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

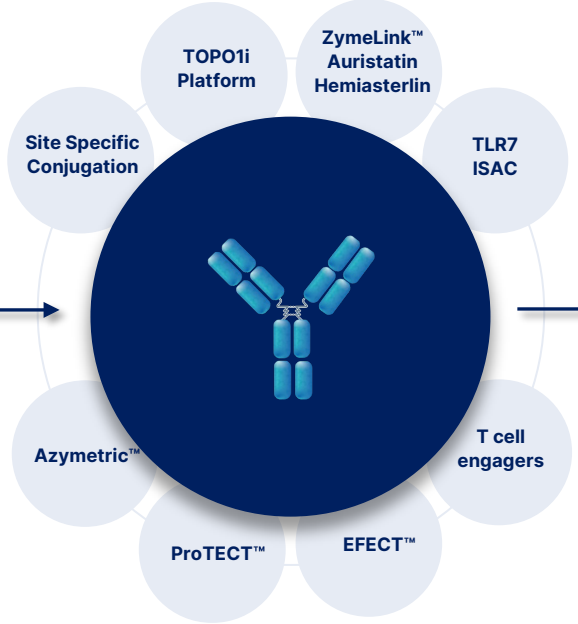
World Bispecific Summit 2024
Wednesday, September 4th

**Paul Moore, PhD**
Chief Scientific Officer

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

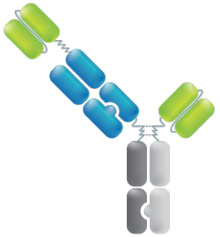
## Select Difficult-to-Treat Cancers & Target


## 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

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

# 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:

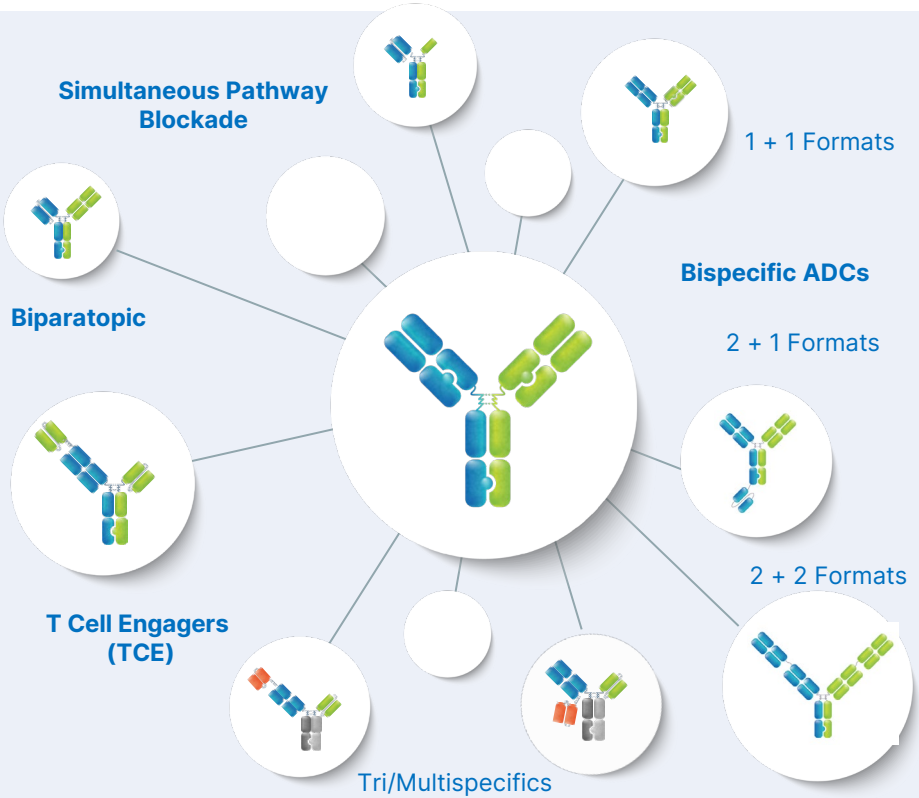
- With existing antibody paratopes; human (IgG1, IgG2A, IgG4) and mouse frameworks; other CH2 and glyco-engineering approaches

## High-throughput screening:

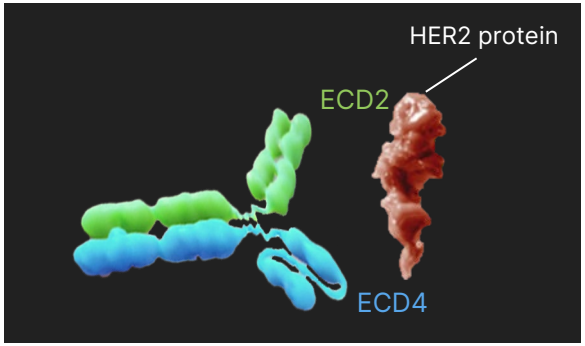
- Best-in-class activity requires screening of alternative targets, epitopes, sequences, target engagement geometries, and mechanisms of action (blocking, lytic, ADC)

## Highly manufacturable

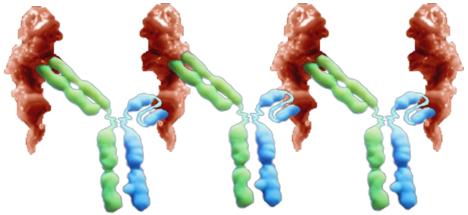
- Antibody like yields/stability; leveraged by multiple pharma/biotech with various clinical stage programs in development



# Zanidatamab: A Bispecific Antibody for HER2-Expressing Cancers

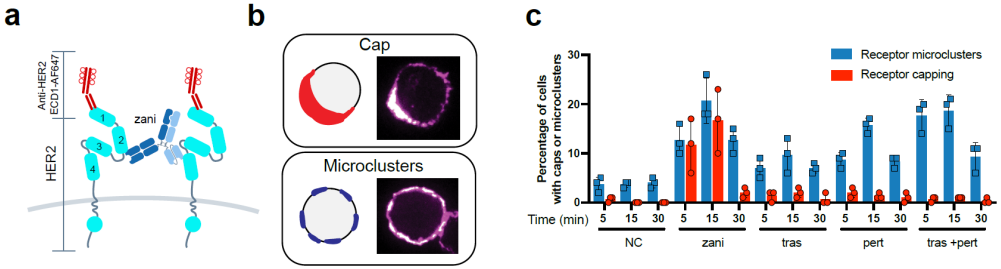



**Zanidatamab binds two distinct epitopes of HER2**



**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*

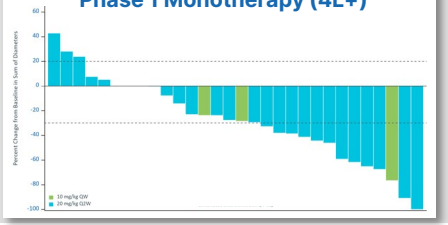
# Zanidatamab Exhibits Robust Clinical Activity across Multiple HER2+ Cancer Types

zymeworks

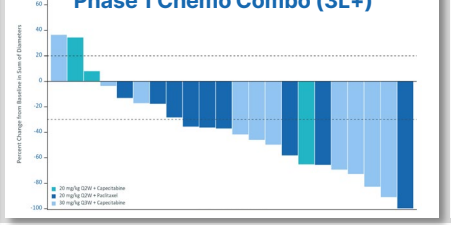
BeiGene Jazz Pharmaceuticals

## Gastroesophageal

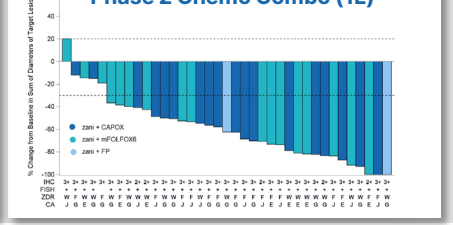
### Phase 1 Monotherapy (4L+)



