zymeworks

Making a Meaningful Difference

Building Differentiated & Next Generation T Cell Engagers to Improve Responses in Difficult-to-Treat Tumors

Nicole Afacan, PhD
Principal Scientist, Multispecific Antibody Therapeutics

Engineering Multispecifics and ADCs to Adapt to Different Tumor Environments

We select difficult-to-treat cancers

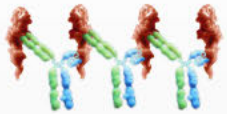
Current focus: Gynecological cancers, NSCLC, and gastrointestinal cancers

We engineer biotherapeutics with multiple in-house complementary technologies

The foundation antibody is selected using Azymetric™ to screen multiple geometric formats

Zymeworks technology used to design different parts of the antibody:
EFECT™ ; ProTECT™; T-cell engagers; TOPO1i Platform; ZymeLink™ Auristatin/Hemiasterlin; TLR7, ISAC; site-specific conjugation

We customize the modalities for the target + tumor microenvironment



Geometry prevents it from binding to the same HER2 molecule

Zanidatamab: Lead multispecific in clinical trials. HER2 biparatopic antibody engineered to overcome challenges in heterogenous tumors under

- FDA Priority Review (action date of Nov 29, 2024)
- Licensed to Jazz and BeiGene
- Positive pivotal data 2L+ BTC (*Lancet Oncology*)
- Phase 3, 3-arm RCT: 1L GEA topline ~ late 2024

Multispecifics

- Multiple MOAs in single molecule
- Synergistic biology (understand the TME)
- Precision targeting through multivalency

Antibody-drug conjugates

- Antibody design (mono, bispecific, etc)
- Payload (4+ in-house developed payloads)
- DAR (select according to need: 2, 4, 8)

1L first line; 2L second line; ADC antibody-drug conjugate; BLA Biologics License Application; BTC biliary tract cancer; DAR drug-antibody ratio; GEA gastroesophageal adenocarcinoma; HER2 human epidermal growth factor receptor 2; ISAC immunestimulating antibody conjugate; MOA mechanism of action; NSCLC non-small cell lung cancer; RCT randomized clinical trial; TME tumor microenvironment; TOPO1i topoisomerase-1 inhibitor

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-BC-303[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			
TriTCE Co-Stimulatory Trispecific T Cell Engager	Under active evaluation	TAA x CD3 x CD28		Pilot toxicology 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

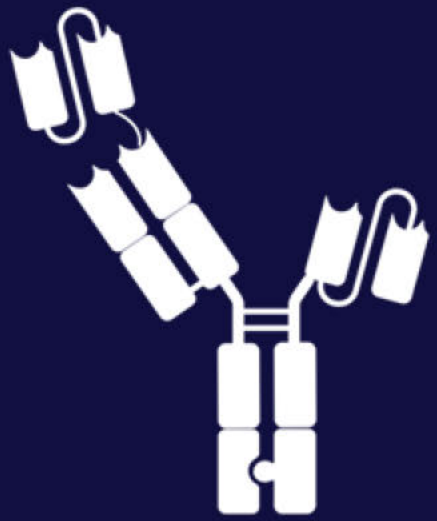
BC breast cancer; BTC biliary tract cancers; CD3 cluster of differentiation 3 protein complex and T cell co-receptor; CD28 cluster of differentiation 28; CLDN claudin; GEA gastroesophageal adenocarcinoma; HER2 human epidermal growth factor receptor 2; KLK2 kallikrein-related peptidase 2; MSLN mesothelin; NSCLC non-small cell lung cancer; OVCA ovarian cancer; PD-L1 programmed cell death ligand 1; TAA tumor associated antigen; TriTCE trispecific T cell engager.
1. Trial initiation expected in the second half of 2024.

Multispecific T-Cell Engagers

Technology and Expertise to Overcome the Current Key Challenges Observed in Clinic

Key Challenges	Proposed Zymeworks Solutions
1 Narrow therapeutic window and toxicity due to CRS associated with Gen 1 TCE in solid tumors	**2+1 T-Cell Engager (ZW171)** Mitigate CRS with low-affinity T-cell binding and enhanced efficacy and selectivity with avidity-driven tumor antigen binding
2 Limited T-cell intratumoral availability and T-cell anergy in solid tumors	**TriTCE co-stimulation: in development** Increase T-cell fitness, activation, and proliferation via tumor-dependent T-cell co-stimulation
3 Immunosuppressive tumor microenvironment limiting T-cell responses in solid tumors	**TriTCE checkpoint inhibitor: in development** Increase T-cell responses through simultaneous checkpoint blockade and avidity-driven binding

CRS cytokine release syndrome; TCE T-cell engager; TriTCE trispecific T-cell engager



ZW171

MSLN x CD3 Multispecific

A bispecific T cell engager expected to commence Phase 1 studies in the second half of 2024

Opportunity

- Optimized 2+1 format and geometry with enhanced mesothelin (MSLN)-dependent anti-tumor activity[1]
- MSLN has a slow turnover rate making it suitable for TCE targeting[2]

Rationale

- Moderate to high membranous expression is frequent in ovarian cancer, non-small cell lung cancer (NSCLC), mesothelioma and other cancers[3]
- Preliminary antitumor activity supports utility of T-cell targeted therapies in treatment of MSLN-expressing solid tumors[4]

Progress

- ZW171 exhibits MSLN-dependent cytotoxicity in MSLN-expressing cancer cell lines[1]
- Superior *in vitro* and *in vivo* anti-tumor activity compared to clinical benchmark in preclinical studies[1]
- IND cleared by the FDA

IND investigational new drug; MSLN mesothelin; scFV single-chain variable fragment

1. Afacan N et al., Abstract #2942 presented at AACR 2023; 2. Quanz et al Oncotarget, 2018;9, (75) 34103-34121; 3. Chang K, Pastan I, Proc Natl Acad Sci U S A. 1996;93(1)136-40; 4. Hassan R, et al. Nat Med. 2023;29 2089-2109

Designed to Widen the Therapeutic Window: Enhanced Safety + Anti-Tumor Activity

Azymetric™ and EFECT™ KO Fc

Variety of formats, geometries and paratope affinities were generated and screened prior to lead candidate final design →development of ZW171.

Antibody Format

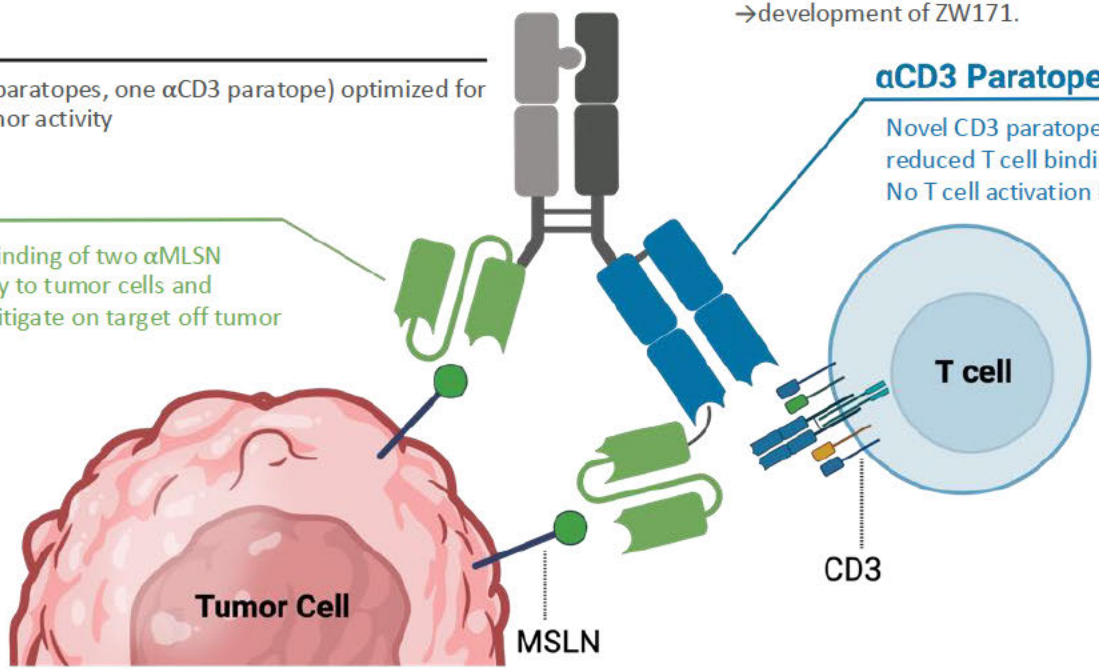
2 +1 format (two αMSLN paratopes, one αCD3 paratope) optimized for tumor-dependent antitumor activity

