



Leveraging Azymetric™ to Optimally Format T Cell Engagers and Bispecific ADCs

World Bispecific 2024
Wednesday, Sept 4th

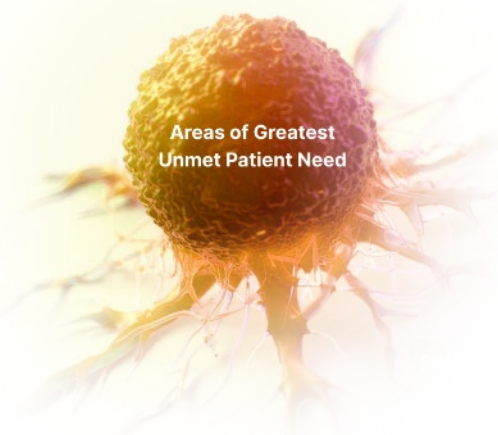
Paul Moore, PhD
Chief Scientific Officer

Nasdaq: ZYME | zymeworks.com

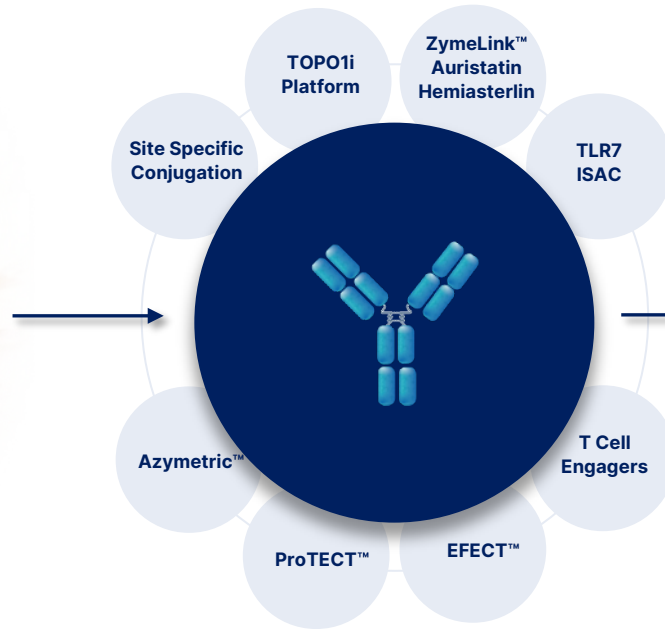


Zymeworks: Pushing the Boundaries of Antibody Based Therapeutics through Multispecifics and Drug Conjugates

Select Difficult-to-Treat Cancers & Target



Design with Complementary Technology

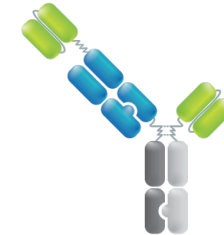


Optionality with Two Foundational Fit-for-Purpose Modalities



Antibody-drug Conjugates

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



Multispecifics

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

Azymetric™ – Adaptable to Different Formats and Applications

Engineering:

- Set of transferable mutations supporting pure and stable Fc heterodimer formation with exclusive chain pairing during co-expression
- Libraries of constant domain Fab mutations available for kappa/kappa, kappa/lamda and lambda/lambda bispecific LC combinations

Compatibility:

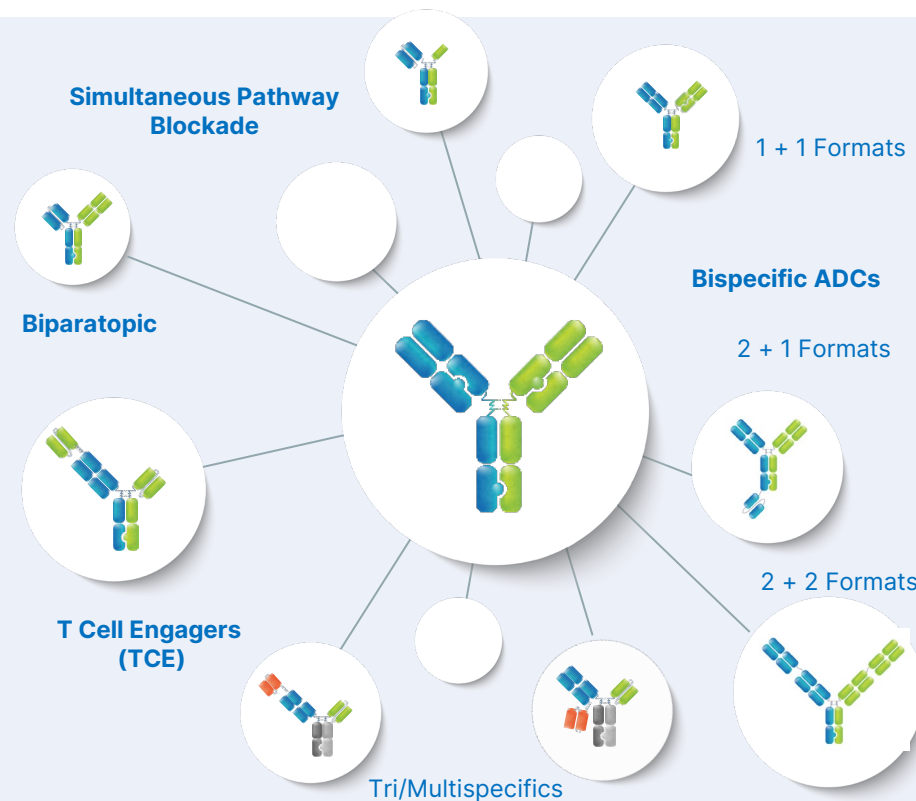
- With existing antibody paratopes; human (IgG1, IgG2A, IgG4) and mouse frameworks; other CH2 and glyco-engineering approaches

High-throughput screening:

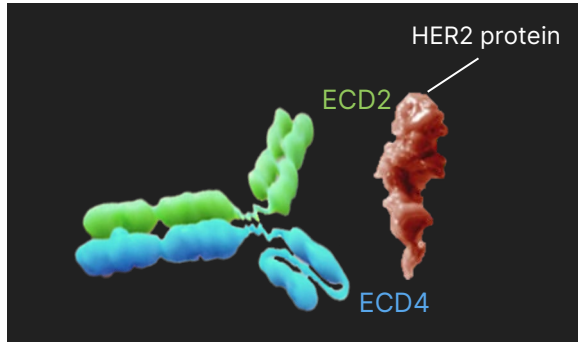
- Best-in-class activity requires screening of alternative targets, epitopes, sequences, target engagement geometries, and mechanisms of action (blocking, lytic, ADC)

Highly manufacturable

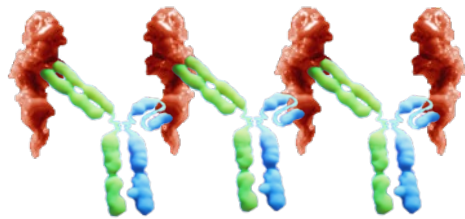
- Antibody like yields/stability; leveraged by multiple pharma/biotech with various clinical stage programs in development