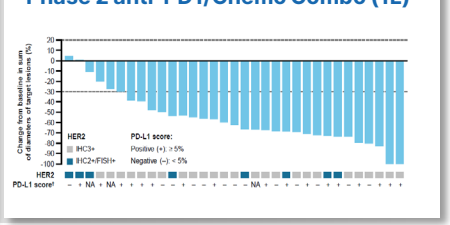
### Phase 1 Chemo Combo (3L+)



### Phase 2 Chemo Combo (1L)



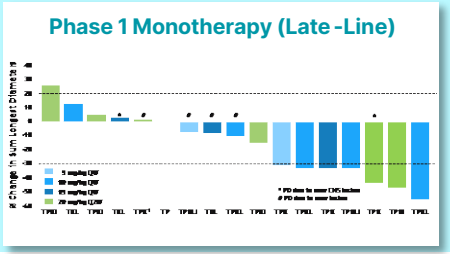

### Phase 2 anti-PD1/Chemo Combo (1L)



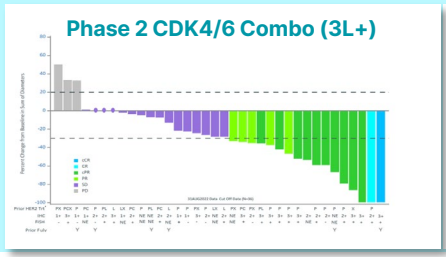

HERIZON-GEA-01: Randomized Phase 3 Study Ongoing

## Breast Cancer

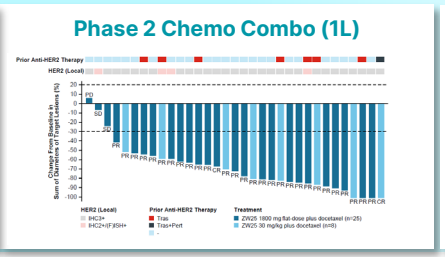
### Phase 1 Monotherapy (Late -Line)



### Phase 2 CDK4/6 Combo (3L+)



### Phase 2 Chemo Combo (1L)

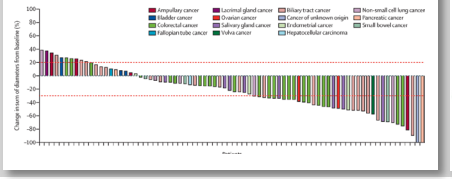



### Phase 3: EMPOWHER Breast Cancer Patients Progressed on T-Dxd

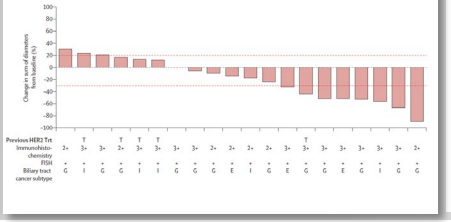
**Initiated**

## BTC & Others

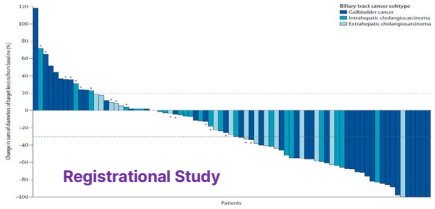
### Other Cancers: Phase 1 Monotherapy (Late-Line)



### BTC: Phase 1 Monotherapy (2L+)



### BTC: Phase 2b Monotherapy (2L)



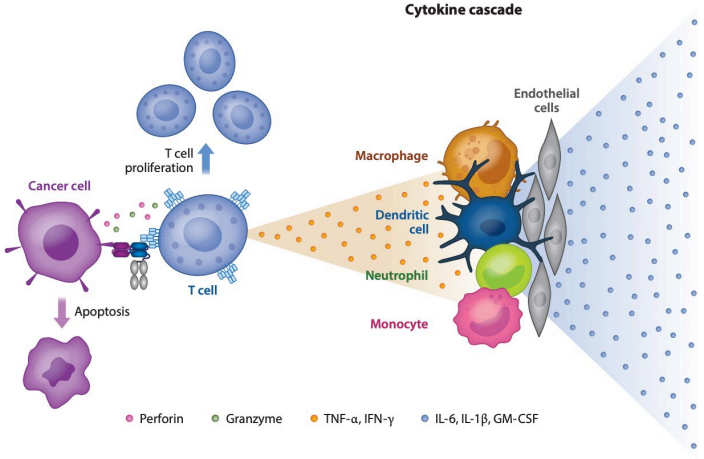

cORR: 41.3%
mDOR: 14.9mo
OS: 15.5mo IHC3+: 18.1mo

BLA Application accepted
Priority Review

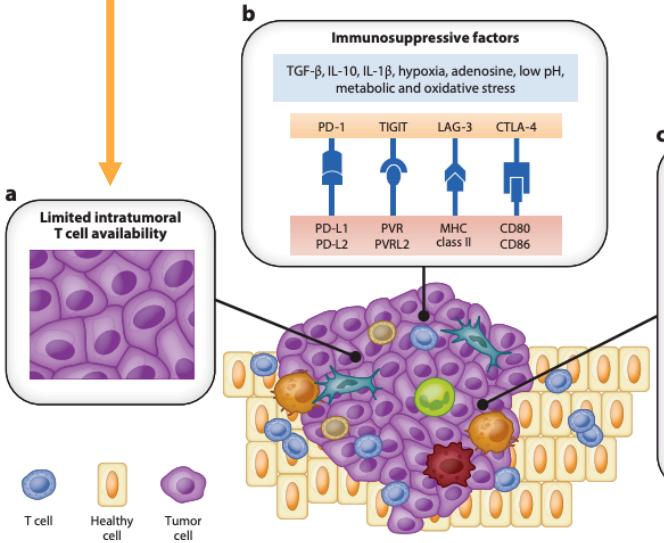
Phase 3 (1L) Trial Initiated

# 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

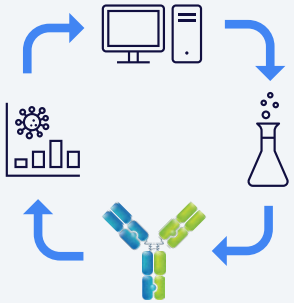



**Key Problem 3:**
Immunosuppressive tumor microenvironment

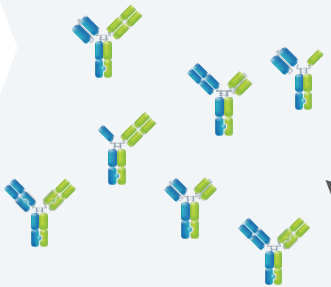
Arvedson T et al Ann Rev Cancer Biol 2022

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

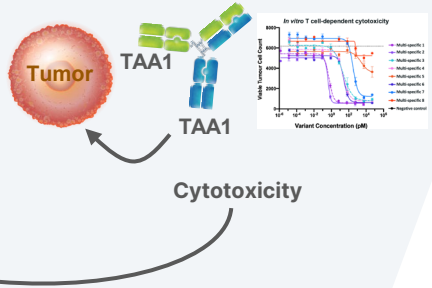
Paratope screening & optimization, *in silico* affinity engineering



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



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




**Single lead optimized to:**

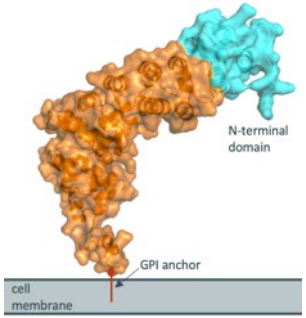
- Target TAA over-expressing cells
- Improve T cell responses
- Maximize therapeutic index
- Modulate cytokine release

- 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 Several Cancers and is an Attractive TCE Target