αCD3 Paratope

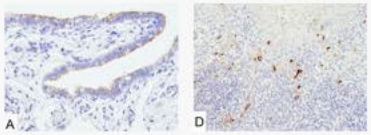
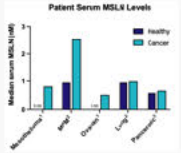
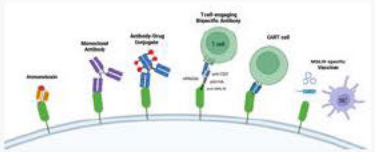
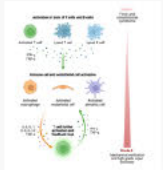
Novel CD3 paratope with low affinity and reduced T cell binding to mitigate CRS
No T cell activation in absence of tumor

αMSLN Paratopes

Avidity dependent MSLN binding of two αMLSN paratope drives cytotoxicity to tumor cells and spares normal tissues to mitigate on target off tumor toxicity



4 Key Challenges to Overcome in The Design of a MSLN Targeting T Cell Engager

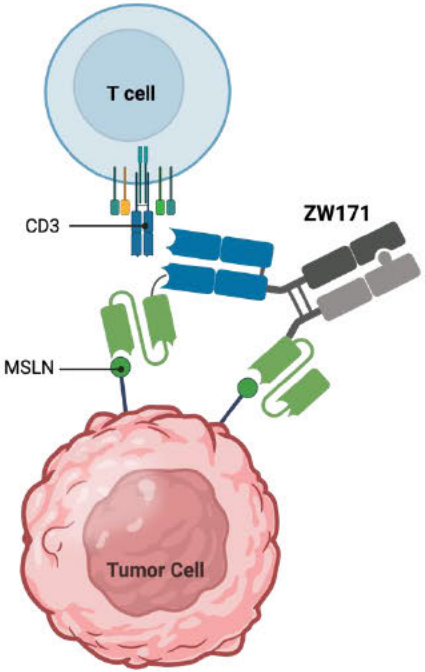
Challenge	ZW171 Design Solution
Normal tissue expression could lead to off tumor on target toxicity[1] 	**Optimized 2 +1 format and geometry enables** avidity dependent MSLN binding of two αMLSN paratopes and **selective cytotoxicity to tumor cells versus normal tissues** and **reduce impact of soluble MLSN** on potency
Soluble MSLN in serum may bind and neutralize targeted therapy[2,3,4,5] 	
Limited anti-tumor activity with past MSLN-targeted agents highlights need to optimize anti-tumor activity 	**Optimized 2 +1 format and geometry** (two αMSLN scFv paratopes, one αCD3 Fab paratope) with **enhanced MSLN-dependent anti-tumor activity**
Cytokine release syndrome elicited by T cell targeting therapies limits therapeutic window[6] 	**Novel CD3 paratope** with **low affinity** and **reduced T cell binding** to mitigate CRS, avoid T cell activation in the absence of tumor, and support effective MSLN-dependent tumor cell killing

α anti; DC dendritic cell; Fab fragment antigen-binding region scFv single chain variable fragment

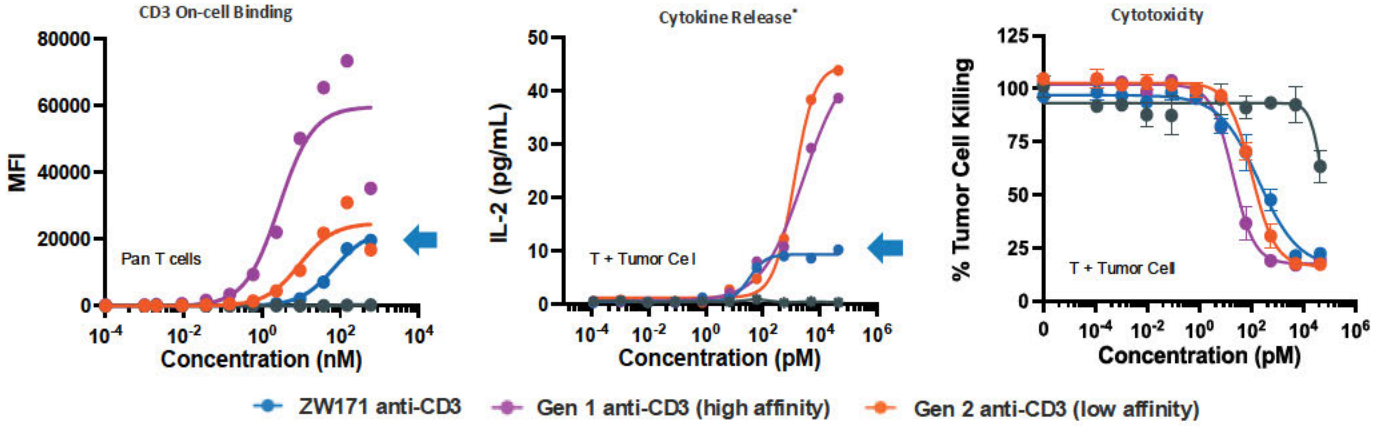
1. Inaguma S, et al., Oncotarget. 2017; 8 26744-26754 2. Hassan et al. Clin Cancer Res. 2006; 12(2) 447-53; 3. Smith KER, et al., JCO 2024; 42, 2565-2565; 4. Holevoet et al. Am J Respir Crit Care Med. 2010;181(6) 620-5; 5. Sharon et al. Clin Chem Lab Med. 2012;50(4) 721-5; 6. Shmabukuro-Vornhagen, A., *et al. j. immunotherapy cancer* 2018; 6, 56

Designed for Safety Both in T Cell and Tumor Cell Engagement

- Novel anti-CD3 paratope engages CD3 at a different epitope than prior anti-CD3 antibodies utilized in T-cell engagers



