriTCE co-stim therapeutic program

Enhanced cytotoxicity at low effector-to-target ratios



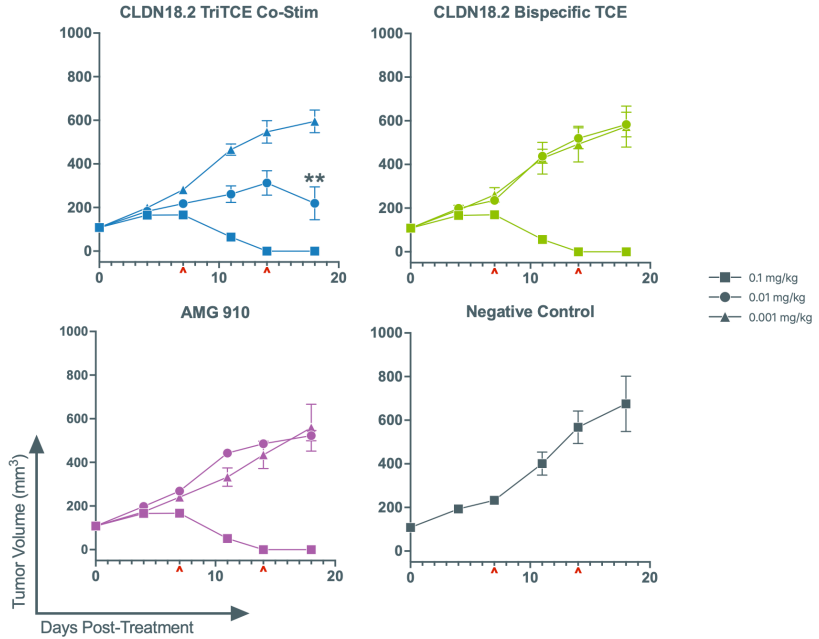
TriTCE Co-Stim may show superior activity in 'cold', poorly infiltrated tumors

Improved TAA-dependent T cell proliferation and survival



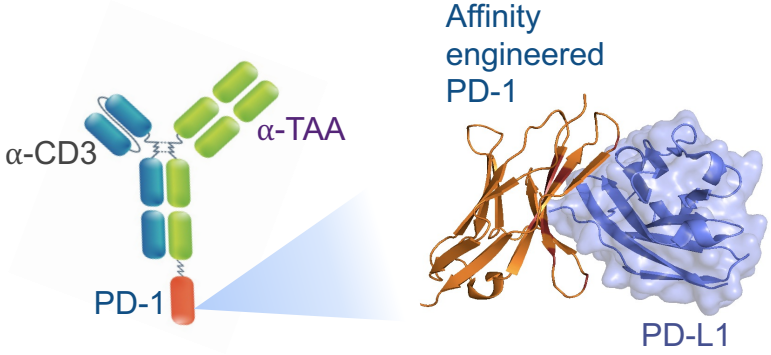
TriTCE co-stim may provide more durable responses in solid tumors

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

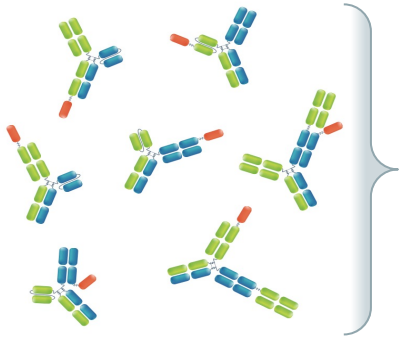


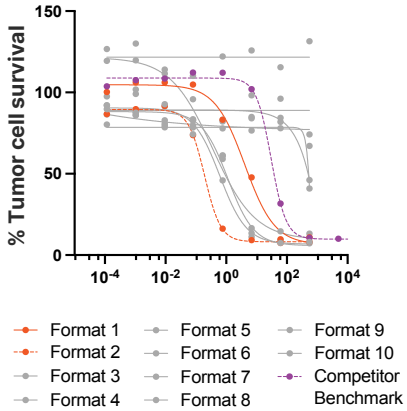
AACR 2023 #5121

TriTCE-CPI: Trispecific T cell engagers with checkpoint inhibition for the treatment of solid tumors