## MSLN Plays a Role Tumor Development

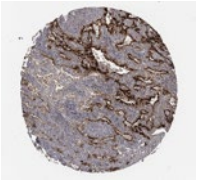



- Glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein[1]
- Binds MUC16 and plays a role in cell adhesion, tumor progression, metastasis, CAF biology and chemo-resistance [1,2]
- MSLN is expressed at low levels in the mesothelium, as well as tissues such as the fallopian tubes and tonsils[3,4]
- MSLN has a slow turnover rate making it suitable for TCE targeting[5]
- 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 tumors[7]

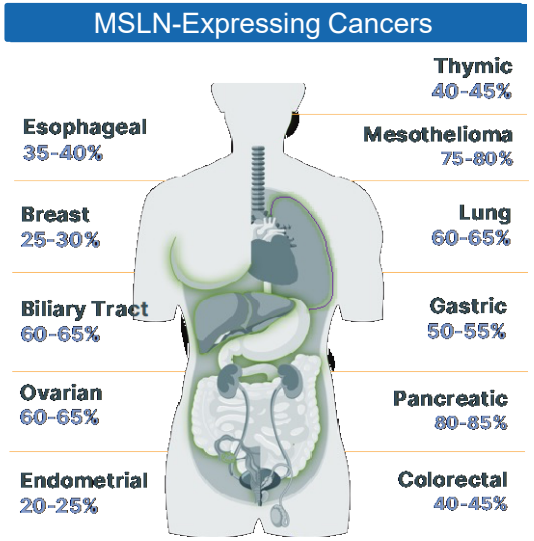
## MSLN is Expressed in Various Human Cancers

MSLN IHC

Ovarian cancer[4]



Lung cancer[6]

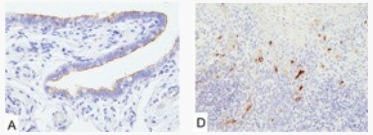
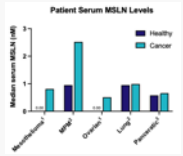
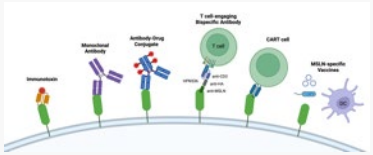
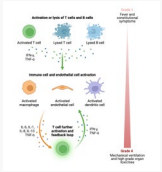



Adapted from Morello et al Cancer Discov 2016; 6: 133–146 and Inaguma et al Oncotarget. 2017; 8 :26744-26754

- Moderate to high membranous expression is frequent in ovarian cancer, NSCLC, mesothelioma and other cancers[4]

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 | | ZW171 Design Solution |
|---|---|---|
| 1 | Normal tissue expression could lead to off tumor on target toxicity[1] |  | Avidity dependent MSLN binding of two αMLSN paratopes to enable **selective cytotoxicity to tumor cells versus normal tissues** and **reduce impact of soluble MLSN** on potency |
| 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 |  | **Optimized 2 +1 format and geometry** (two αMSLN scFv paratopes, one αCD3 Fab paratope) with **enhanced MSLN-dependent anti-tumor activity** |
| 4 | Cytokine release syndrome elicited by T cell targeting therapies limits therapeutic[6] |  | **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

**Azymetric™ and EFECT™ KO Fc**

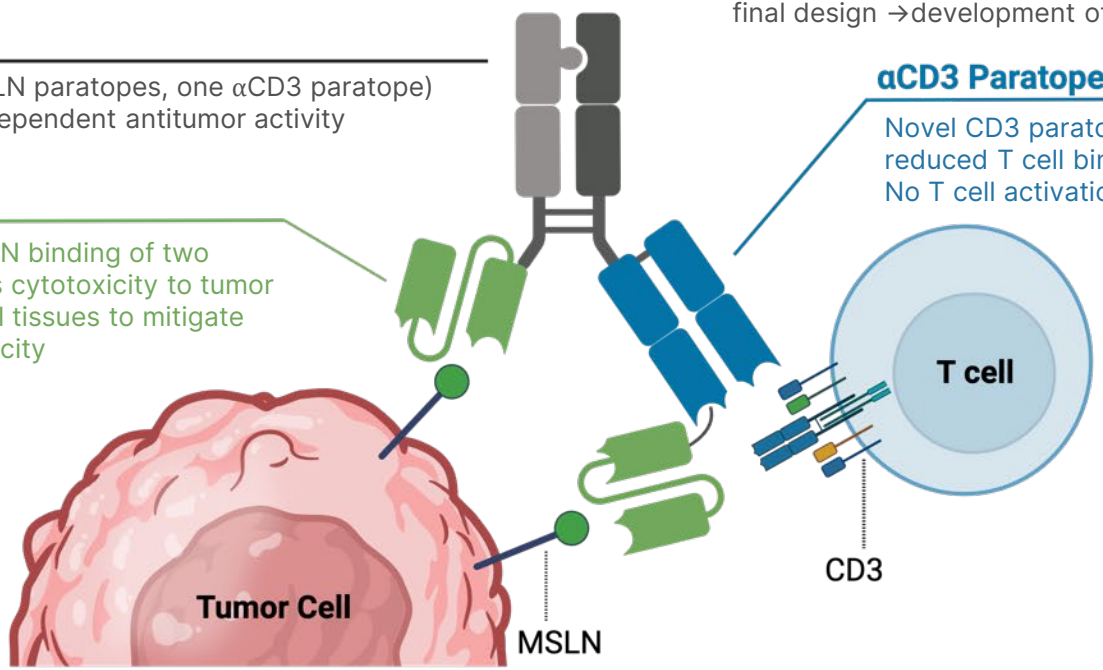
Variety of formats, geometries and paratope affinities were generated and screened prior to lead candidate final design →development of ZW171.

**Antibody Format**

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

**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

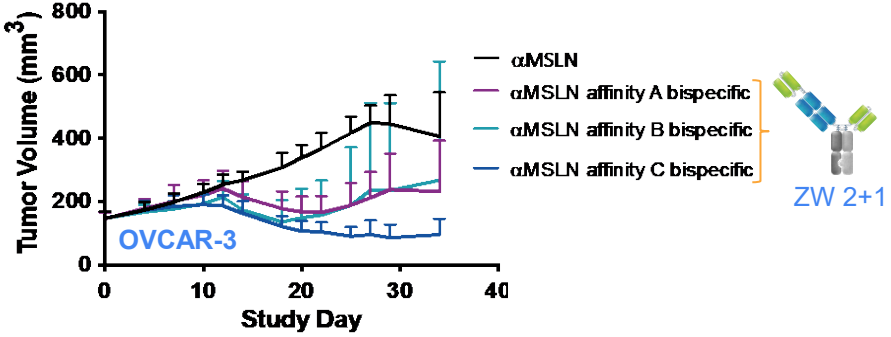



**αCD3 Paratope**

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

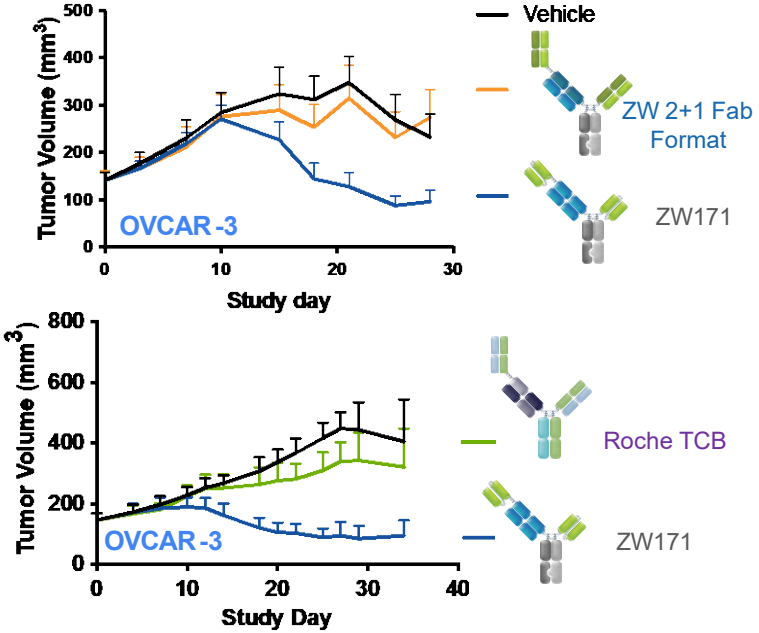
# Geometry Format of ZW171 Critical for Activity in Tumor Models