Zanidatamab: A Bispecific Antibody for HER2-Expressing Cancers

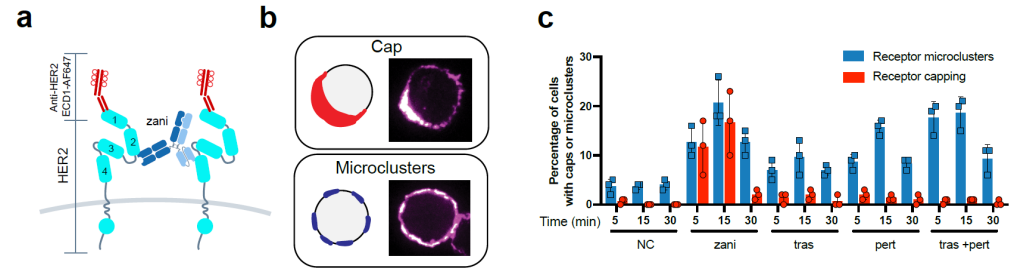


Zanidatamab binds two distinct epitopes of HER2

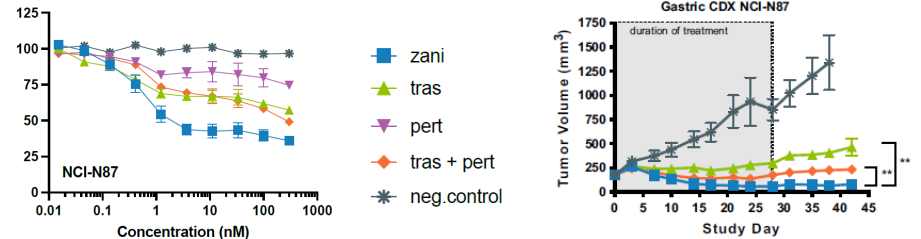


The geometry of zanidatamab prevents it from binding to the same HER2 molecule

Zanidatamab induces HER2 capping and cluster formation on cell surface



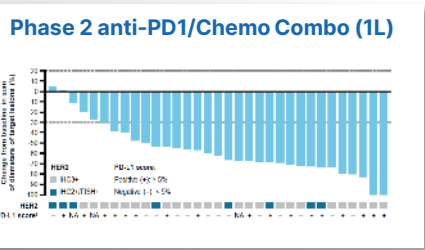
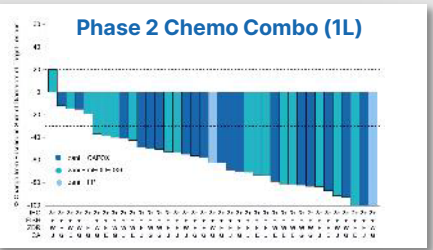
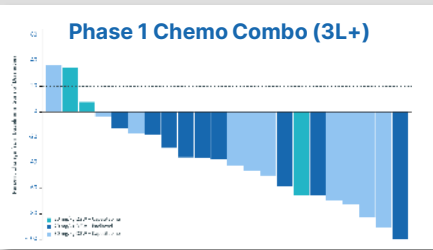
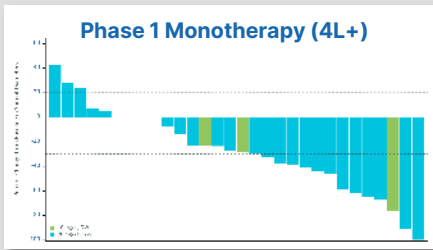
Zanidatamab inhibits tumor cell proliferation and growth



Weisser et al, Nature Communications 2023

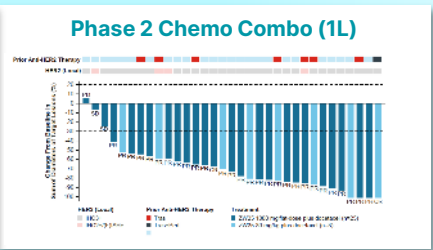
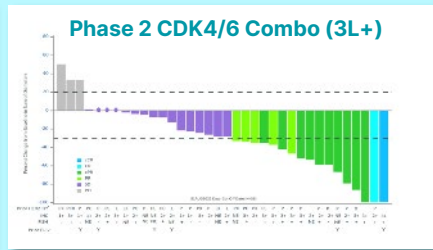
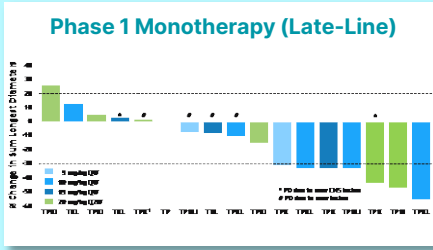
Zanidatamab Exhibits Robust Clinical Activity across Multiple HER2+ Cancer Types

Gastroesophageal



HERIZON-GEA-01: Randomized Phase 3 Study Ongoing

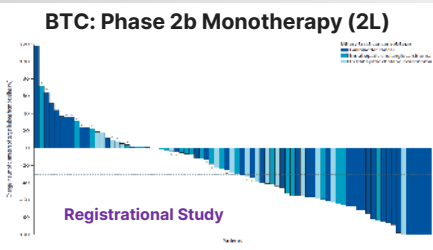
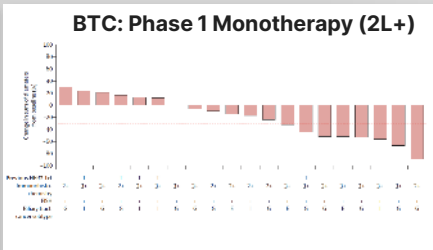
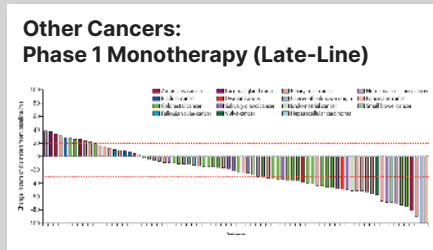
Breast Cancer



Phase 3: EMPOWER Breast Cancer Patients Progressed on T-DXd

Initiated

BTC & Others



cORR: 41.3%
mDOR: 14.9mo
OS: 15.5mo IHC3+: 18.1mo

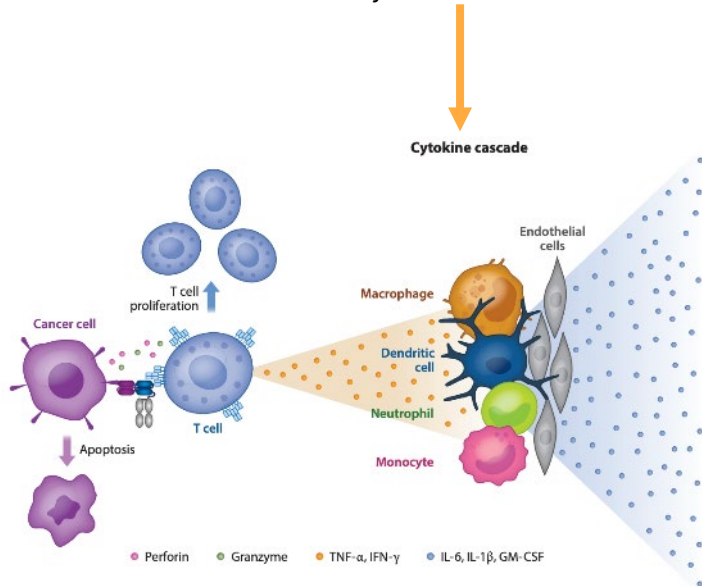
BLA Application accepted Priority Review

Phase 3 (1L) Trial Initiated

Designing Next Generation T Cell Engagers to Overcome Key Challenges

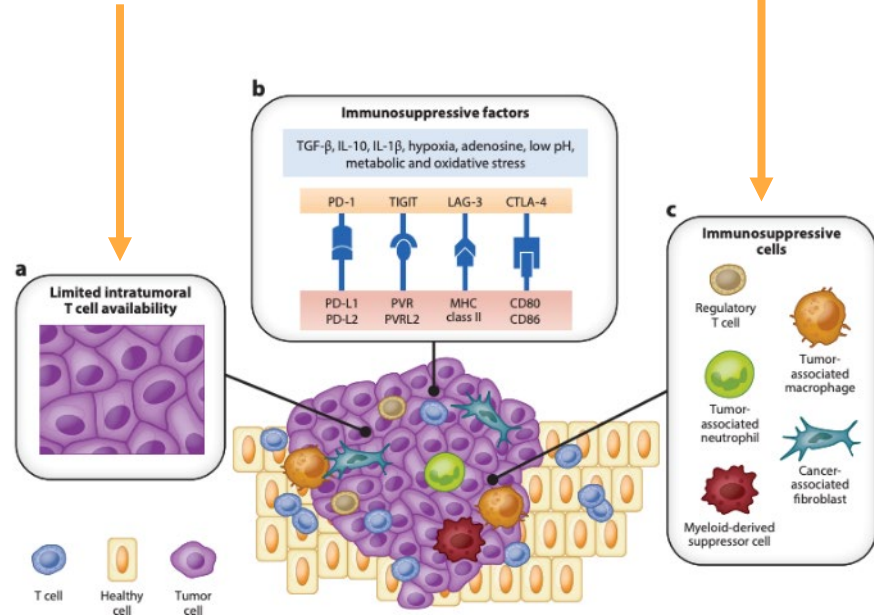
Key Problem 1:

Narrow therapeutic window and limitations due to concomitant cytokine release



Key Problem 2:

Low T cell infiltration
T cell anergy

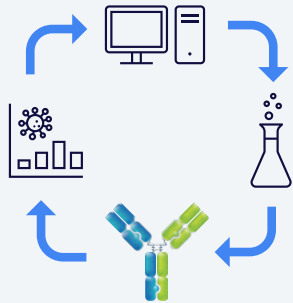


Key Problem 3:

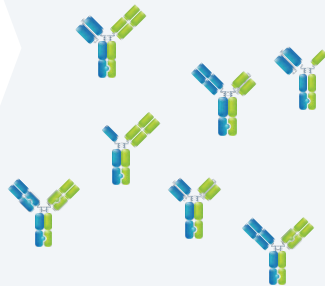
Immunosuppressive tumor
microenvironment

Core Competency of Protein Engineering & Flexibility of Azymetric™ Platform Enables Screening of Multiple Parameters in Parallel

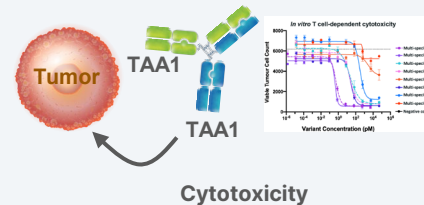
Paratope screening & optimization, *in silico* affinity engineering



Generate panel of extensively engineered antibodies: valency, geometry & affinity



In vitro & *in vivo* biophysical and functional characterization of multispecific antibodies



Single lead optimized to:

