



# **ZW209: A DLL3 Targeted Trispecific T Cell Engager with Conditional and Obligate cis CD28 Co-stimulation to Improve Responses in DLL3-Expressing Tumors**

T Cell Engager Summit  
June 26, 2025

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Senior Director, Multispecific Antibody Therapeutics

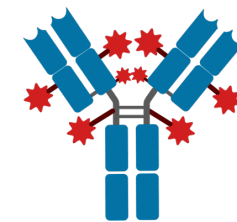
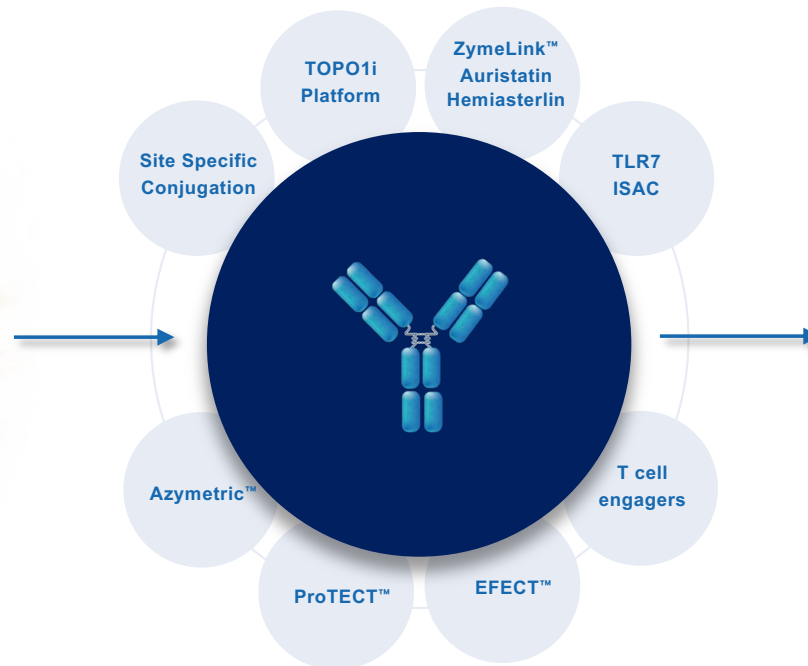
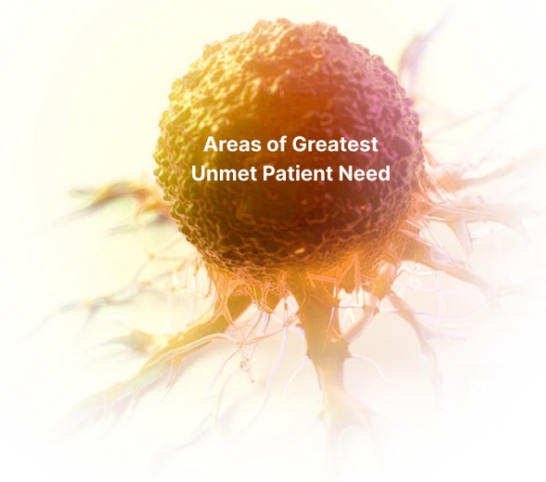
Nasdaq: ZYME | [zymeworks.com](https://zymeworks.com)

# Unique Capabilities in Protein Engineering Provide Opportunity for Differentiated Pipeline of ADCs and Multispecific Antibodies

Select Difficult-to-Treat  
Cancers and Target

Design with  
Complementary Technology

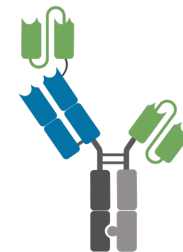
Optionality with Two Foundational  
Fit-for-Purpose Modalities



## Antibody-Drug Conjugates

Customization:

- Antibody properties
- Antibody format
- Payload
- DAR



## Multispecifics

Customization:

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

# Differentiated Pipeline of Multifunctional Therapeutics

Program	Technology	Target	Indication	Discovery	Preclinical	Phase 1	Phase 2	Phase 3
Solid Tumor Oncology: Antibody-Drug Conjugates (ADC)								
<b>ZW191</b> Topo1i ADC   DAR 8   Fc WT	ZD06519 Payload	FRα	Gynecological Thoracic	<div>NCT06555744</div>				
<b>ZW220</b> Topo1i ADC  DAR 4   Fc Mut	ZD06519 Payload	NaPi2b	Gynecological Thoracic	<div></div>				
<b>ZW251</b> Topo1i ADC   DAR 4   Fc WT	ZD06519 Payload	GPC3	Digestive System (HCC)	<div></div> Anticipated IND mid 2025				
Solid Tumor Oncology: Multispecific Antibody Therapeutics (MSAT)								
<b>Zanidatamab</b> Bispecific	Azymetric™	HER2	Multiple indications	Development partners: Jazz Pharmaceuticals and BeOne				
<b>ZW171</b> Trivalent TCE   2+1 Format	Azymetric™ Novel anti-CD3	MSLN x CD3	Gynecological Thoracic	<div>NCT06523803</div>				
<b>ZW209</b> Trispecific TCE   Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	DLL3 x CD3 x CD28	Thoracic	<div></div> Anticipated IND 1H 2026				
<b>ZW239</b> Trispecific TCE   Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	CLDN18.2 x CD3 x CD28	Digestive System	<div></div>				
Autoimmune & Inflammatory Diseases								
<b>ZW1528</b> Dual Cytokine Blocker	Azymetric™ Hetero-Fab   YTE	IL4Rα x IL-33	<div></div> Anticipated IND* 2H 2026					
<b>ZW1572</b> Dual Cytokine Blocker	Azymetric™ Hetero-Fab   YTE	IL4Rα x IL-31	<div></div>					

# 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

## Flexibility

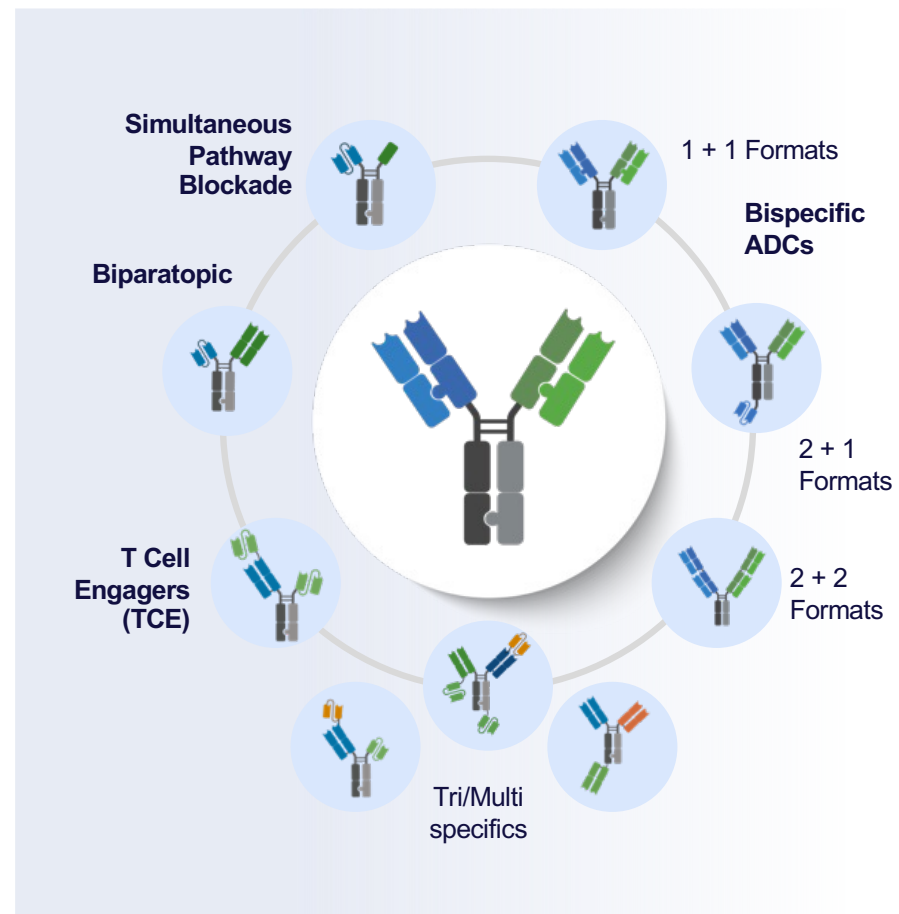
Can employ novel or existing antibody paratopes; human (IgG1, IgG2A, IgG4) and mouse frameworks; other CH2 and glyco-engineering approaches. Compatible with linker/payload conjugation