## Anti-MSLN Paratope Affinity is Critical



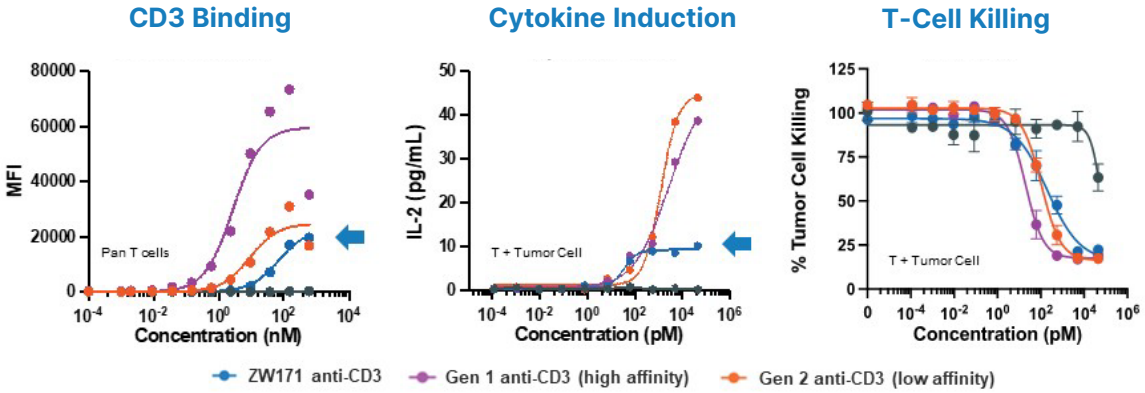

In vivo anti-tumor activity evaluated with established tumor models that have reduced sensitivity compared to co-implantation (tumor + PBMC) models


## 2 + 1 Geometry is Critical



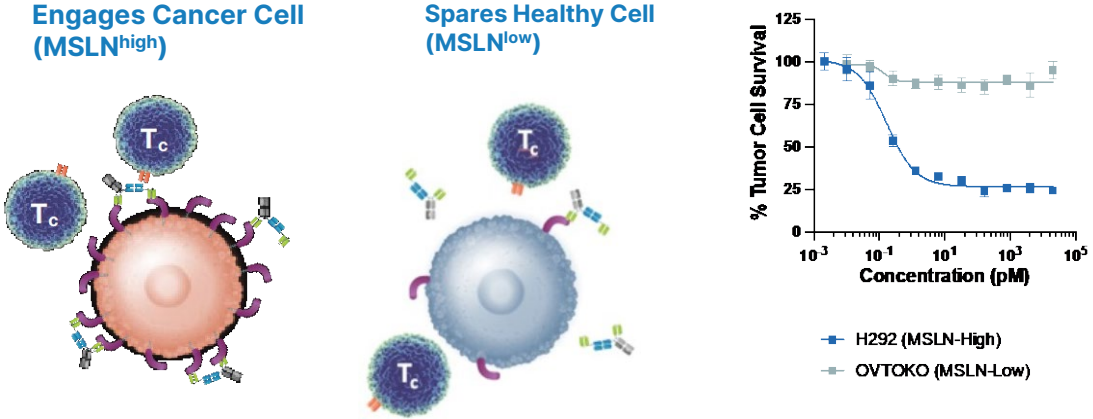

OVCAR-3 tumor fragments were engrafted subcutaneously in NOG mice. After tumors reached 100-200 mm3, mice were humanized with donor PBMC (3 donors) then treated 2QW x4 with test article. HuPBMC = human peripheral blood mononuclear cells

# ZW171 Designed for Safety both in T Cell and Tumor Cell Engagement




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

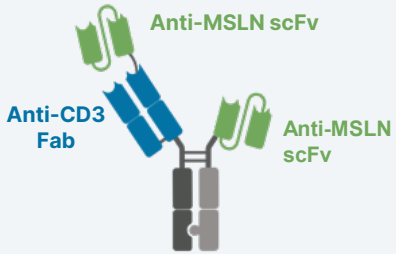
Reduced cytokine induction but maintains full cytotoxicity potential

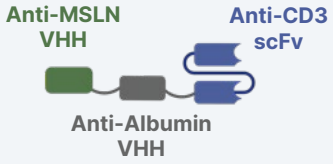



Designed to preferentially bind and target tumor cell lysis versus engagement of normal tissues through avidity dependent MSLN binding

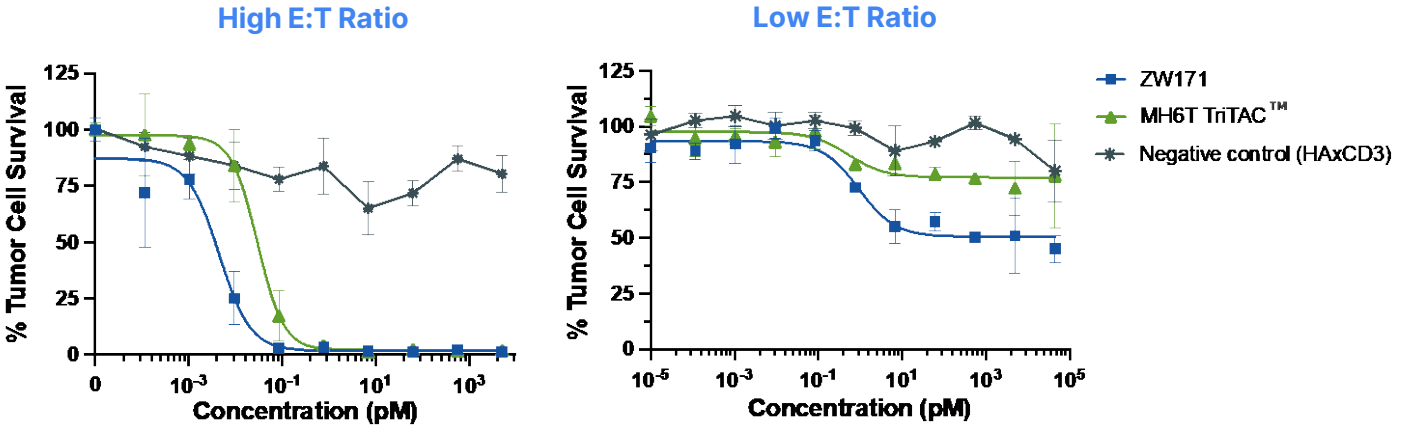
**ZW171 well tolerated in repeat dose NHP study (up to 30 mg/kg)**

# ZW171 Mediates Greater Cytotoxicity against MSLN-Expressing Tumor Cells



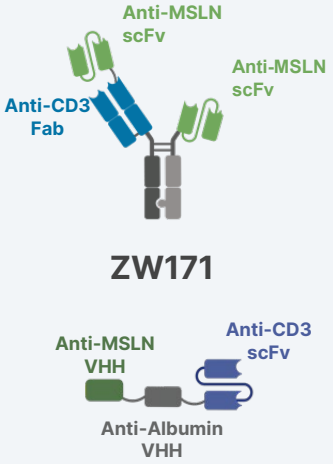




The activity of ZW171 was benchmarked against Harpoon's MSLN targeting MH6T TriTAC™