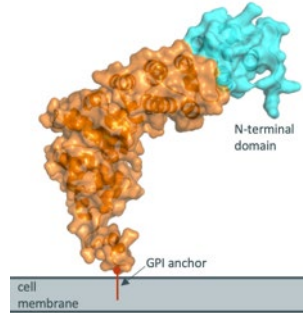
- Target TAA over-expressing cells
- Improve T cell responses
- Maximize therapeutic index
- Modulate cytokine release

- Core competency of protein engineering harnessed to engineer and optimize multiple parameters *in silico*
- Flexibility of Azymetric™ platform enabled extensive screening of antibodies based on valency, geometry, and affinity

TAA: tumor associated antigen

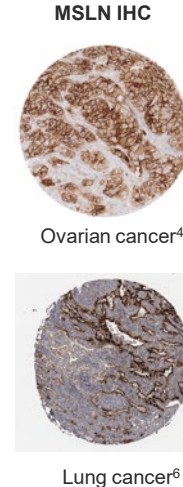
MSLN is Expressed in a Several Cancers and is an Attractive TCE Target

MSLN Plays a Role Tumor Development

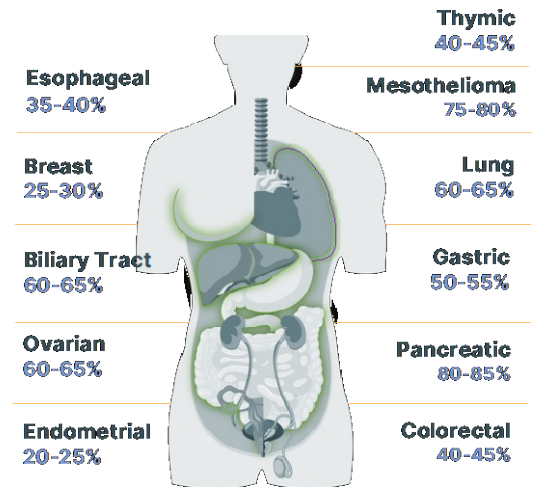


- Glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein¹
- Binds MUC16 and plays a role in cell adhesion, tumor progression, metastasis, CAF biology and chemo-resistance^{1,2}
- MSLN is expressed at low levels in the mesothelium, as well as tissues such as the fallopian tubes and tonsils^{3,4}
- MSLN has a slow turnover rate making it suitable for TCE targeting⁵
- Preliminary anti-tumor activity observed with an engineered T cell therapy (gavo-cel) supports utility of T cell targeted therapies in treatment of MSLN-expressing solid tumors⁷

MSLN is Expressed in Various Human Cancers



MSLN-Expressing Cancers



Adapted from Morello et al Cancer Discov 2016; 6: 133-146 and Inaguma et al Oncotarget. 2017; 8 :26744-26754

- Moderate to high membranous expression is frequent in ovarian cancer, NSCLC, mesothelioma and other cancers⁴

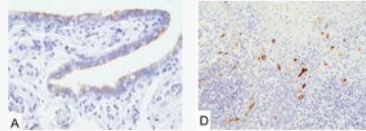
1. Shen J, et al. *Front Oncol.* 2020; 10:1263; 2. Huang H, et al., *Cancer Cell.* 2022; 40(6): 656-673.e7; 3. Chang K, Pastan I, *Proc Natl Acad Sci U S A.* 1996;93(1):136-40; 4. Weidemann S, et al *Biomedicines.* 2021;9(4):397; 5. Quanz et al *Oncotarget.* 2018;9, (75): 34103-34121; 6. Human Protein Atlas, CAB080356; 7. Hassan R, et al. *Nat Med.* 2023;29:2099-2109

Four Key Challenges to Overcome in the Design of a MSLN Targeting TCE

Challenge

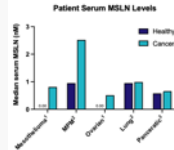
1

Normal tissue expression could lead to off tumor on target toxicity¹



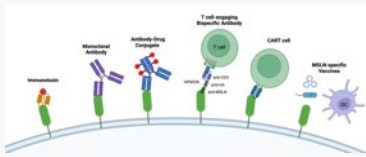
2

Soluble MSLN in serum may bind and neutralize targeted therapy^{2,3,4,5}



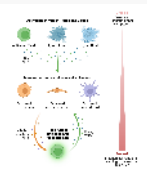
3

Limited anti-tumor activity with past MSLN-targeted agents highlights need to optimize anti-tumor activity



4

Cytokine release syndrome elicited by T cell targeting therapies limits therapeutic⁶



ZW171 Design Solution

Avidity dependent MSLN binding of two α MSLN paratopes to enable **selective cytotoxicity to tumor cells versus normal tissues** and **reduce impact of soluble MSLN** on potency

Optimized 2 +1 format and geometry (two α MSLN scFv paratopes, one α CD3 Fab paratope) with **enhanced MSLN-dependent anti-tumor activity**

Novel CD3 paratope with **low affinity** and **reduced T cell binding**

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. Hollevoet 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. Shimabukuro-Vornhagen, A., et al. J. immunotherapy cancer 2018; 6, 56

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

Antibody Format

2 +1 format (two α MSLN paratopes, one α CD3 paratope) optimized for tumor-dependent antitumor activity

α MSLN Paratopes

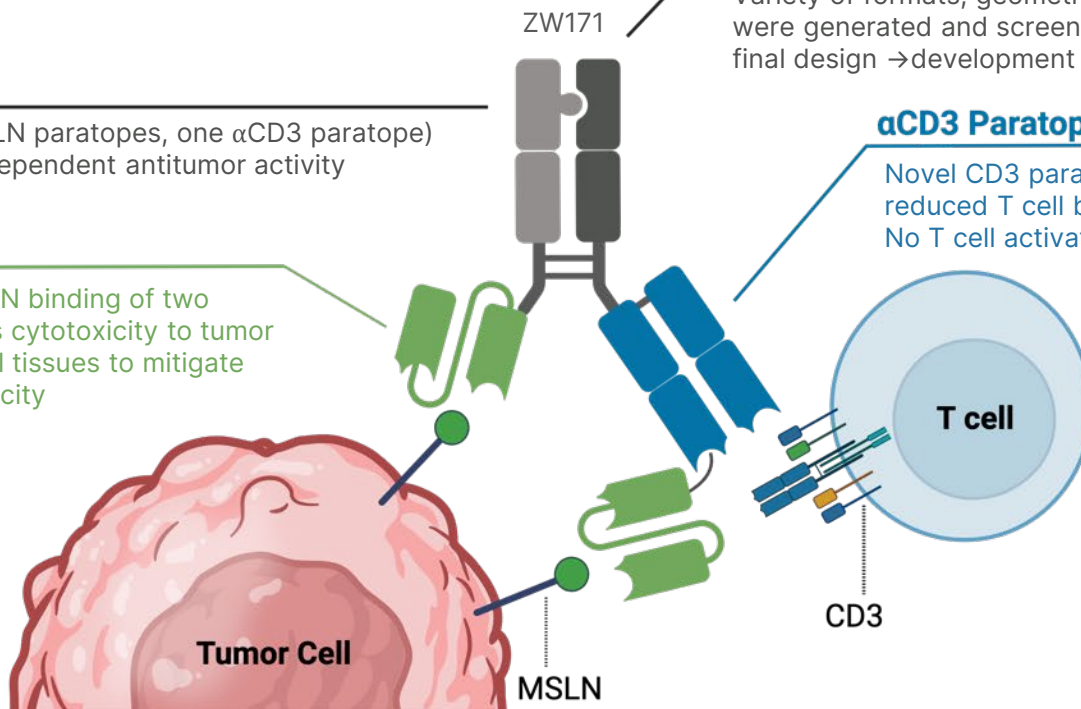
Avidity dependent MSLN binding of two α MSLN paratope drives cytotoxicity to tumor cells and spares normal tissues to mitigate on target off tumor toxicity