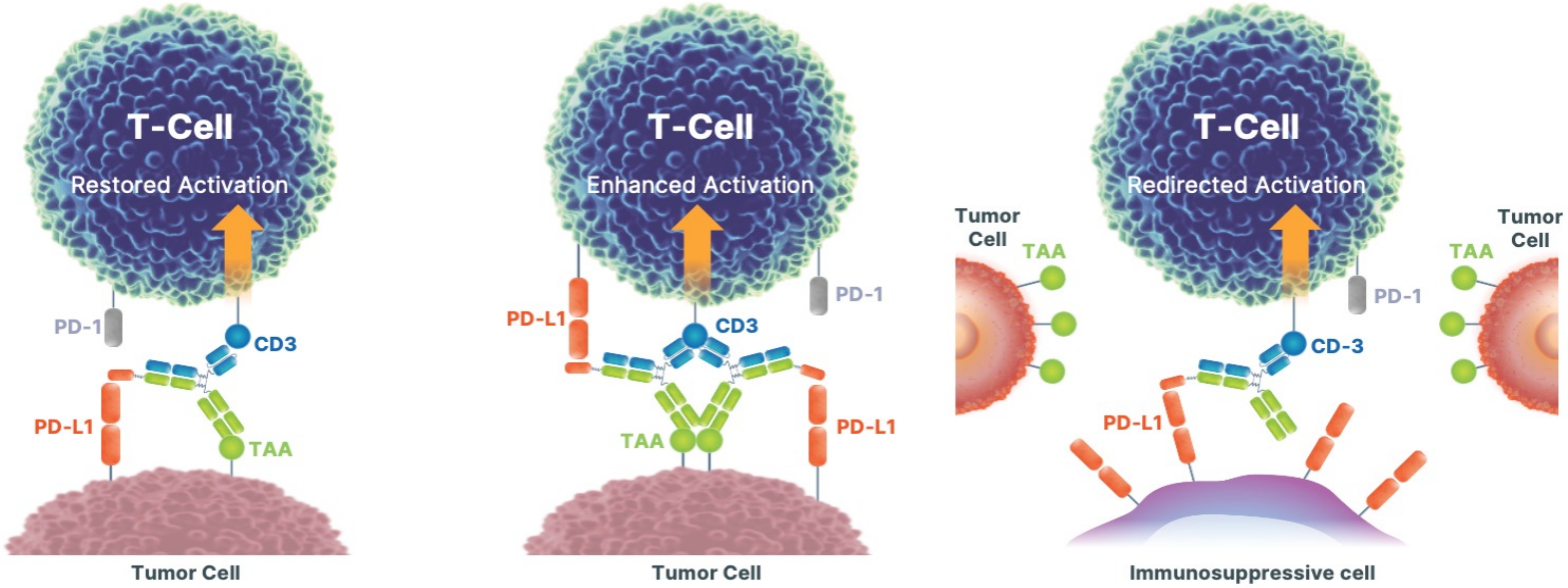
TriTCE-CPI format screening





AACR 2023 #2982

TriTCE-CPI: Trispecific T cell engagers with checkpoint inhibition for the treatment of solid tumors



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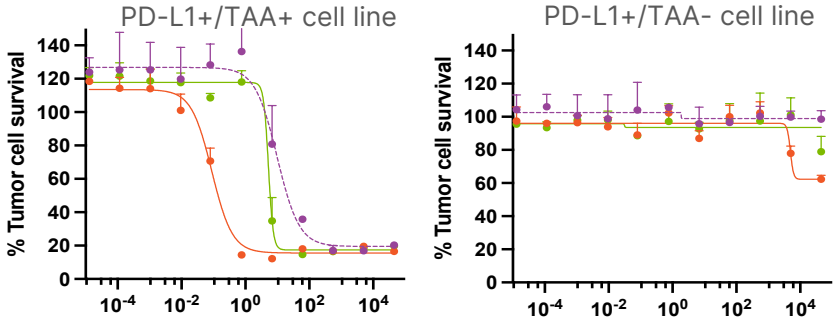
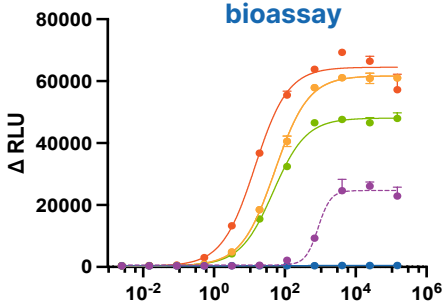
TriTCE-CPI: Trispecific T cell engagers with checkpoint inhibition for the treatment of solid tumors