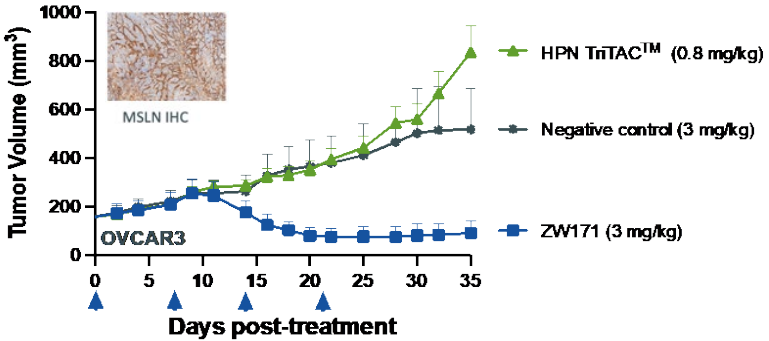

Human PBMCs and OVCAR3 tumor cells were co-cultured at effector-to-target cell ratio of 5:1 (high E:T) or 1:10 (low E:Y) in the presence of ZW171, MH6T TriTAC™ or negative control for 72 hours.
Afacan N, et al. Presented at: AACR. 2023 (abstr #2942)

# ZW171 Mediates Enhanced Anti-tumor Activity in PBMC-Engrafted Xenograft Models



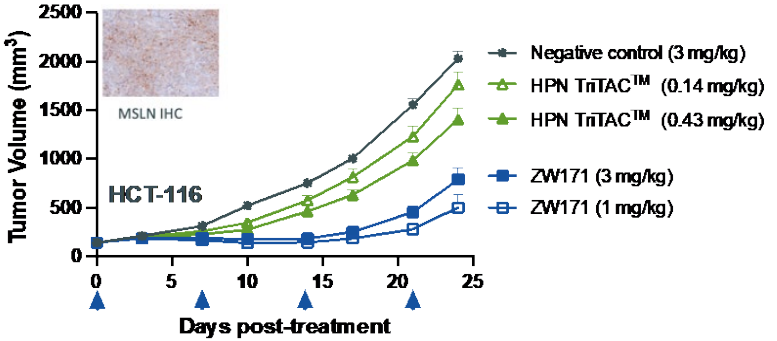

The activity of ZW171 was benchmarked against Harpoon's MSLN targeting MH6T TriTAC™

## 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



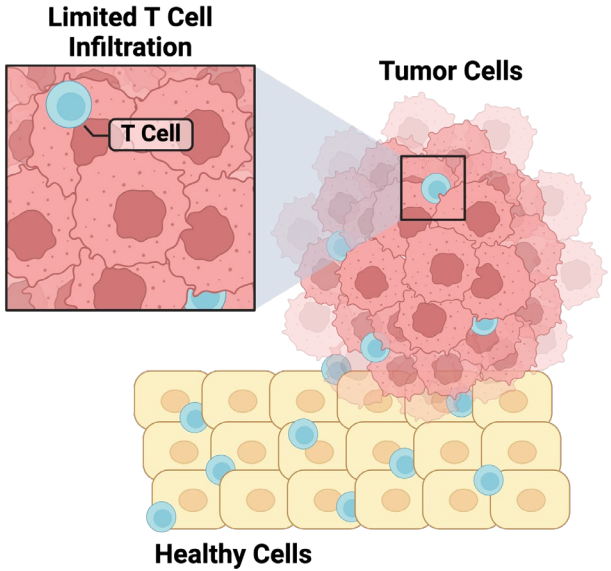

Mice were engrafted with HCT-116 cells and humanized with donor PBMC (3 donors). Mice were dosed i.v. QW x4 with ZW171 or i.p. daily x 18 with HPN TriTAC. Neg control (HAxCD3)

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

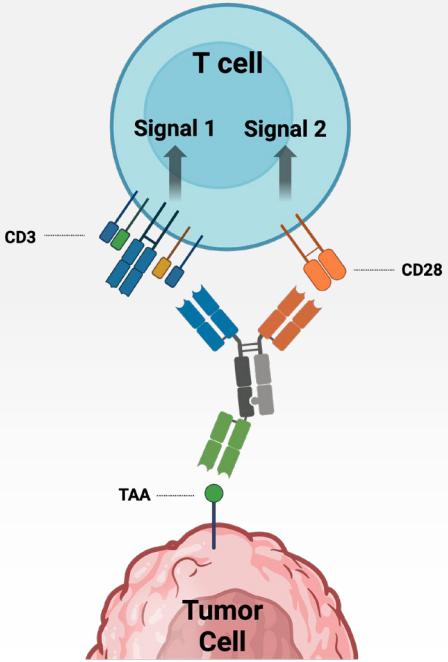
Afacan N, et al. Presented at: AACR. 2023 (abstr #2942)

# 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 anergy remain challenges in the treatment of solid tumors**




## Zymeworks Trispecific Co-stimulatory Program



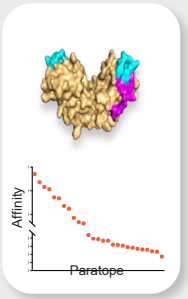

Provides Signal 1 (CD3) and Signal 2 (CD28) in one molecule to **increase T cell activation and proliferation**

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

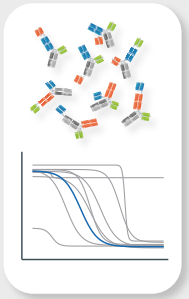
TriTCE Co-stim have the potential to provide **more durable responses** and activateT cell responses in 'cold' tumors with lower T cell infiltration

Arvedson T et al Ann Rev Cancer Biol 2022

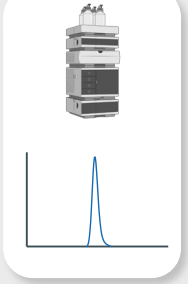
# TriTCE Co-stim Platform and Workflow Established



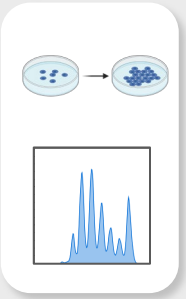

**Paratope Engineering**



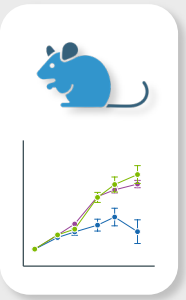
**Format Selection & Screening**



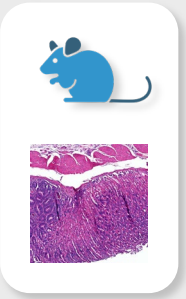
**Biophysical Characterization**



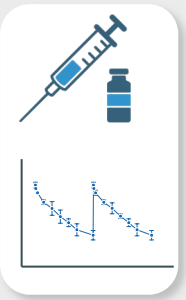
***in vitro* Activity & Differentiation**



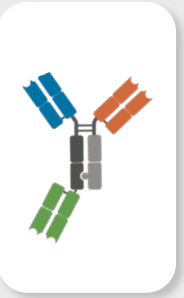
***in vivo* Activity & PK**



**Mouse Tolerability**



**Non-GLP NHP Tox & PK**



**Lead Selection**

**Paratope Libraries Established**

- α CD3 library (6 paratopes)
- αCD28 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**

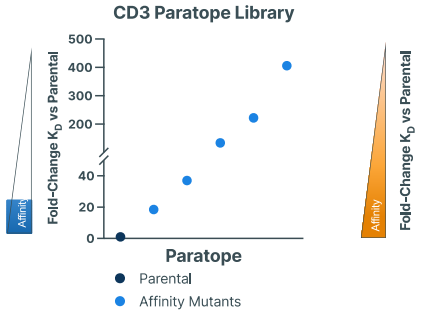
- PBMC engrafted CDX *in vivo* tumor models to interrogate the anti-tumor activity
- PBMC engrafted, non-tumor bearing *in vivo* model used to assess potential for Cytokine Release Syndrome