Azymetric™ and EFACT™ KO Fc

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

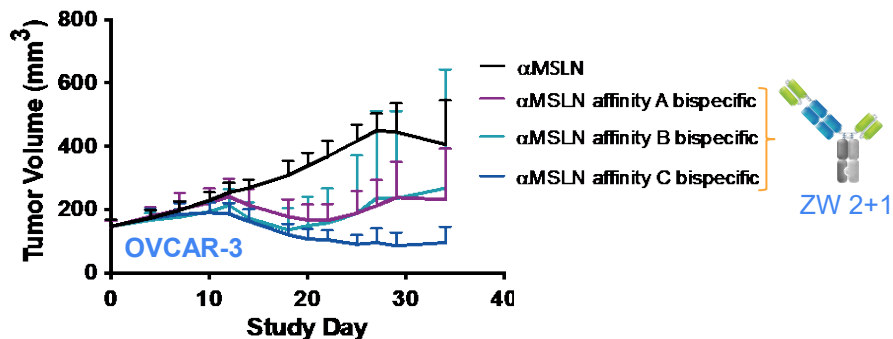
α CD3 Paratope

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



Geometry Format of ZW171 Critical for Activity in Tumor Models

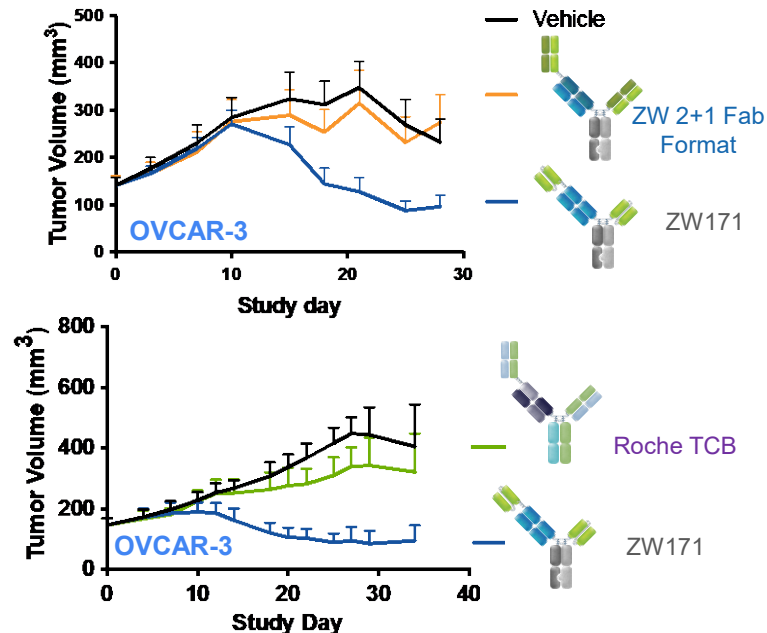
Anti-MSLN Paratope Affinity is Critical



In vivo anti-tumor activity evaluated with established tumor models that have reduced sensitivity compared to co-implantation (tumor + PBMC) models

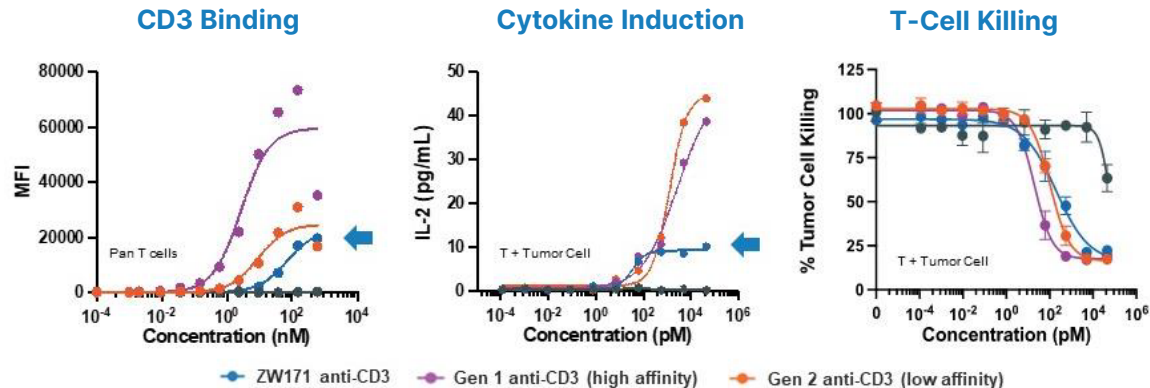


2 + 1 Geometry is Critical



OVCAR-3 tumor fragments were engrafted subcutaneously in NOG mice. After tumors reached 100-200 mm³, mice were humanized with donor PBMC (3 donors) then treated 2QW x4 with test article. HuPBMC = human peripheral blood mononuclear cells

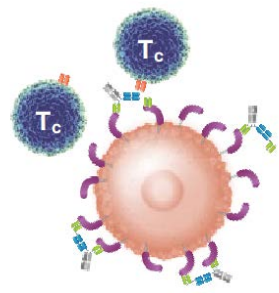
ZW171 Designed for Safety Both in T Cell and Tumor Cell Engagement



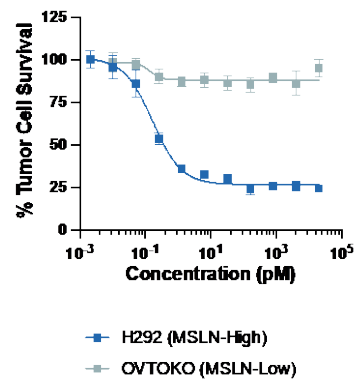
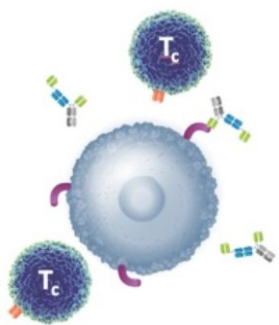
Novel low affinity anti-CD3 paratope (NHP x-reactive)

Reduced cytokine induction but maintains full cytotoxicity potential

Engages Cancer Cell (MSLN^{high})



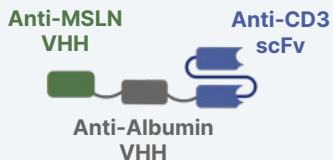
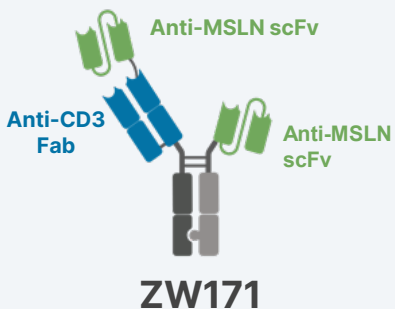
Sparses Healthy Cell (MSLN^{low})



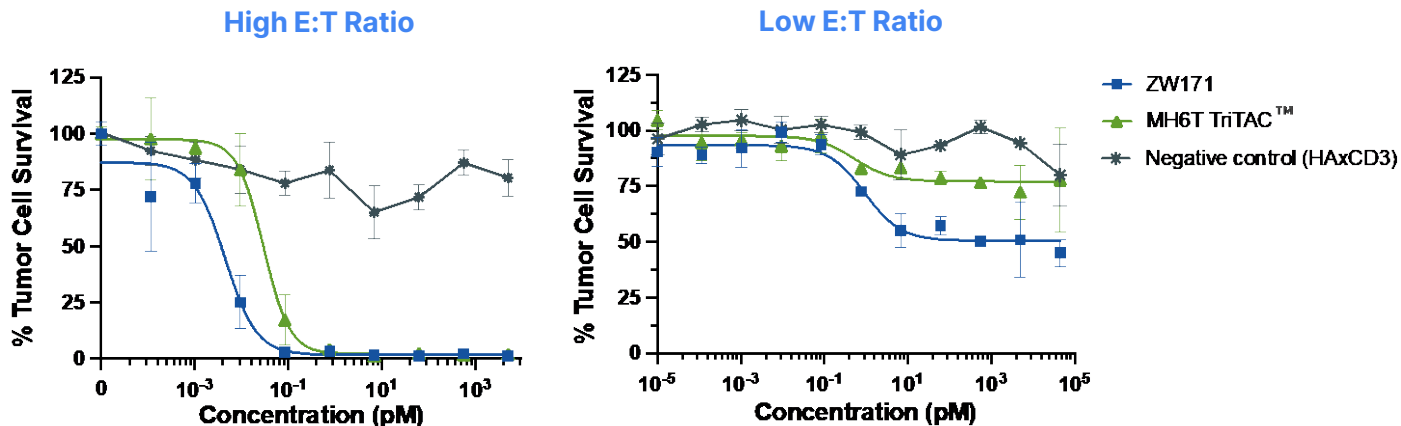
Designed to preferentially bind and target tumor cell lysis versus engagement of normal tissues through avidity dependent MSLN binding

ZW171 well tolerated in repeat dose NHP study (up to 30 mg/kg)

ZW171 Mediates Greater Cytotoxicity against MSLN-expressing Tumor Cells



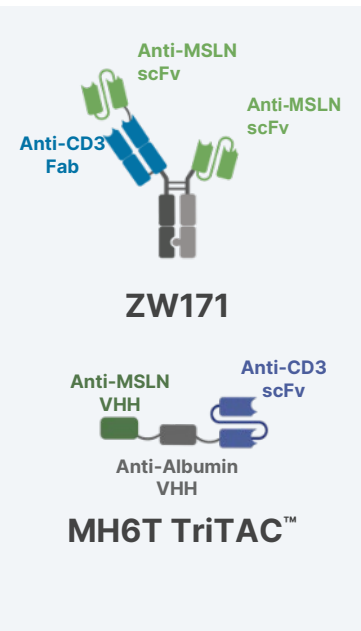
The activity of ZW171 was benchmarked against Harpoon's MSLN targeting MH6T TriTAC™



Human PBMCs and OVCA3 tumor cells were co-cultured at effector-to-target cell ratio of 5:1 (high E:T) or 1:10 (low E:T) in the presence of ZW171, MH6T TriTAC™ or negative control for 72 hours.