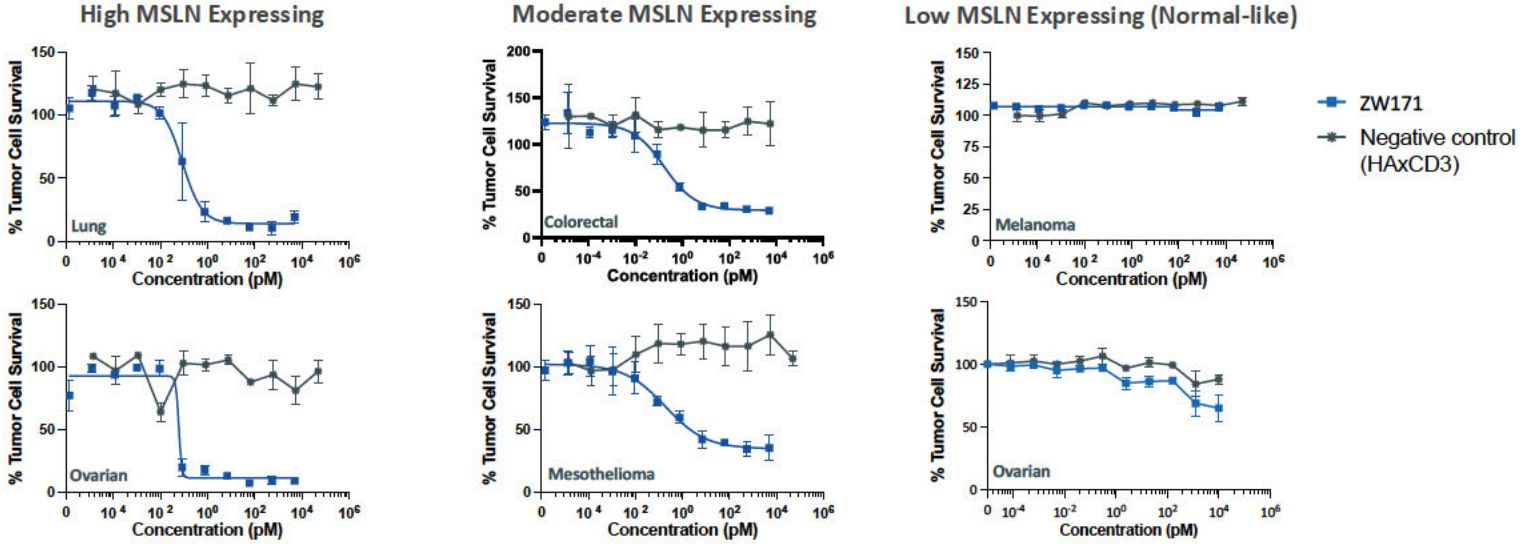
- Exhibits reduced T cell binding and cytokine release but no impact on redirected T cell-mediated lysis of tumor cells
- NHP toxicology data shows ZW171 is well-tolerated up to 30 mg/kg



*Cytokine release from T-cell–dependent cytotoxicity assay with pan T cells and H292 lung tumor cells at 5:1 E:T.
1. Afacan N, et al. Presented at AACR. 2023 (abstr #2942).

ZW171 Mediates Cytotoxicity Against High and Moderate MSLN- Expressing Tumor Cells

Bivalent MSLN binding drives binding to tumor cells that express moderate to high levels of MSLN and spares binding to low MSLN-expressing normal tissue



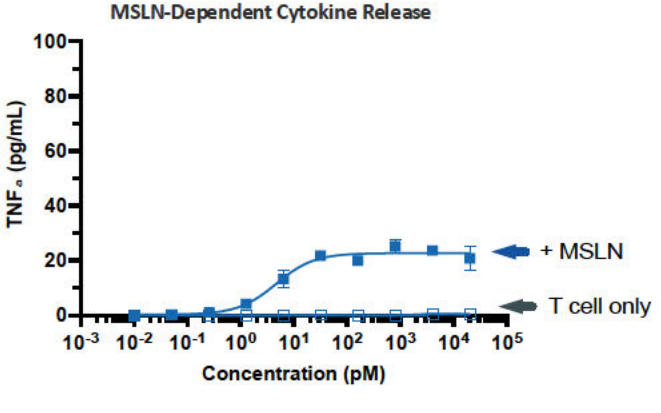
MSLN-dependent cytotoxicity in MSLN$^+$ lung, ovarian, colon, mesothelioma, gastric and pancreatic cancer cell lines

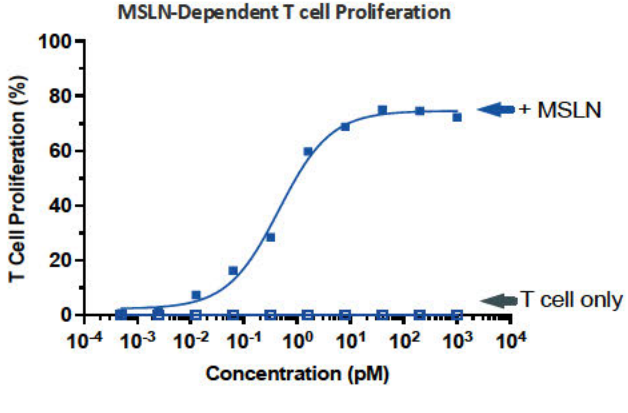
Human pan T cells and tumor cells were co-cultured at an effector-to-target rat o of 5 1 in the presence of ZW171 or negative control for 72 hours. H292 and OVCAR8 MSLN$^{+/+}$; HCT116 and H2452 MSLN$^{+/-}$; OVTOKO and A375 MSLN$^{-/-}$ cell lines

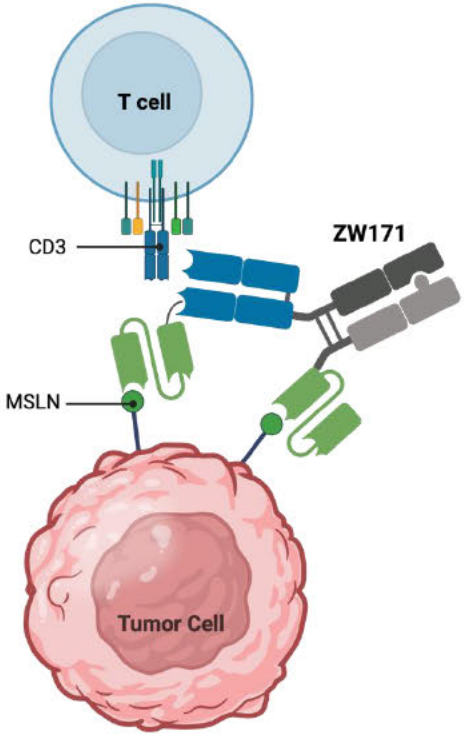
Afacan N, et al. Presented at AACR. 2023 (abstr #2942)

Designed for Safety Both in T Cell and Tumor Cell Engagement

ZW171 mediates MSLN-dependent cytokine release and T cell proliferation







→Mitigates the risk of

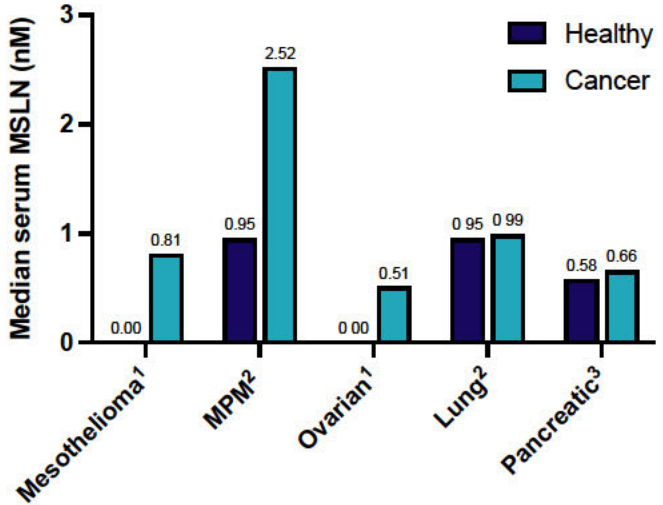
- Peripheral T cell activation
- Cytokine Release Syndrome (CRS)

Coculture of Pan-T + H292 lung tumor cells at 2:1 E:T. TNFa release was measured from collected supernatants by MSD.T cell proliferation assay with pan T cells with/without OVCAR-3 ovarian tumor cells at 10:1 E:T
Afacan N, et al. Presented at AACR. 2023 (abstr #2942)