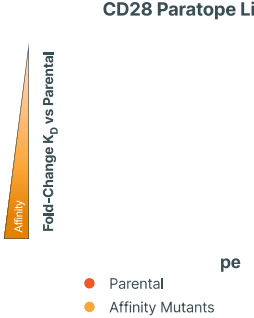
**NHP Tolerability and PK**

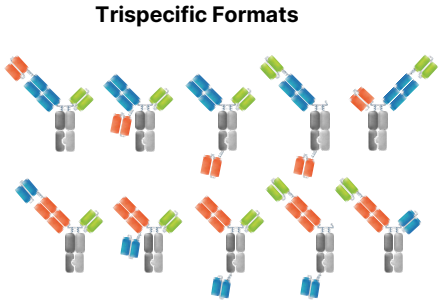
**Repeat dose with cyno surrogate**

**Lead TriTCE Co-stim format identified**

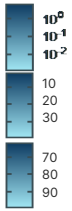
# Lead TriTCE Co-stim Selected Following Extensive Format Screening



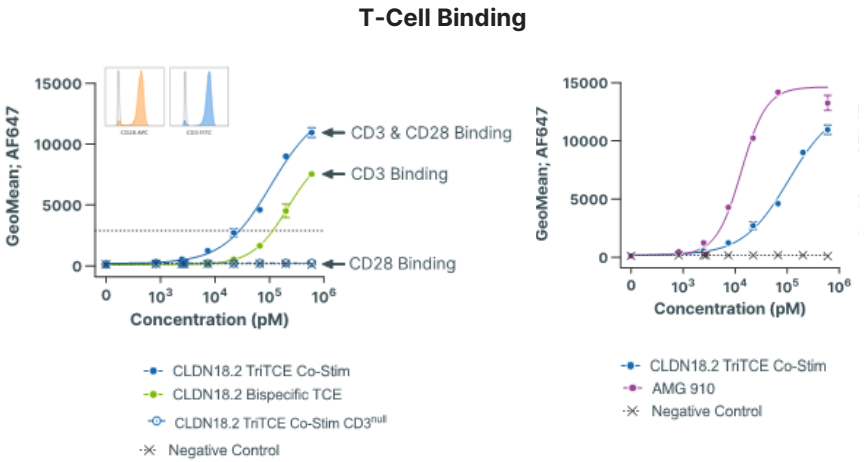

| Antibody Format | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Target-Dependent? | ✓ | ✓ | ✓ | ✗ | ✓ | ✗ | ✗ | ✗ | ✗ | ✗ |
| Potency ($IC_{50}$; pM) | | | | | | | | | | |
| Yield (mg/L) | | | | | | | | | | |
| Thermal Stability ($T_m$; °C) | | | | | | | | | | |




| | Desired | Not Desired |
|---|---|---|
| | Lead TriTCE | Target-Independent TriTCE Co-Stim (Format 10) |
| Periphery (No Target Expression) | ✓ No T Cell Activation<br>✓ No Loss of T cell Viability | × T cell Activation<br>× Loss of T cell Viability |
| Tumor (Target Expression) | ✓ Cytotoxicity of Target Cells<br>✓ T Cell Activation | ✓ Cytotoxicity of Target Cells<br>✓ T Cell Activation |

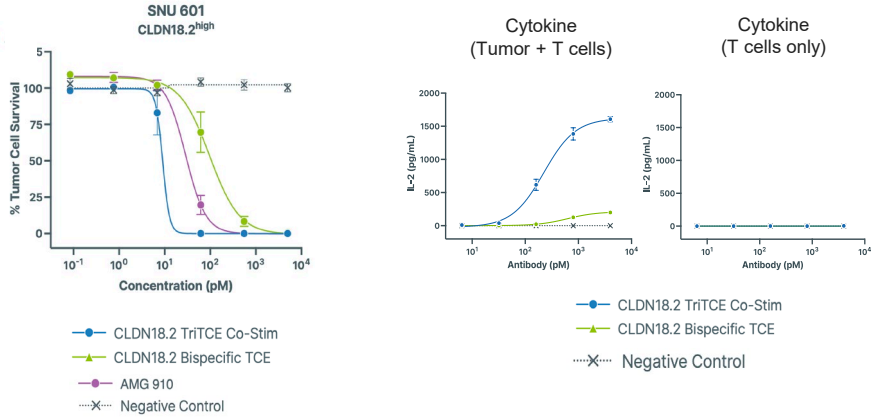
# CLDN18.2 TriTCE Co-stim Exhibits Differential Activity Profile in vitro

## CD28 engagement conditional on CD3 binding




**Reduced T-cell binding relative to benchmark CLDN 18.2 x CD3 Engager (AMG910)**

## Enhanced Tumor Cell Killing Relative to Bispecific



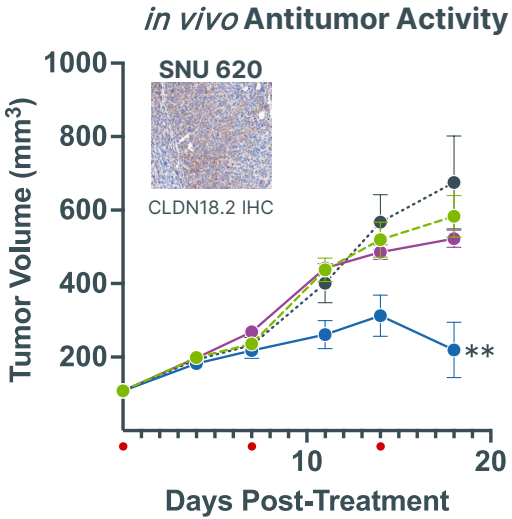

**Cytokine release dependent on Tumor cell engagement**

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

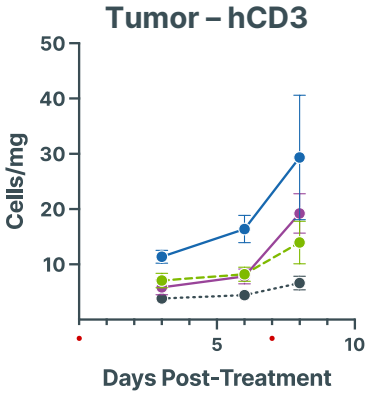
Newhook L et al., TriTCE Co-stim, next generation costimulatory trispecific T cell engagers for the treatment of solid tumors. Abstract #5121 presented at American Association for Cancer Research annual meeting 2023.

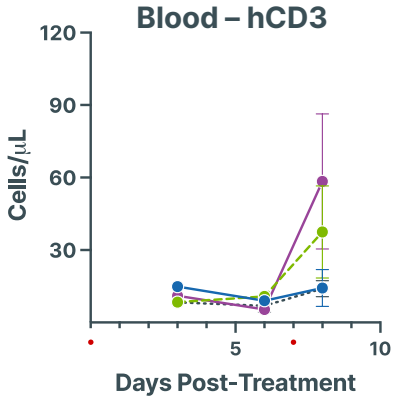
# CLDN18.2 TriTCE Mediates Enhanced Anti-tumor Activity

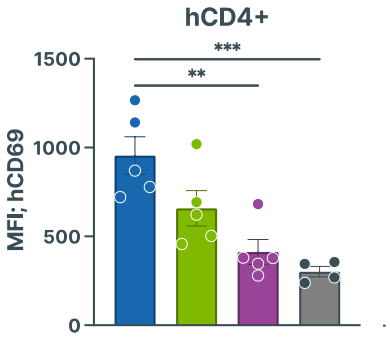
## Enhanced Anti-tumor Activity Associated with Increased T cells in Tumor But Not in Blood