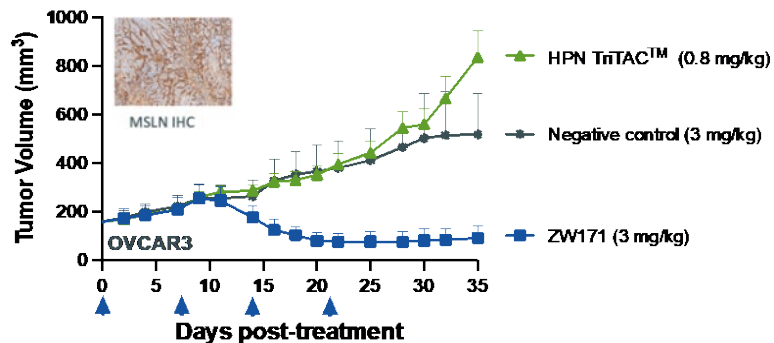
Afacan N, et al. Presented at: AACR. 2023 (abstr #2942)

ZW171 Mediates Enhanced Anti-tumor Activity in PBMC-engrafted Xenograft Models



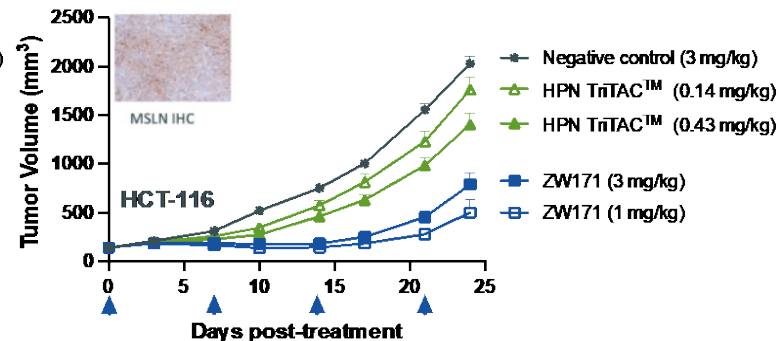
The activity of ZW171 was benchmarked against Harpoon's MSLN targeting MH6T TriTAC™

MSLN^{High}-Expressing Ovarian Cancer Model



OVCAR-3 tumor engrafted mice were humanized with donor PBMC (3 donors) and dosed i.v. QW x4 with ZW171 or i.p. daily x 18 with HPN TriTAC. Neg control (HAxCD3)

MSLN^{Med}-Expressing Colorectal Cancer Model

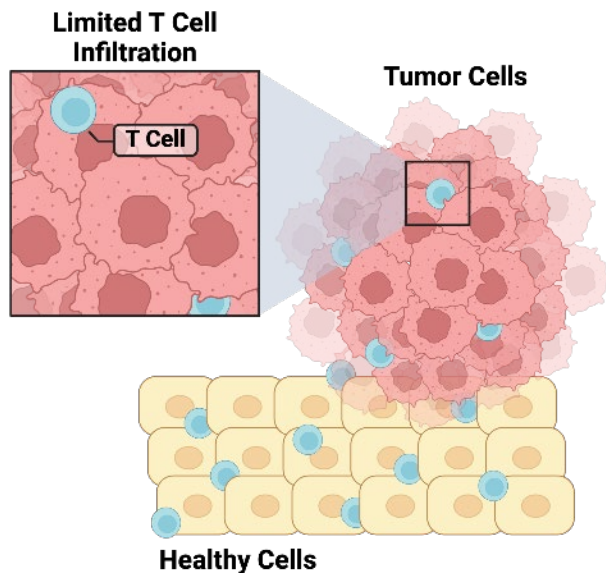


Mice were engrafted with HCT-116 cells and humanized with donor PBMC (3 donors). Mice were dosed i.v. QW x4 with ZW171 or i.p. daily x 18 with HPN TriTAC. Neg control (HAxCD3)

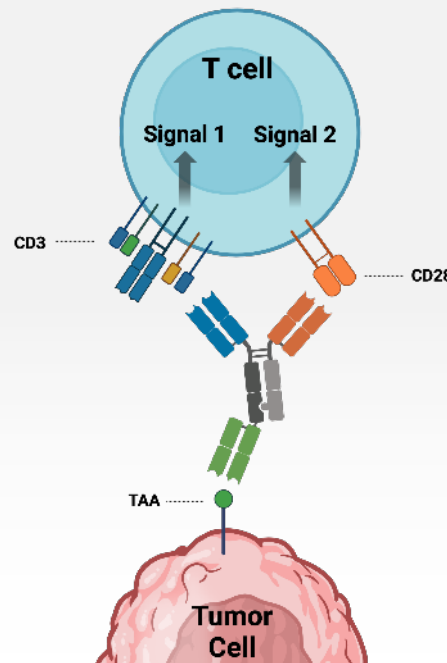
ZW171 IND cleared by the FDA; Expected to commence Phase 1 studies in the second half of 2024 (NCT06523803)

Zymeworks TriTCE Co-stim: Overcoming Lack of Efficacy and Durability of Responses in Solid Tumors by Optimized Co-delivery of Signal 1 and 2

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



Zymeworks Trispecific Co-stimulatory Program

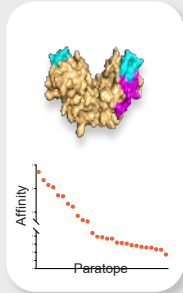


Provides Signal 1 (CD3) and Signal 2 (CD28) in one molecule to **increase T cell activation and proliferation**

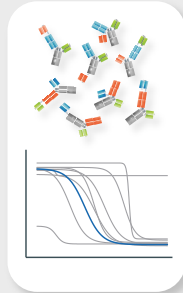
Engineered to balance signal 1 and 2 for optimized **TAA-dependent T cell activation** and expansion

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

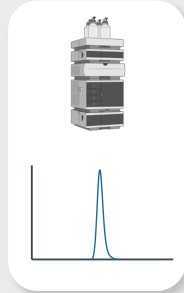
TriTCE Co-stim Platform and Workflow Established



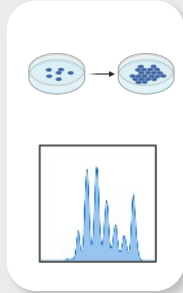
Paratope Engineering



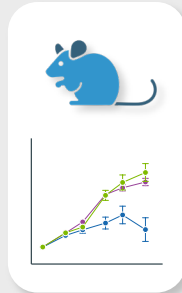
Format Selection & Screening



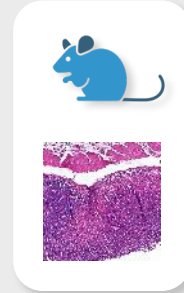
Biophysical Characterization



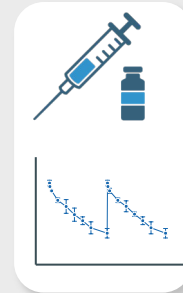
***in vitro* Activity & Differentiation**



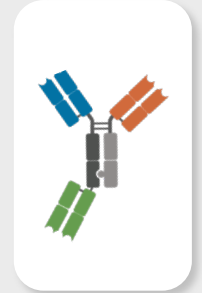
***in vivo* Activity & PK**



Mouse Tolerability



Non-GLP NHP Tox & PK



Lead Selection

Paratope Libraries Established

- α CD3 library (6 paratopes)
- α CD28 library (40 paratopes)

Formats Engineered and Screened

- Ten trispecific formats generated using the Azymetric™ and EFECT™ Platforms

Developability Confirmed

- High monomer purity
- High melting temperature
- No developability flags

Complex Primary Cell Assays Developed

- T cell Phenotyping
- BCL-xL (Survival)
- T cell Viability
- Cytokine Release Assay
- Serial Rechallenge
- T cell Bridging