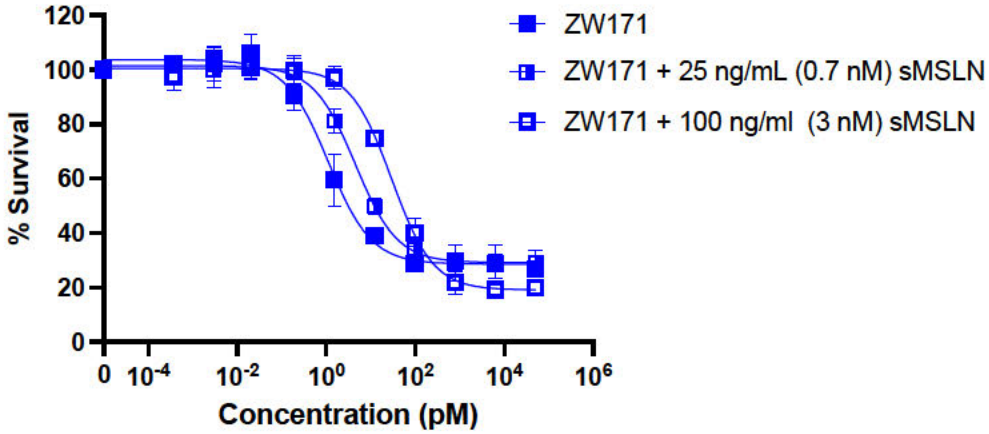
ZW171 Maintains Cytotoxic Potency in Presence of Clinically Relevant MSLN Concentrations Observed in Patient Serum Samples

- Soluble serum MSLN levels are elevated is some, but not all, MSLN-expressing cancers
- Serum MSLN levels are elevated in mesothelioma and ovarian cancer patients, but remain comparable to healthy controls in lung and pancreatic cancer patients

Patient Serum MSLN Levels

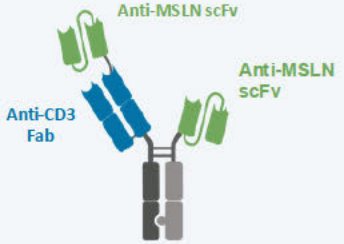


Cytotoxicity in the presence of soluble MSLN



MPM Malignant pleural mesothelioma
Figure adapted from 1 Hassan et al. Clin Cancer Res. 2006;12(2) 447-53; 2 Hollevoet et al. Am J Respr Crit Care Med. 2010;181(6) 620-5; 3 Sharon et al. Clin Chem Lab Med. 2012;50(4) 721-5
Zhang x, et al. Transl Oncol. 2022; 21 101440

ZW171 Mediates Greater Cytotoxicity Against MSLN-Expressing Tumor Cells Compared to Benchmark

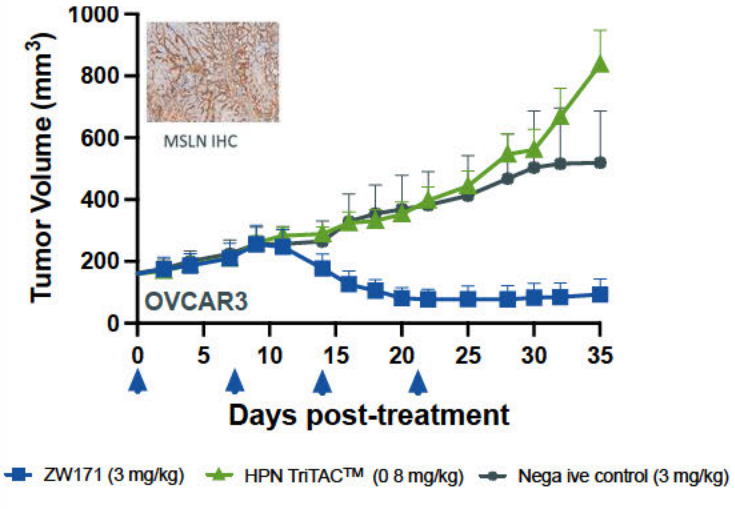


ZW171

MH6T TriTAC™

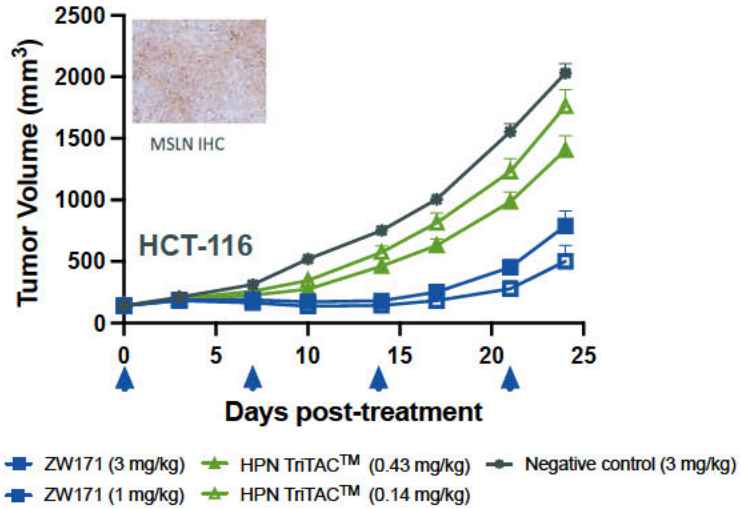
The activity of ZW171 was benchmarked against Harpoon's MSLN targeting MH6T TriTAC™

MSLN^{High}-Expressing Ovarian Cancer Model



OVCAR-3 tumor engrafted mice were humanized with donor PBMC (3 donors) and dosed i.v. QWx4 with ZW171 or i.p. daily x18 with HPN TriTAC. Neg control (HAxCD3)

MSLN^{Med}-Expressing Colorectal Cancer Model



Mice were engrafted with HCT-116 cells and humanized with donor PBMC (3 donors). Mice were dosed i.v. QWx4 with ZW171 or i.p. daily x 18 with HPN TriTAC. Neg control (HAxCD3)

Afacan N, et al. Presented at AACR. 2023 (abstr #2942)

ZW171: A Differentiated MSLN x CD3 Bispecific T Cell Engager

Widening the therapeutic window of bispecific T-cell engagers

Therapeutic Rationale

MSLN is a **clinically validated target** with high **expression in many solid tumor types** that represent a **high unmet medical need**

Investigational MSLN-targeted biologics have demonstrated clinical activity in MSLN-expressing cancers

Product Differentiation

Engineered for optimal format, paratope affinity, and stability

Reduced anti-CD3 affinity and 2+1 avidity-driven format expected to translate to **improved safety profile and widened therapeutic index**

Opportunity

First and best-in class treatment for MSLN-expressing cancers

Improved anti-tumor activity in MSLN-expressing in vivo tumor models **compared to clinical benchmark**

Next Milestones

The Company has received IND clearance by the FDA to commence clinical studies for ZW171 and commence Phase 1 studies in the second half of 2024

GLP Good Laboratory Practices; GMP Good Manufacturing Practices IND Investigational New Drug Application; MSLN mesothelin