## High-throughput Screening

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

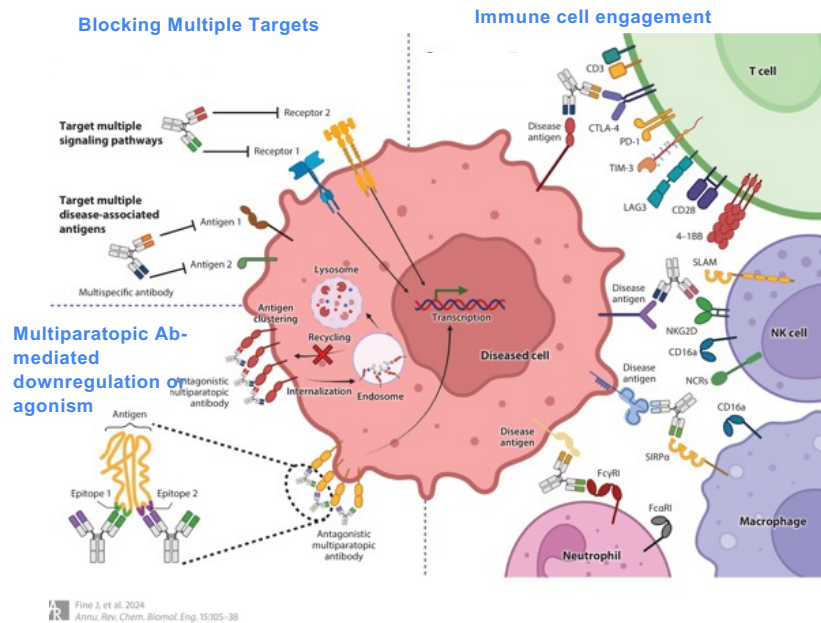
## Highly Manufacturable

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

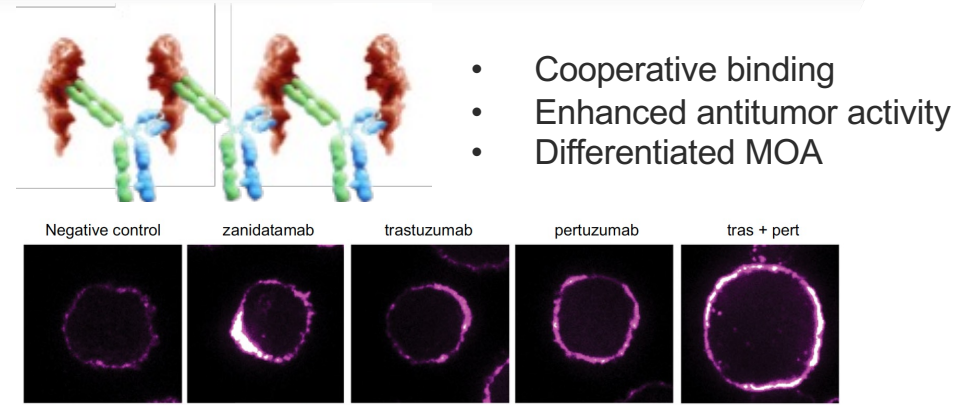


# Multispecific Antibodies Have the Ability to Unlock Novel Biologies

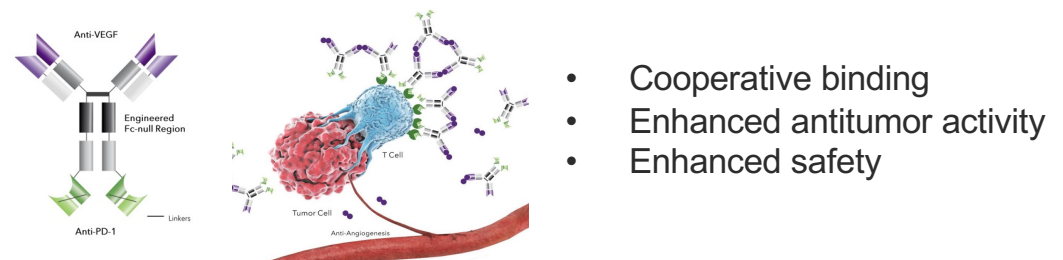
Engaging multiple targets with a single molecule can enable novel therapeutic mechanisms of action that are not possible with mAbs alone or in combination



## zanidatamab<sup>1</sup>: Anti-HER2 biparatopic



## Ivonescimab<sup>2</sup>: Anti-PD1 x VEGF



## Format Matters! Unique Formats Drive Novel Biology

- Interrogating a broad geometric and format space is critical to identifying differentiated candidates
- Traditional multispecific antibody screens often limited in number of formats tested