Humanized Efficacy and Tolerability Mouse Models Developed

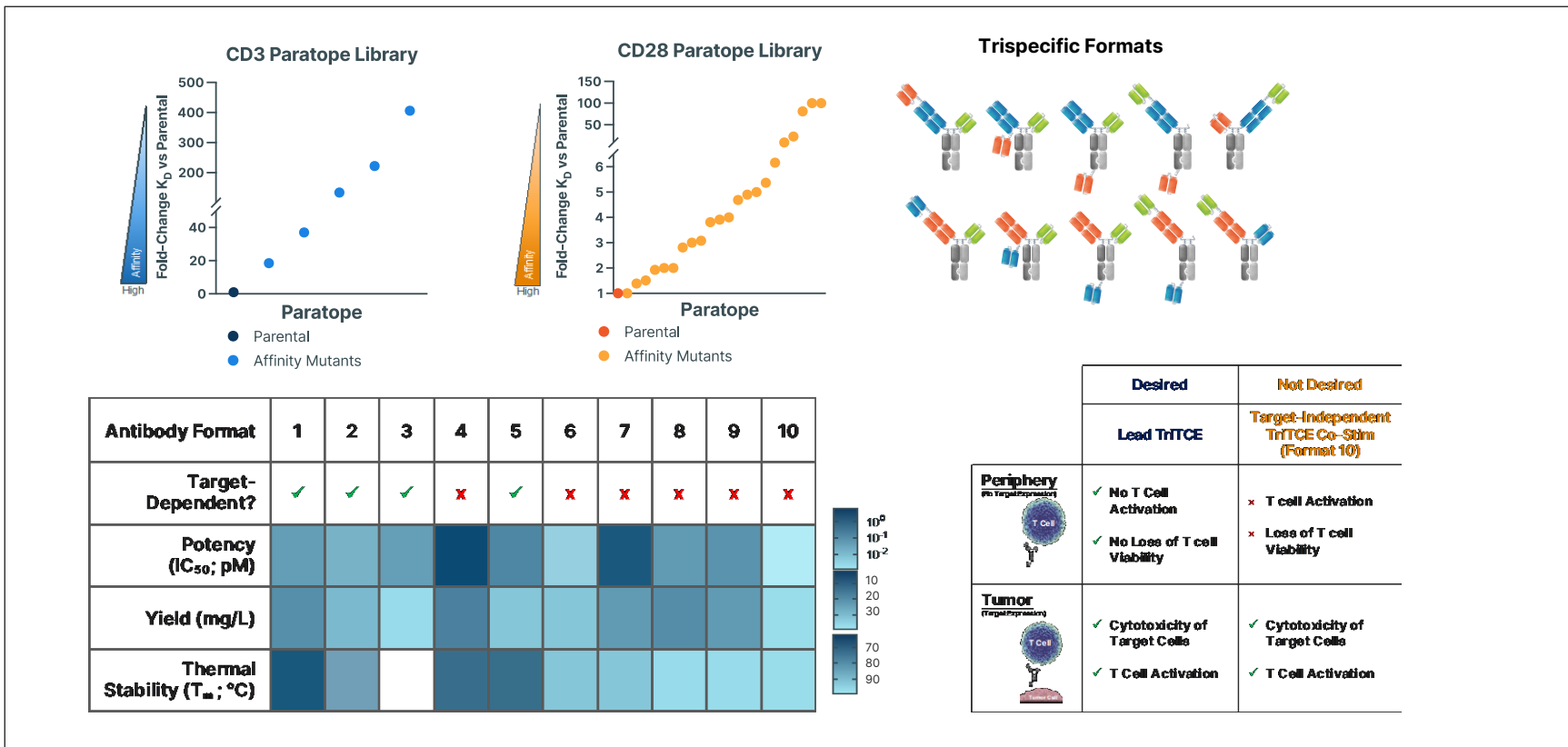
- PBMC engrafted CDX *in vivo* tumor models to interrogate the anti-tumor activity
- PBMC engrafted, non-tumor bearing *in vivo* model used to assess potential for Cytokine Release Syndrome

NHP Tolerability and PK

Repeat dose with cyno surrogate

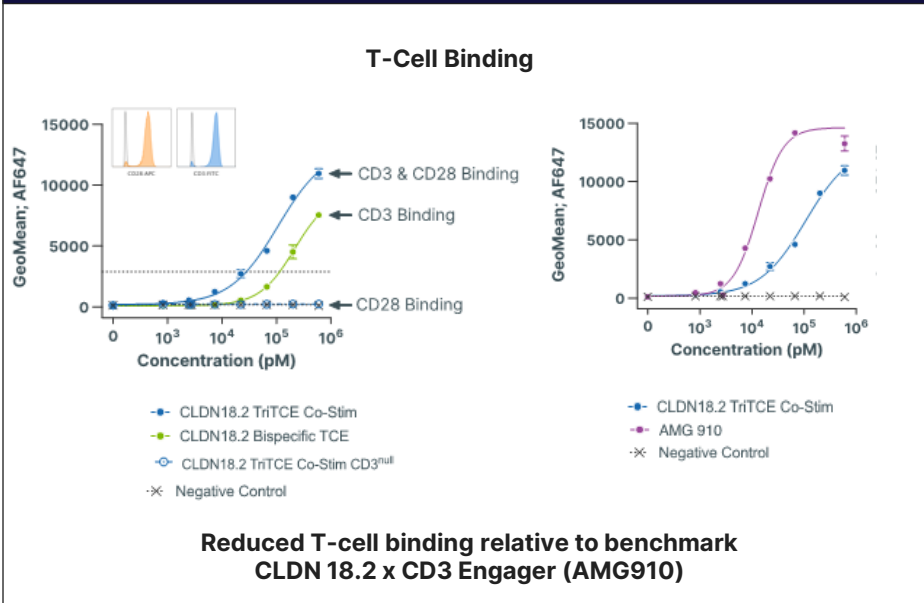
Lead TriTCE Co-stim format identified

Lead TriTCE Co-stim Selected Following Extensive Format Screening

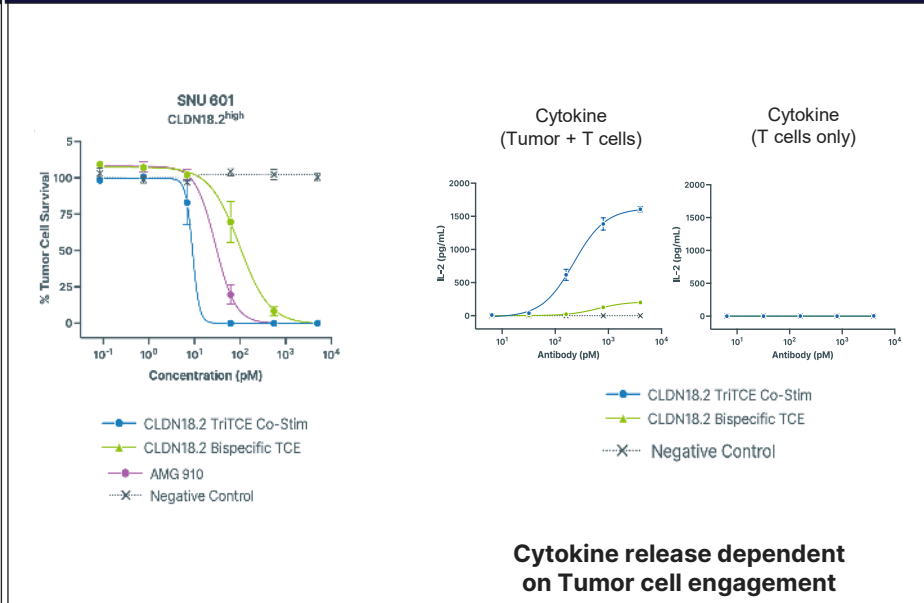


CLDN18.2 TriTCE Co-stim Exhibits Differential Activity Profile In Vitro

CD28 engagement conditional on CD3 binding



Enhanced Tumor Cell Killing Relative to Bispecific



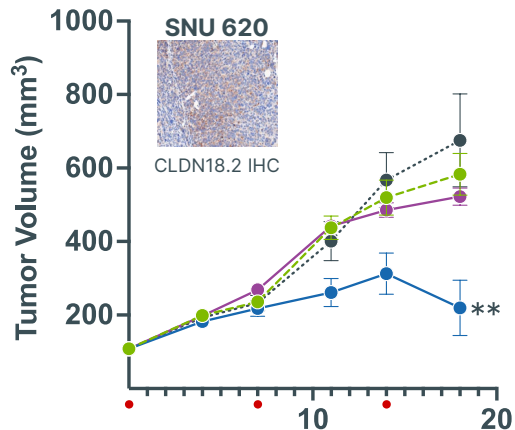
TAA: tumor-associated antigen; TCE: t cell engager

Newhook L et al., TriTCE Co-stim, next generation costimulatory trispecific T cell engagers for the treatment of solid tumors. Abstract #5121 presented at American Association for Cancer Research annual meeting 2023.

CLDN18.2 TriTCE Mediates Enhanced Anti-tumor Activity

Enhanced Anti-tumor Activity Associated with Increased T cells in Tumor but not in Blood

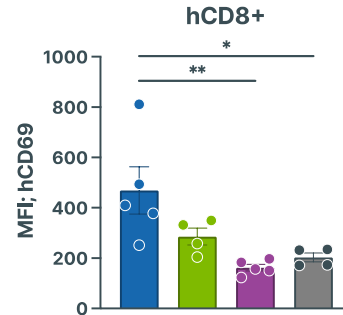
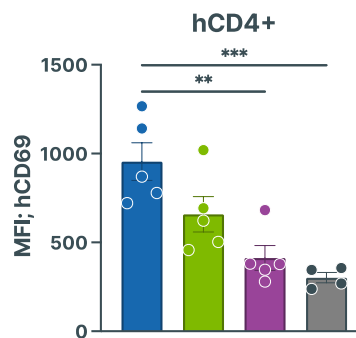
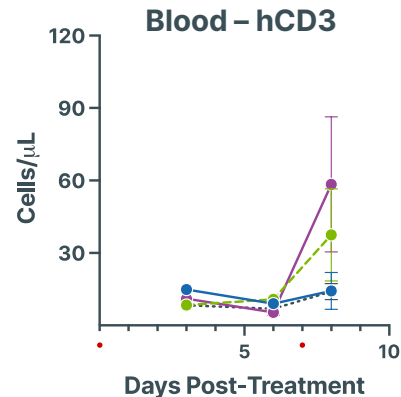
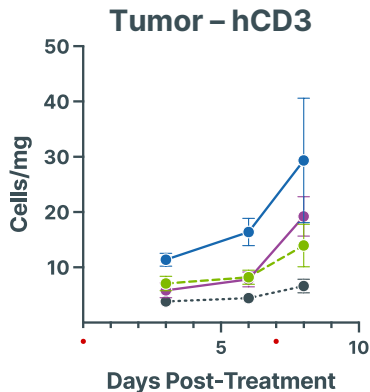
in vivo Anti-tumor Activity