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

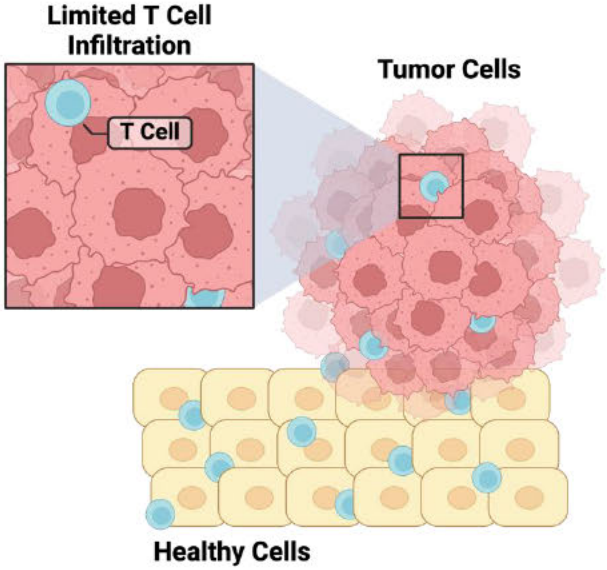
- Novel approach of modular geometry and avidity screening of trispecifics to optimize T cell activation by Signal 1 and Signal 2
- TriTCE Co-stim show superior anti-tumor activity to bispecific benchmarks and exhibit no activation of T cells in absence of tumor cells

Next Milestones

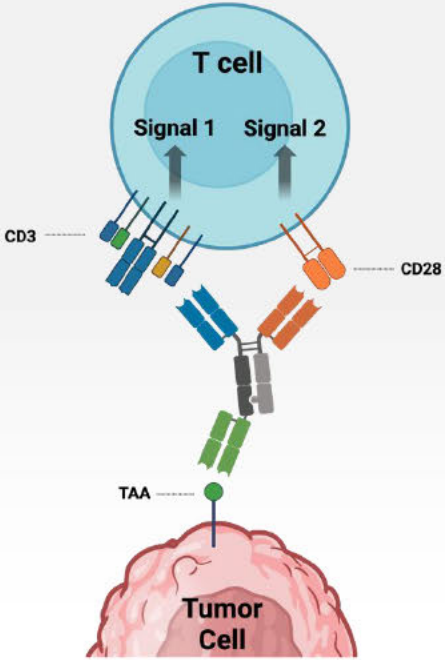
- Expand utility to additional tumor targets

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



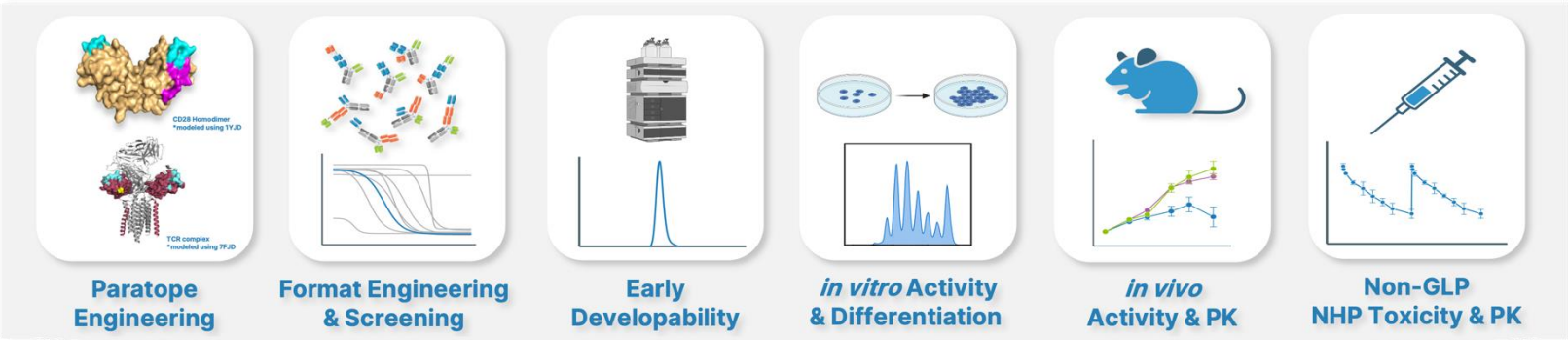
Provides Signal 1 (CD3) and Signal 2 (CD28) in one molecule to **increase T cell activation and proliferation**

Engineered to balance signal 1 and 2 for optimized **TAA-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

Established a Workflow for TriTCE Co-Stim Platform Lead Format Selection

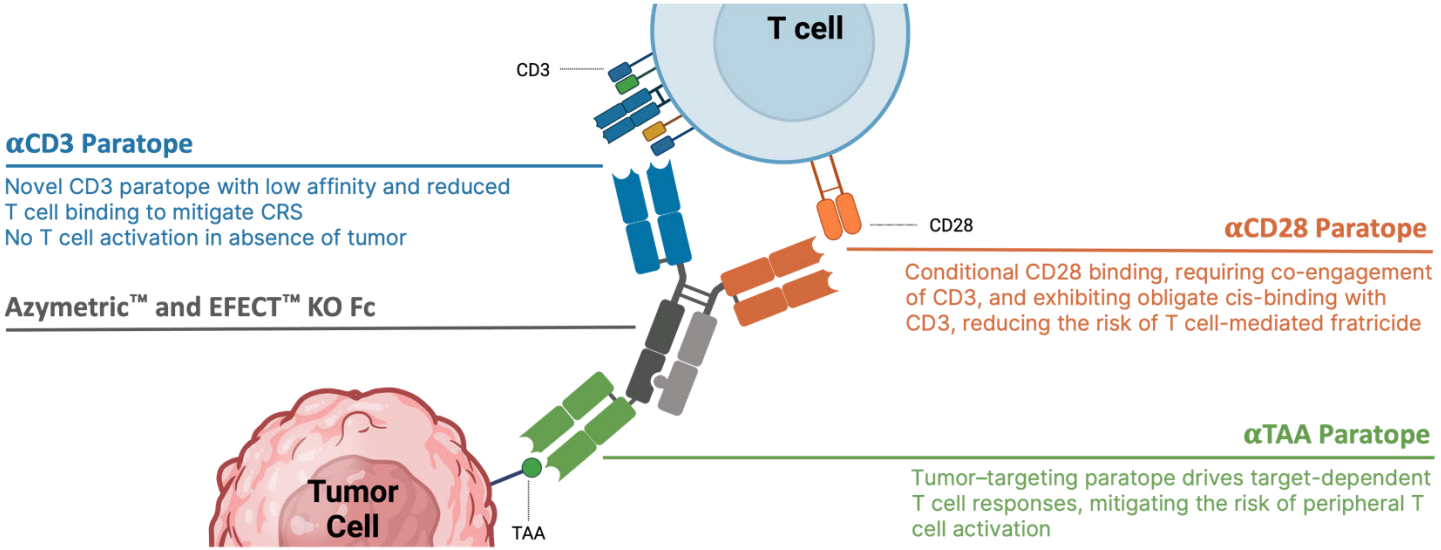


Lead TriTCE Co-Stim Format with Desired Characteristics

- ✓ Target-Dependent Activity
  - ✓ Cytotoxicity of Target Cells
  - ✓ T Cell Activation
- ✓ No Loss of T cell Viability
- ✓ No T cell:T cell Bridging

TriTCE Co-Stim Engineered for Enhanced T Cell Functionality, Antitumor Activity and Tolerability