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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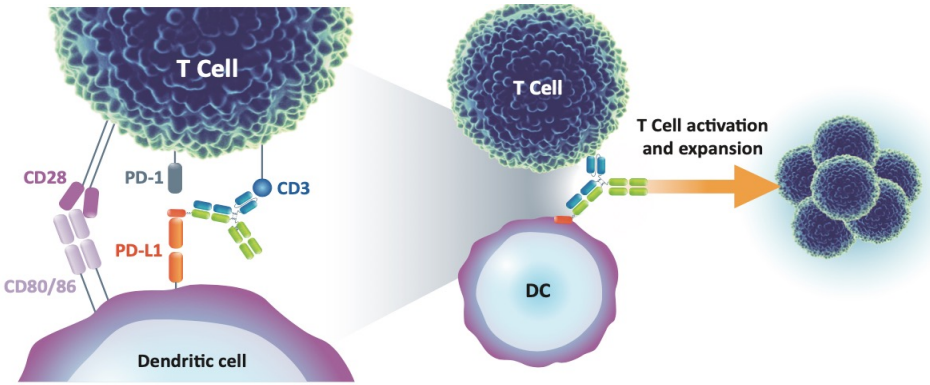
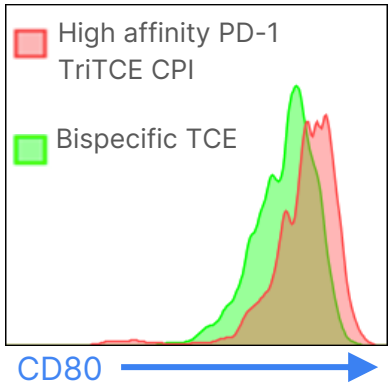
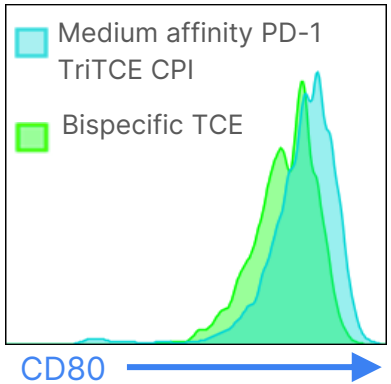
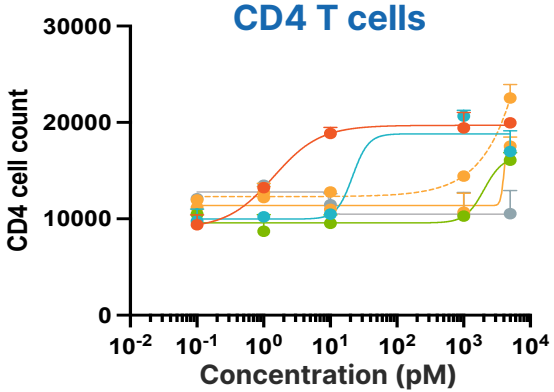
TriTCE CPI formats can be tuned to optimize dendritic cell (DC)-dependent T cell activation

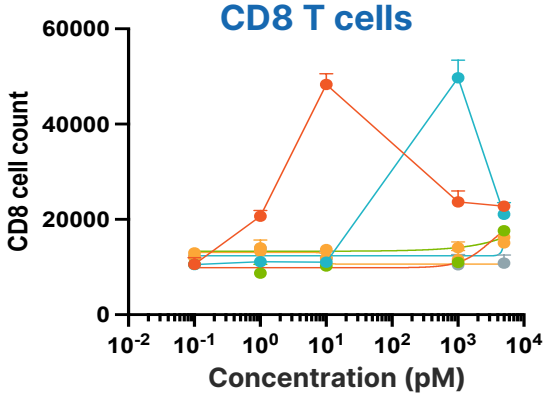


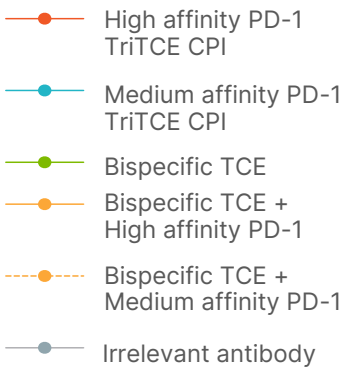
DC - T cell co-culture CD80 expression











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