Days Post-Treatment

- CLDN18.2 TriTCE Co-Stim
- CLDN18.2 Bispecific TCE
- AMG 910*
- Negative Control

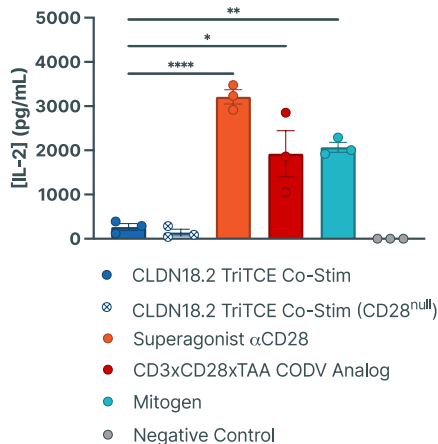
Test articles dosed at 0.01 mg/kg



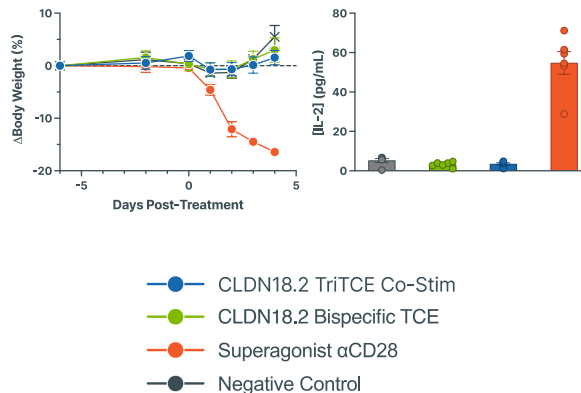
CLDN18.2 TriTCE Co-stim has a Favorable Safety Profile

No Cytokine Activation with PBMCs Alone

Solid-Phase Cytokine Release Assay

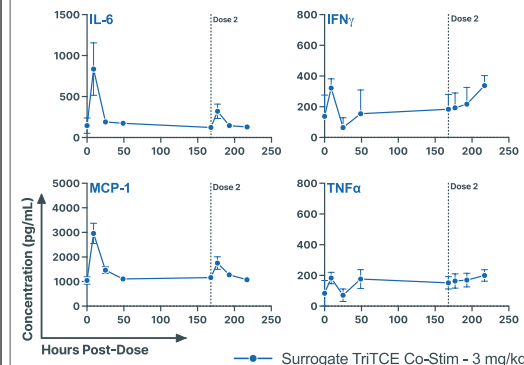


No Systemic Cytokine Release in Humanized Mouse Model



Well-tolerated in NHP

Transient, Minor Increase in Serum Cytokine Post-Dosing

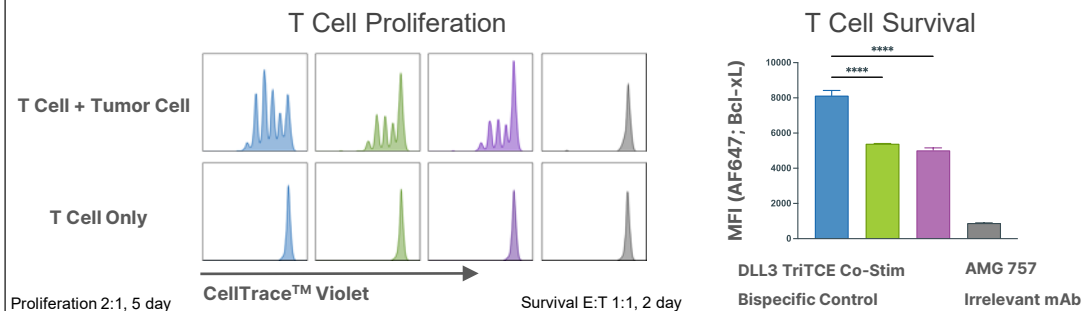


No histopathological changes observed in the stomach, where CLDN18.2 is expressed (Türeci et al., 2011)

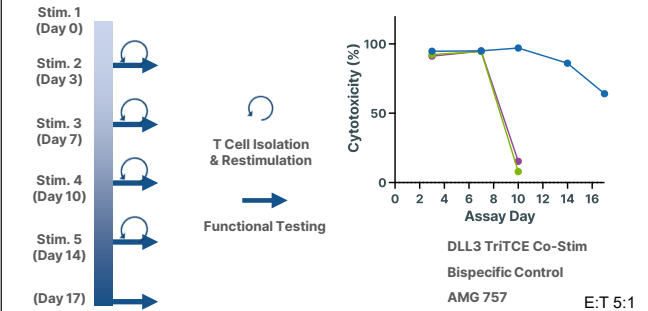
TriTCE Co-stim Applicable to Additional Targets

DLL3 TriTCE Co-stim: CD3 x CD28 x DLL3

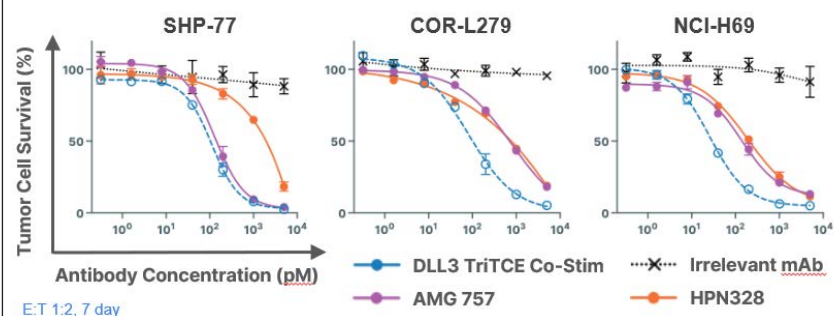
Improved T Cell Proliferation and Survival



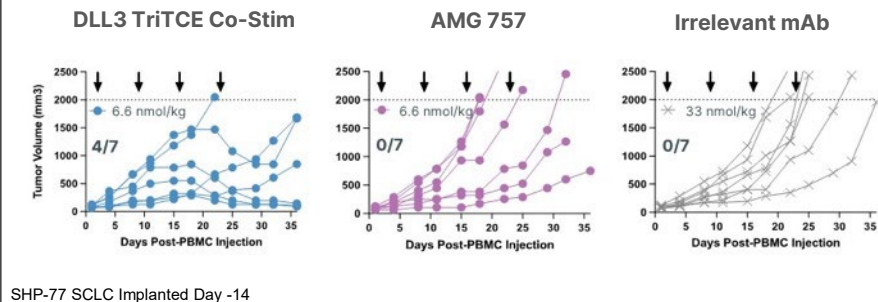
Sustained Cytotoxicity



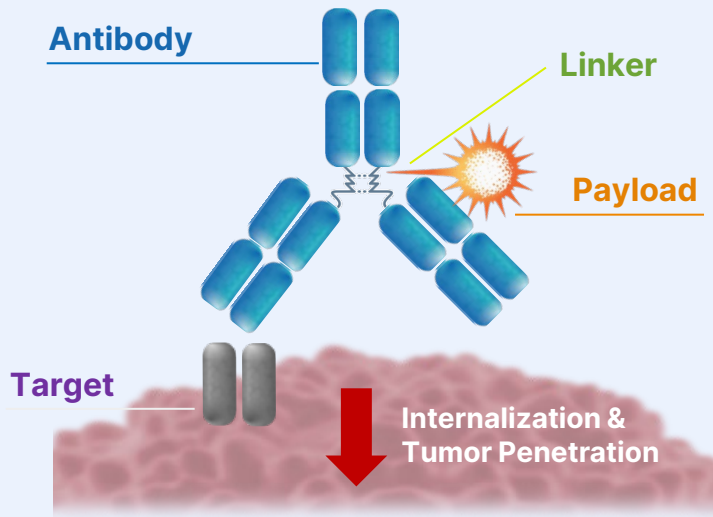
Increased Cytotoxicity vs. Benchmarks at Low E:T



Anti-tumor Activity in Established Tumor Xenograft Model



Core Competencies Utilized in Next Generation ADC Design



- Leveraging a combination of validated and novel targets. Validated targets provides opportunity for benchmarking in preclinical development and expected clinical differentiation; novel targets anticipated to increase over time and address additional patients
- Exploiting our **proprietary TOP01i payload (ZD06519)** while exploring alternate mechanisms of action for longer-term development
- Leveraging validated **peptide-cleavable linkers** and **stochastic conjugation**. New chemistries under development to complement novel payloads
- Optimizing **antibody properties** for the ADC mechanism, such as target-mediated binding and **enhanced internalization**. Biparatopic and bispecific ADC formats may also provide future differentiated therapeutics
- Utilize 3D cancer cell line spheroid models to select optimal ADC antibodies based on **tumor spheroid penetration and cytotoxicity**