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

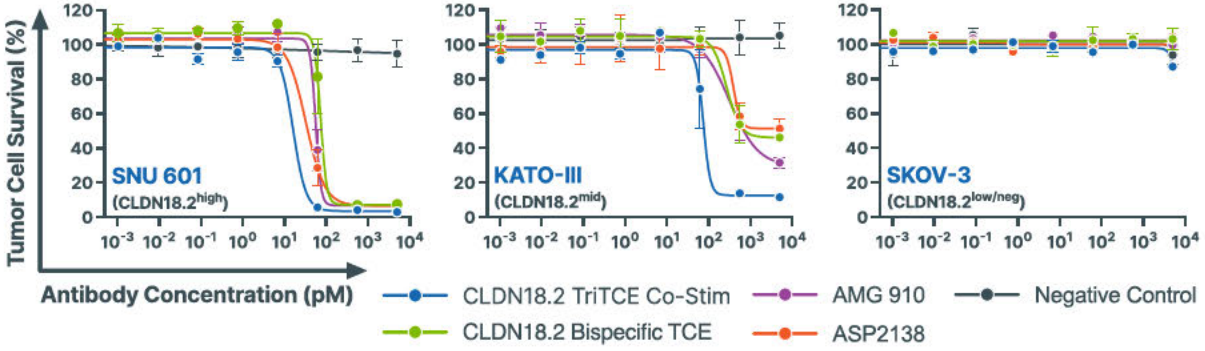


TriTCE Co-Stim platform tested with several targets including CLDN18.2[1] and DLL3[2]

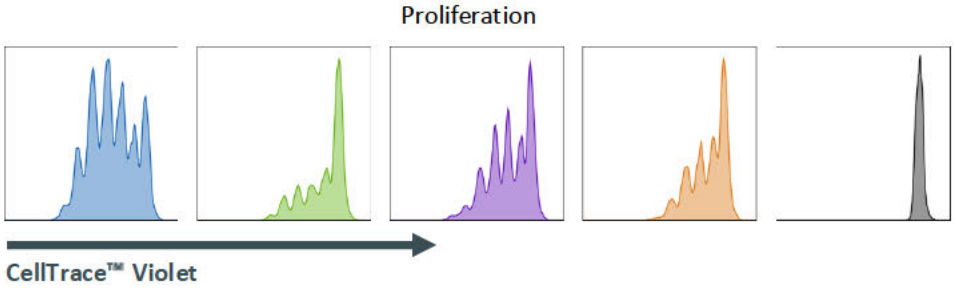
CRS cytokine release syndrome; KO Fc knocked out fragment crystalizable region of antibody (Fc).
1. Newhook L, et al. Presented at SITC Annual Meeting. 2023 (abstr # 1372) 2. Newhook L, et al. Presented at AACR Annual Meeting. 2024 (abstr # 6719)

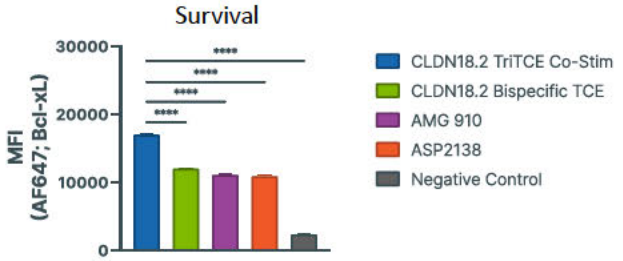
CLDN 18.2 TriTCE Co-Stim Enhances T cell Responses and Antitumor Activity Versus Benchmark Bispecific TCEs

Enhanced Cytotoxicity at Low E:T



Improved T cell Proliferation and Survival

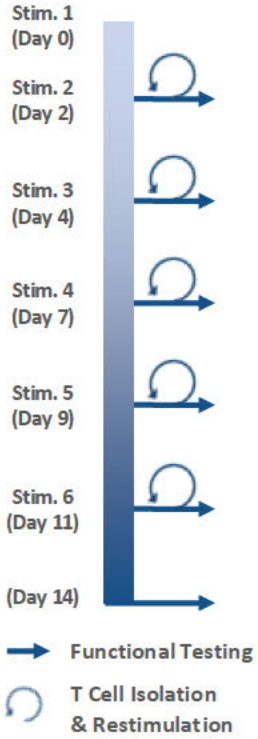


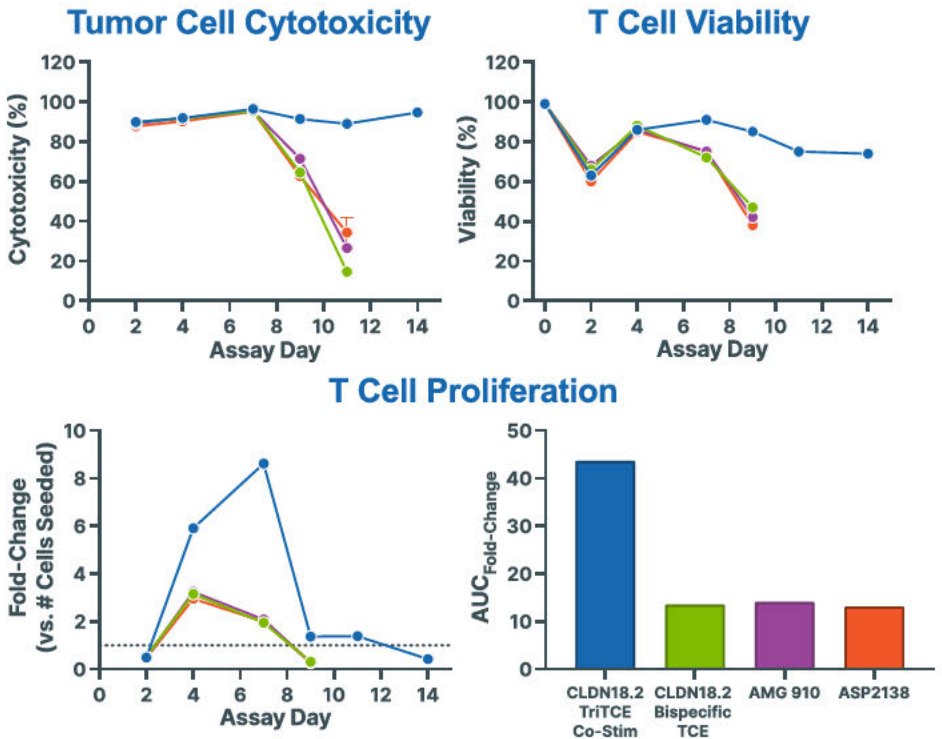


Newhook L, et al. Presented at AACR Annual Meeting. 2024 (abstr # 6719)

CLDN 18.2 TriTCE Co-Stim Displays Sustained T Cell Fitness and Antitumor Activity in a Serial Repeat Challenge Assay

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



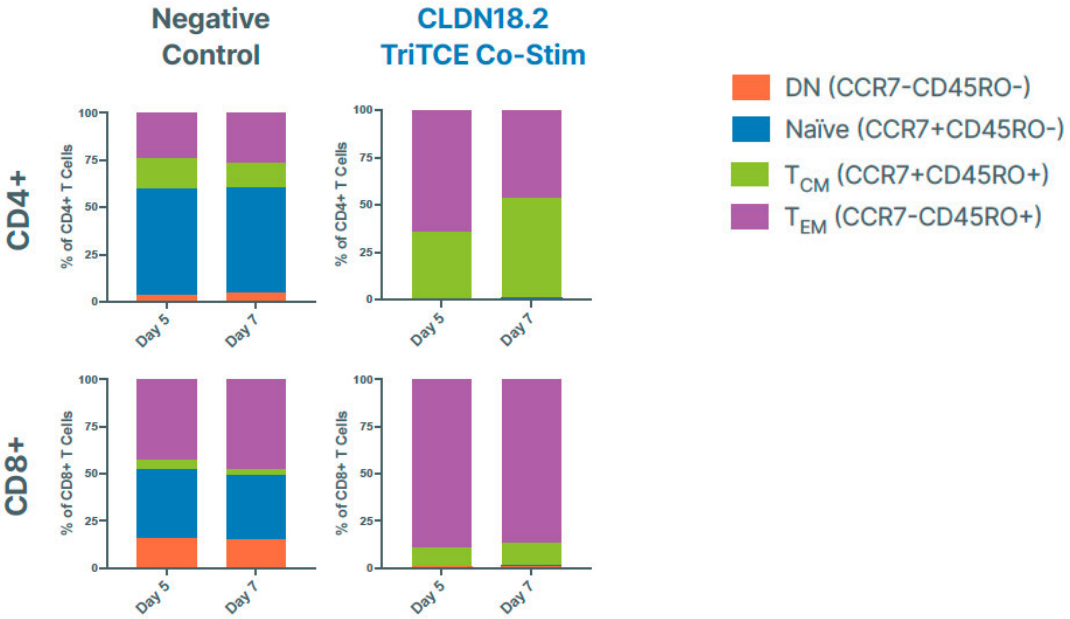


CD28 costimulation mediates sustained T cell activity *in vitro* relative to bispecific TCE, which may translate to more durable antitumor responses