Azymetric™ facilitates efficient heterodimeric antibody assembly

→ Allows for HTP production and screening of multiple formats

→ Allows for identification of geometries with novel biology

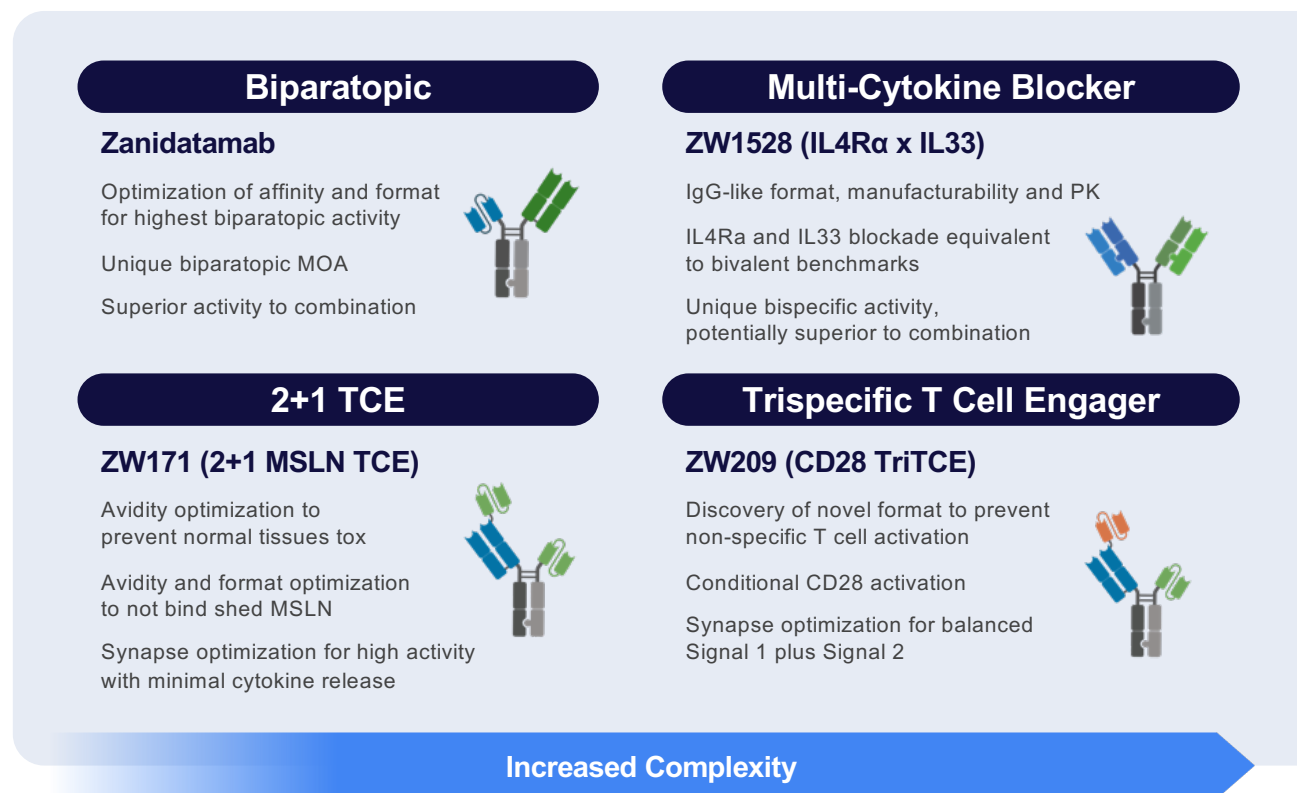


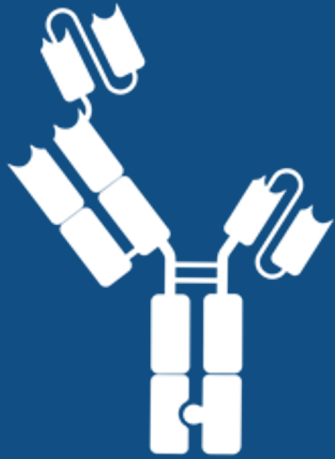
## Zymeworks' Engineering Approach: Key Expertise in Format and Geometry Screening to Identify Differentiated Activity

**Potential best-in-class activity** requires screening of epitopes, affinities and target engagement geometries

**Unique flexibility of Azymetric™** enables format and affinity optimization for potential best-in-class attributes

**Discovery of unique biology** and differentiation to combination approaches





# ZW171

Bispecific Antibody Designed to Target Gynecological, Thoracic, and Digestive System Cancers

Initiated Phase 1 clinical trial in 2H 2024 (NCT06523803)

## Optimized Design<sup>1</sup>

- T cell-engaging bispecific antibody for the treatment of MSLN-expressing solid tumors, built with Azymetric™.
- Unique geometry: Two single-chain fragment variable arms targeting MSLN; one Fab arm targeting the CD3 component of the T cell receptor, redirecting the body's immune system to fight cancer cells.