Targets with Complementary Expression Profile Provide Opportunity to Broaden Responsive Patient Population through Combination Targeting

Inter-patient Target Expression

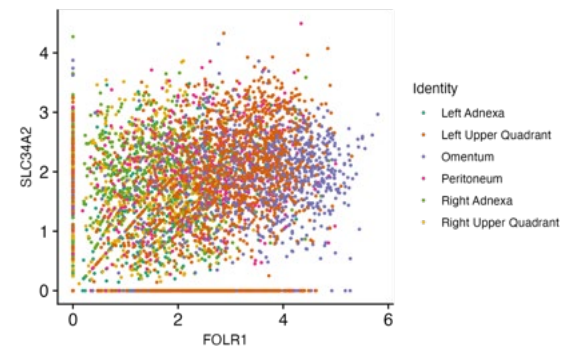


		FR α			
		IHC Score	0	1+	2+
NaPi2b	3+	0	5	8	20
	2+	6	6	12	13
	1+	0	11	7	12
	0	2	2	2	5

Example Pair: FR α and NaPi2b

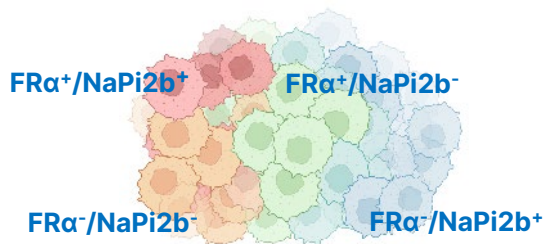
Intra-patient Target Expression Variability

Target expression within individual patient can vary dependent on tumor location

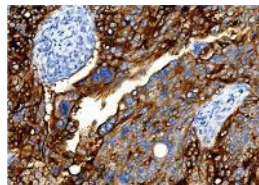


Single cell RNA analysis of HGSOC patient tumor samples (Vazquez-Garcia et al 2022 Nature 612: 778)

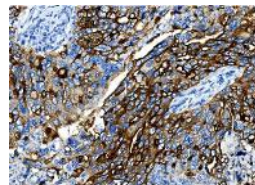
Intra-tumor Lesion Expression



FR α






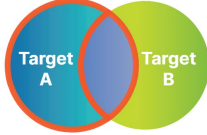

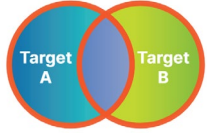
NaPi2b



IHC of FR α and NaPi2b from the same patient sample and same region

Bispecific Antibody-drug Conjugates: Modulation of Format Support

Various Opportunities to Enhance Therapeutic Window

<p>1+1 Bispecific Format</p>		 <p><i>Enhanced specificity Reduced patient population</i></p>
<p>2+1 Bispecific Format</p>		 <p><i>Enhanced ADC function Patient population is target1 + % overlap</i></p>
<p>2+2 Bispecific Format</p>		 <p><i>Enhanced ADC function Enhanced patient population</i></p>

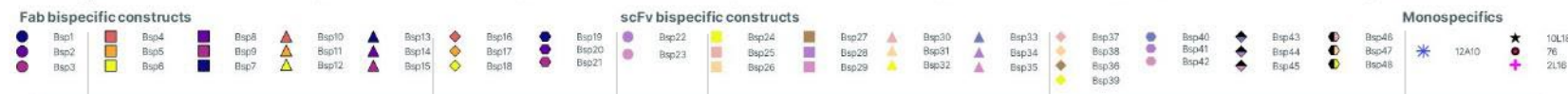
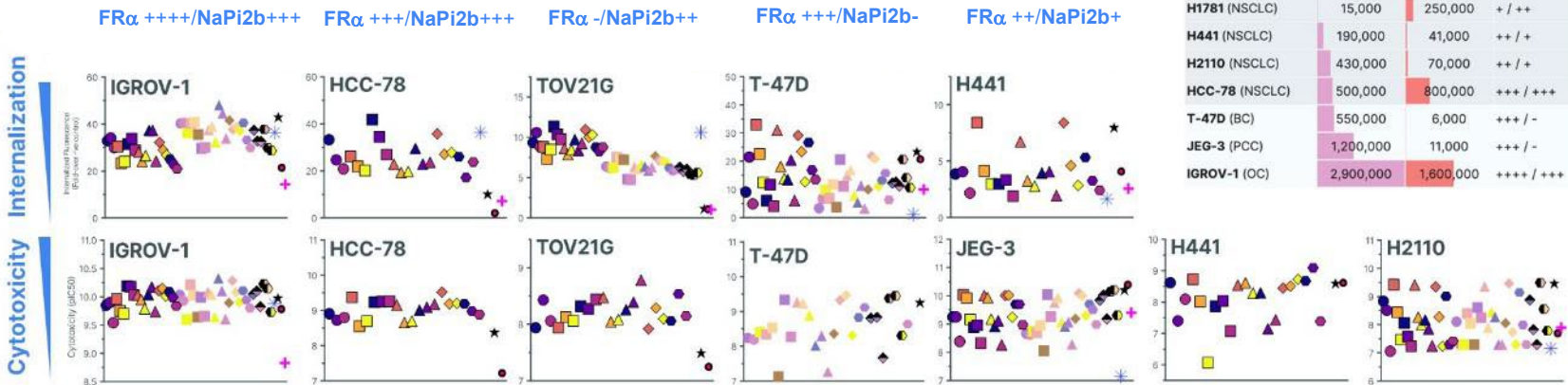
To identify optimal Bispecific format(s) to broaden tumor coverage performed proof of concept study with FR α and NaPi2b

- 48 bispecific ADCs produced: 3 different valencies (1+1, 2+1, 2+2); 11 different formats (geometry and Fab/scFv components); several paratopes; 'model' payload (ZymeLink™ Auristatin)
- Evaluated for binding, internalization, and cytotoxicity (in cell lines representative of different relative expression scenarios)

Biological Screening of FR α x NaPi2b Bispecific ADC Library

In vitro Screening Across Panel of Cancer Cell Lines with Range in FR α /NaPi2b Expression

Cell line	FR α /cell	NaPi2b/cell	FR α /NaPi2b
EBC-1 (NSCLC)	0	0	- / -
TOV21G (OC)	6,000	350,000	- / ++
H1781 (NSCLC)	15,000	250,000	+ / ++
H441 (NSCLC)	190,000	41,000	++ / +
H2110 (NSCLC)	430,000	70,000	++ / +
HCC-78 (NSCLC)	500,000	800,000	+++ / +++
T-47D (BC)	550,000	6,000	+++ / -
JEG-3 (PCC)	1,200,000	11,000	+++ / -
IGROV-1 (OC)	2,900,000	1,600,000	+++ / +++



10L18 is superior FR α paratope
 12A10 NAPI2b paratope most active as bivalent

2+2 and 2+1 formats > 1+1 bispecific formats across broader range of cell lines

2+2 N-term Fab bispecific format > 2+2 N+C Fab Format

Similar functional trends observed across Fab-only and scFv-containing bispecifics

→ Lead Bispecific ADCs selected for in vivo modeling and PK analyses

Leveraging Azymetric™ to Optimally Format T Cell Engagers and Bispecific ADCs

Presentation Summary

Azymetric provides a robust Fc heterodimer solution enabling high throughput functional screening of multispecific antibody panels to select those optimally formatted for a therapeutic application.

The most advanced Azymetric molecule, zanidatamab, a bi-paratopic anti-HER2 antibody undergoing pivotal clinical studies, exemplifies the opportunity afforded to enhance antitumor responses beyond that achieved through combination of antibody components. In this presentation, data illustrating the utility of Azymetric for two additional applications will be shared:

Development of multi-functional T cell engagers including incorporation of conditional co-stimulation

Format screening of bispecific antibodies to support the delivery of small molecule payloads simultaneously to two independently expressed cancer targets.

Acknowledgements - A Global Team Effort

ZW171, a T Cell-Engaging, Bispecific Antibody for the Treatment of Mesothelin-Expressing Solid Tumors

Nicole Afacan , Chayne Piscitelli, Patricia Zwierzchowski , Siran Cao, Janessa Li , Wingkie Wong, Kara White-Moyes, **Thomas Spreter von Kreudenstein**, **Nina E. Weisser**

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

Screening novel format antibodies to design bispecific ADCs that address target heterogeneity

Stuart D. Barnscher, Dunja Urosev, Kevin Yin, Andrea Hernandez Rojas, Sam Lawn, Vincent Fung, Jodi Wong, Araba Sagoe-Wagner, Lemlem Degefie, Ali Livernois, Catrina Kim, Paul A. Moore, **Jamie R. Rich**



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