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

Treatment with CLDN 18.2 TriTCE Co-Stim 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



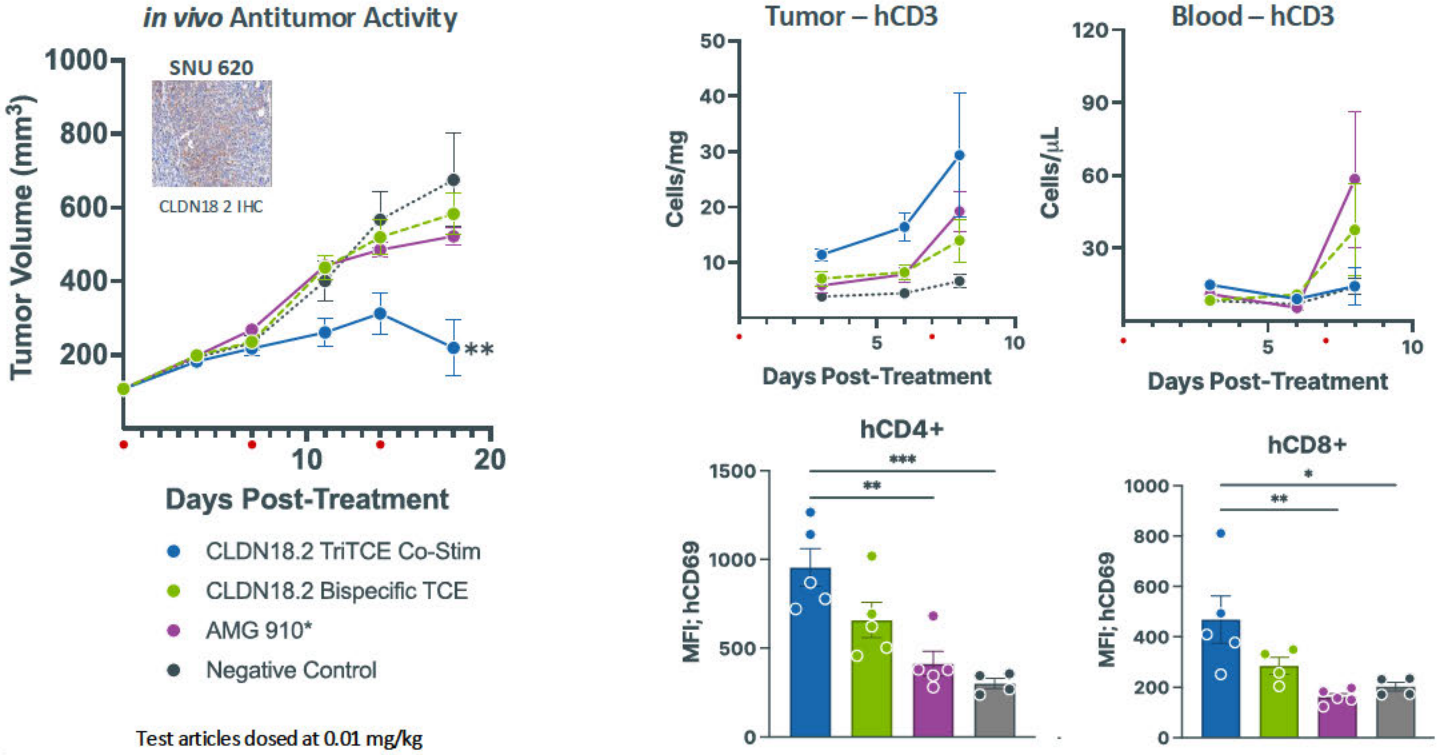
Test articles incubated with PBMCs co-cultured with CLDN18.2-expressing SNU 601 target cells and assessed for expansion of memory subsets. Memory subsets of CD4+ or CD8+ T cells were analyzed by flow cytometry after 5 and 7 days of co-culture at an E:T of 1:1.

T_{CM} T central memory; T_{EM} T effector memory;
Newhook L, et al. Presented at SITC Annual Meeting. 2023 (abstr # 1372)

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

zymeworks

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



Test articles dosed at 0.01 mg/kg

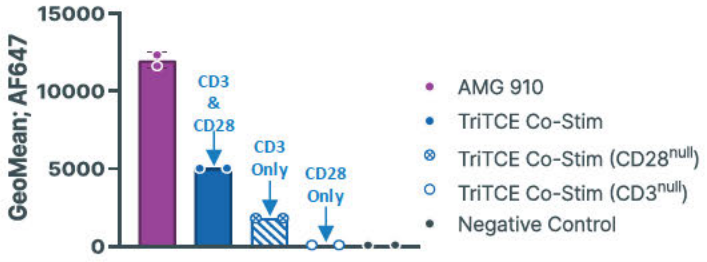
TCE T cell engager

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

CLDN 18.2 TriTCE Co-Stim Exhibits Conditional CD28 Binding and Obligate Cis T Cell Engagement

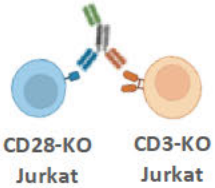
Conditional Binding of CD28, Requiring Co-engagement of CD3

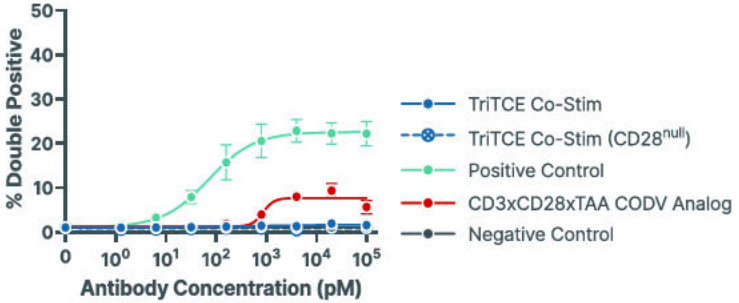
T Cell Binding



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

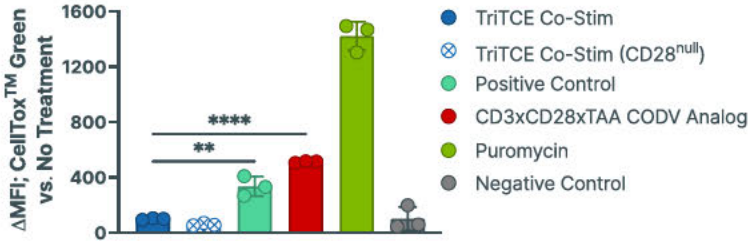
T Cell: T cell Bridging





No Reduction of T Cell Viability

T Cell Monoculture Viability



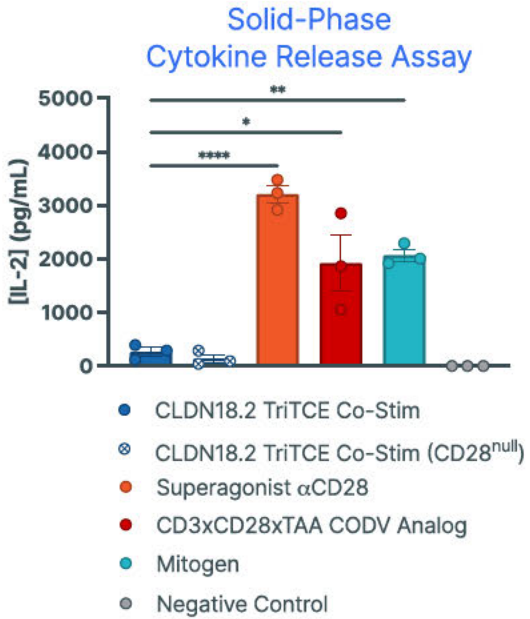
- TriTCE Co-Stim does not mediate T cell:T cell bridging
  - Cell bridging by immune cell-engaging antibodies has the potential to mediate effector cell fratricide, ultimately depleting cells required for therapeutic efficacy (Wang et al., 2018).