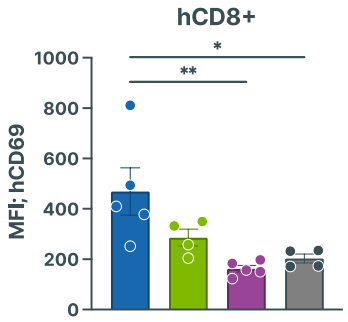



Test articles dosed at 0.01 mg/kg



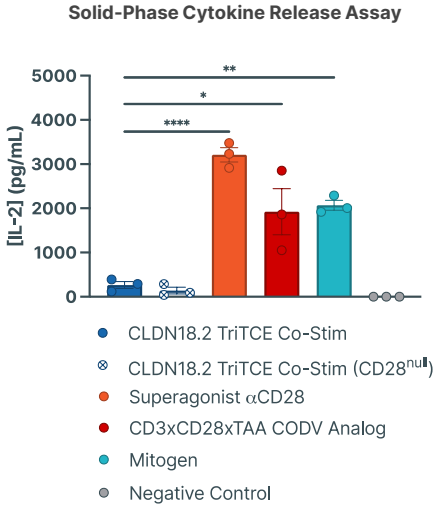



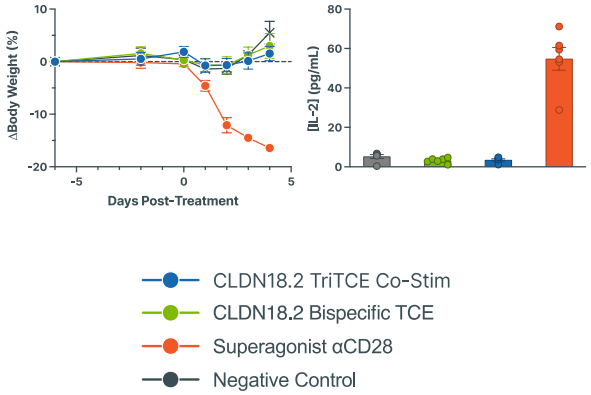



# CLDN18.2 TriTCE Co-stim has a Favorable Safety Profile

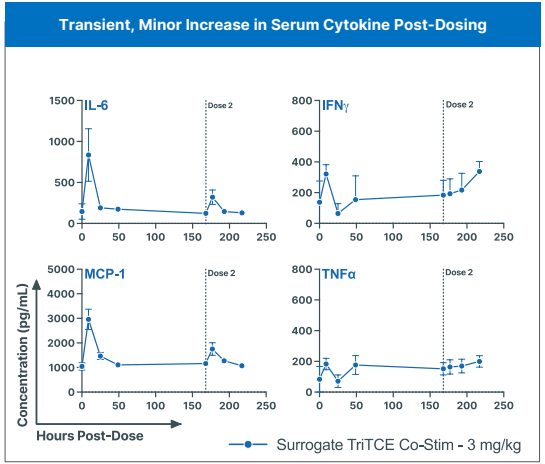
## No Cytokine Activation with PBMCs Alone




## No Systemic Cytokine Release in Humanized Mouse Model




## Well Tolerated in NHP

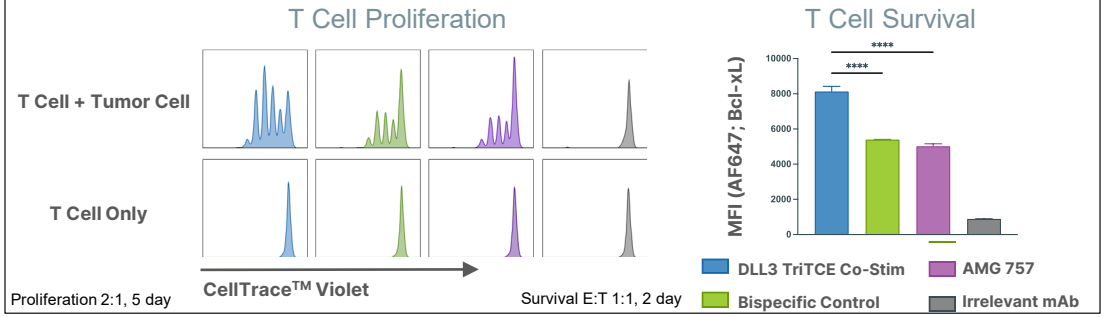



No histopathological changes observed in the stomach, where CLDN18.2 is expressed (Türeci et al., 2011)

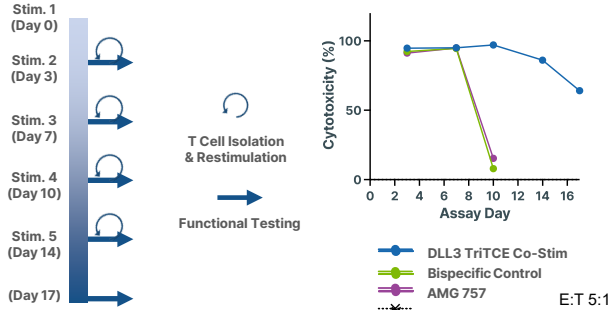
# TriTCE Co-stim Applicable to Additional Targets

DLL3 TriTCE Co-stim: CD3 x CD28 x DLL3

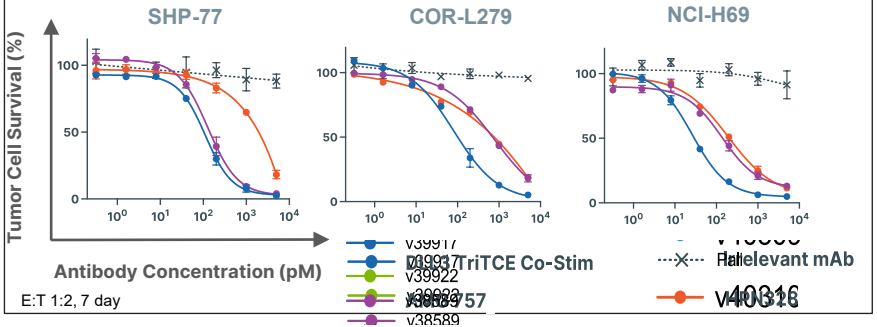
## Improved T Cell Proliferation and Survival



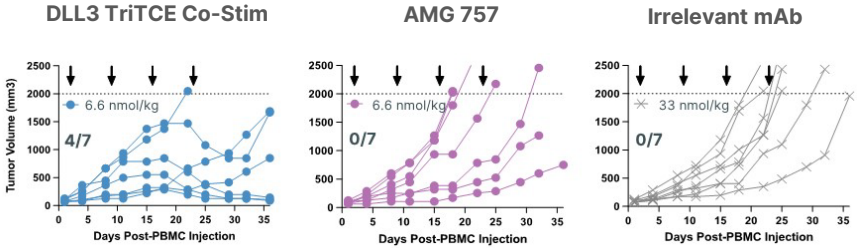

## Sustained Cytotoxicity




## Increased Cytotoxicity vs. Benchmarks at Low E:T

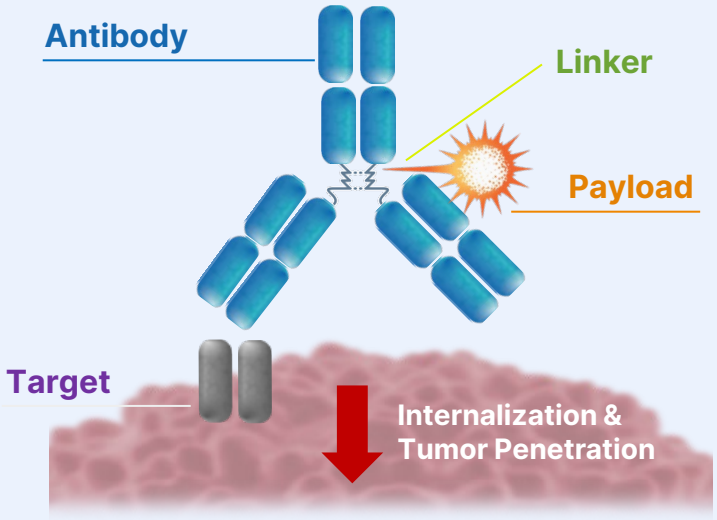



## Antitumor Activity in Established Tumor Xenograft Model




SHP-77 SCLC Implanted Day -14

# 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 TOPO1i 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**

1. Colombo R, Rich JR. Cancer Cell 2022 (40), 1255-1263; 2. Colombo R, Barnscher SD, Rich, JR. Cancer Res 2023, 83 (7). Abstract #1538 presented at AACR 2023.