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

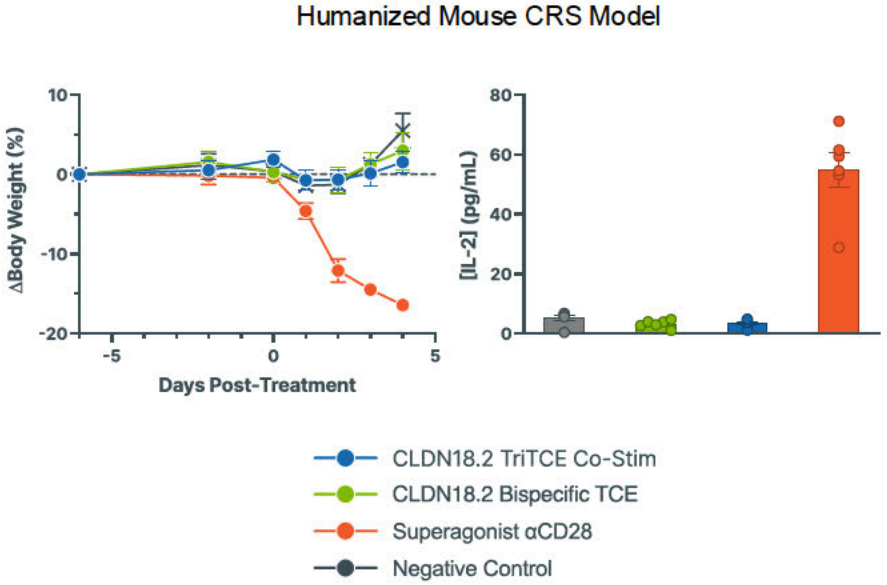
CLDN 18.2 TriTCE Co-Stim Has a Favorable Safety Profile *In Vitro* and in a Mouse CRS *In Vivo* Model

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

No Cytokine Release *in vitro* with Human PBMC Only



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

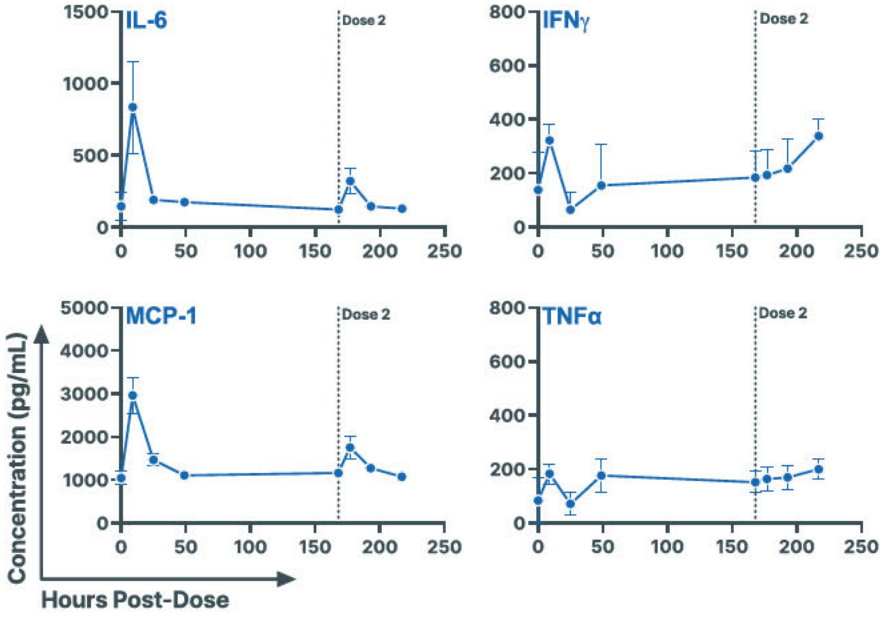


CRS Cytokine release syndrome

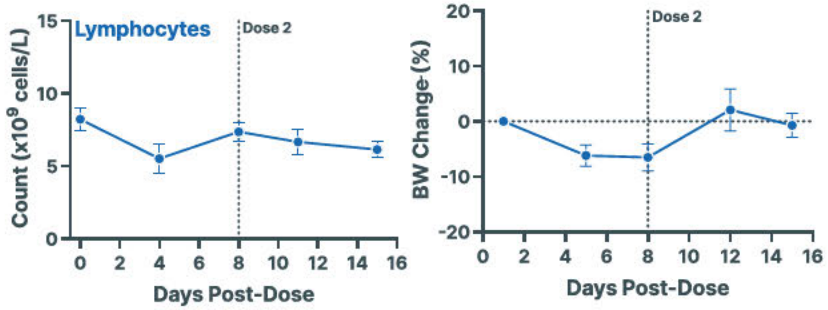
Newhook L, et al. Presented at SITC Annual Meeting. 2023 (abstr # 1372), Newhook L, et al. Presented at AACR Annual Meeting. 2024 (abstr # 6719)

CLDN 18.2 TriTCE Co-Stim 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)

Summary

1. Next generation multispecific T cell engagers with additional modalities can address existing challenges limiting the efficacy of TCE in solid tumors.

2. TriTCE Co-stim approach results in differentiated anti-tumor activity in low E:T settings and has potential to improve outcome for patients, especially those with poorly infiltrated tumors, by increasing the depth and durability of response.

3. Demonstrated *in vitro* and *in vivo* activity across multiple programs, including CLDN 18.2 and DLL3 targeted TriTCE, with a favorable safety profile.

Newhook L et al., Abstract #6719 presented at AACR Annual Meeting 2024.
Repenning P et al., Abstract #6716 Presented at AACR Annual Meeting 2024.

Acknowledgements

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Patricia Zwierzchowski
Wingkie Wong
Janessa Li
Lisa Newhook
Purva Bhojane
Peter Repenning
Diego Perez
Polly Shao
Alec Robinson
Matteo Zago
Nichole Escalante
Maya Poffenberger
Anna von Rossum
Kesha Patel
Alexandra Livernois
Madeline Fung

Catherine Wu
Marylou Vallejo
Richard Kunze
Gavin Storoschuk
Desmond Lau
Aditi Deshmukh
Diana Canals Hernaez
John Zhang
Mariana de Souza Rocha
Jan-Philip Meyer
Kurt Stahl
Michelle Chakraborti
Diego Alonzo
Begonia Silva Moreno
Ritesh Korat

Nicole Afacan
Chayne Piscitelli
Nina Weisser
Thomas Spreter von Kreudenstein
Charles Chen
Pranshul Chauhan
Paul Moore

National Research Council (NRC) Canada

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