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

zymeworks

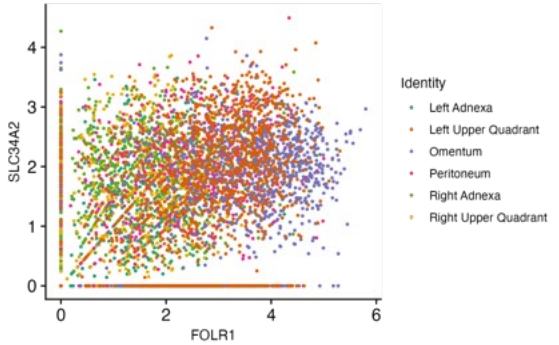
## Inter-Patient Target Expression


| NaPi2b IHC Score | FRα 0 | FRα 1+ | FRα 2+ | FRα 3+ |
|---|---|---|---|---|
| 3+ | 0 | 5 | 8 | 20 |
| 2+ | 0 | 6 | 12 | 12 |
| 1+ | 0 | 11 | 7 | 12 |
| 0 | 2 | 2 | 2 | 5 |

Example Pair: FRα and NaPi2b

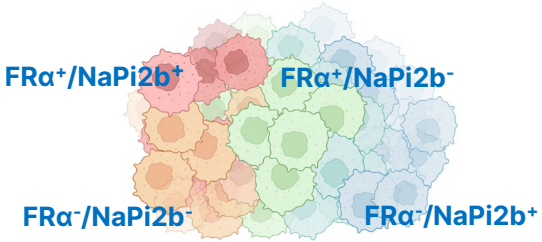
## Inter-Patient Target Variability

**Target expression within individual patient can vary dependent on tumor location**

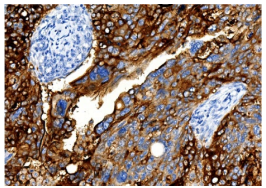



Single cell RNA analysis of HGSOC patient tumor samples (Vazquez-Garcia et al 2022 Nature 612: 778)

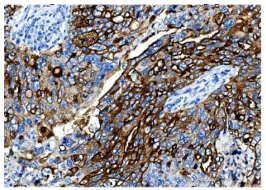
## Intra-Tumor Lesion Expression




FRα

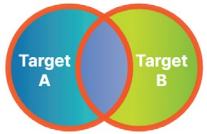


NaPi2b



IHC of FRα and NaPi2b from the same patient sample and same region

# Bispecific Antibody-Drug Conjugates: Modulation of Format Support Various Opportunities to Enhance Therapeutic Window

| | | | |
|---|---|---|---|
| **1+1 Bispecific Format** |  |  | *Enhanced specificity*<br>*Reduced patient population* |
| **2+1 Bispecific Format** |  |  | *Enhanced ADC function*<br>*Patient population is target1 + % overlap* |
| **2+2 Bispecific Format** |  |  | *Enhanced ADC function*<br>*Enhanced patient population* |

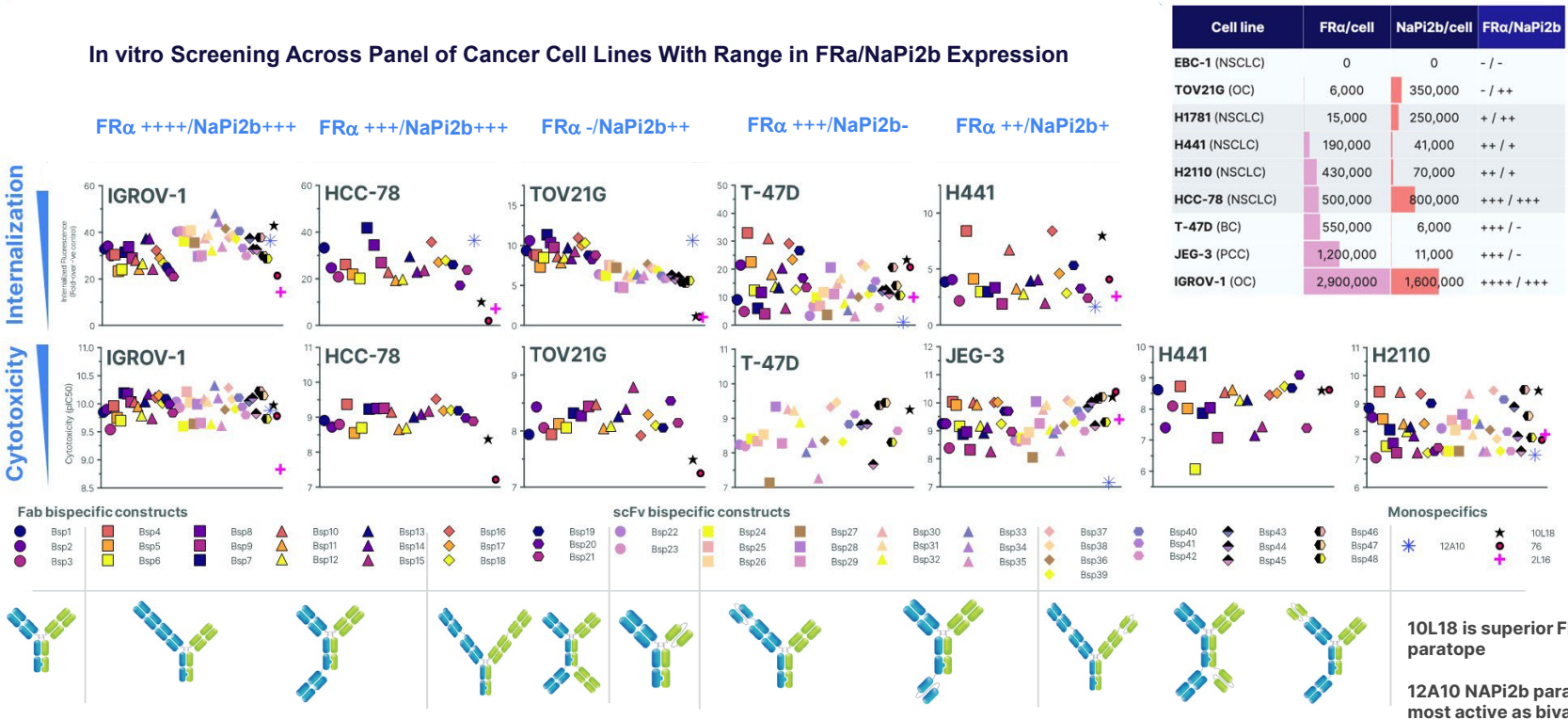
**To identify optimal Bispecific format(s) to broaden tumor coverage performed proof of concept study withFRα and NaPi2b**

- 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 FRα x NaPi2b Bispecific ADC Library

### In vitro Screening Across Panel of Cancer Cell Lines With Range in FRa/NaPi2b Expression

| Cell line | FRα/cell | NaPi2b/cell | FRα/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 FRa 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 Bispecifc 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:

1. **Development of multi-functional T-cell engagers including incorporation of conditional co-stimulation**
2. **Format screening of bispecific antibodies to support delivery of small molecule payloads simultaneously to two independently expressed cancer targets.**

# Acknowledgements: A Global Team Effort

## Zymeworks Publications:

### 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**