## Differentiated Profile<sup>1</sup>

- Enhanced anti-tumor activity and safety profile in preclinical models supports opportunity to overcome clinical limitations of prior MSLN-directed therapies.

## Significant Patient Need

- Strong expression of MSLN in ovarian cancer (~84%) and moderate to strong expression in NSCLC (~36%).<sup>2</sup>
- In the U.S. in 2024<sup>3</sup>:
  - 19K+ new cases of ovarian cancer
  - 234K+ new cases of lung cancer
  - 353K+ new cases of digestive system cancers

MSLN: mesothelin; NSCLC: non-small cell lung cancer; scFV: single-chain variable fragment.

1. Afacan N et al., Abstract #2942 presented at AACR 2023.

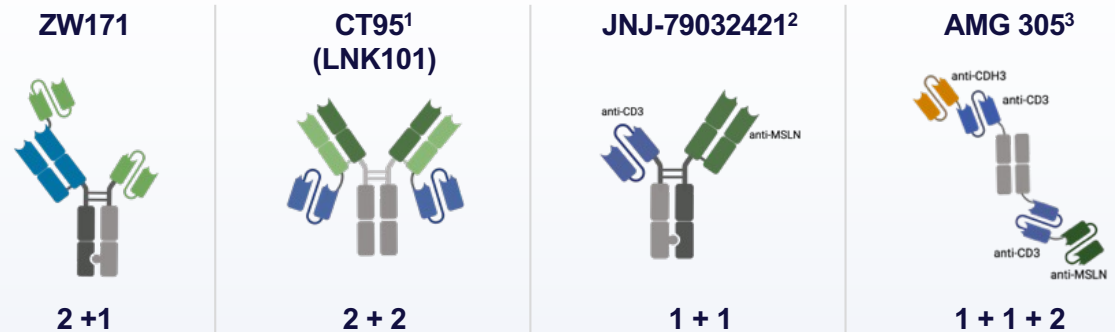
2. Weidemann, S. et al. Biomedicine 2021, Apr 7;9(4):397.

3. <https://acsjournals.onlinelibrary.wiley.com/doi/10.3322/caac.21820>

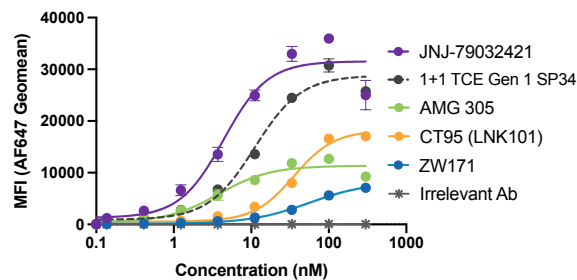


# ZW171 Exhibits a Wider Therapeutic Window Compared to Next Gen MSLN TCEs

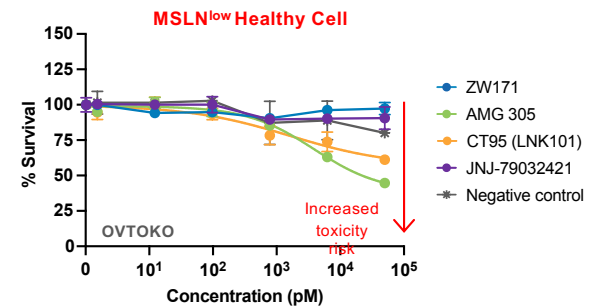
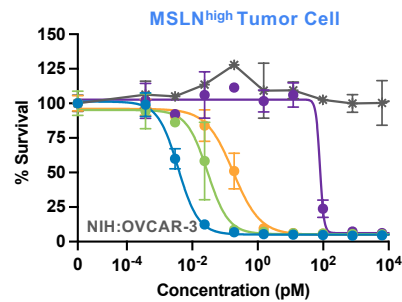
- Enhanced tumor selective cytotoxicity
- No targeting of normal tissues
- Low affinity CD3 binding to mitigate peripheral T cell binding and cytokine release
- Maintains potency in the presence of soluble MSLN



## Low Binding to T cells

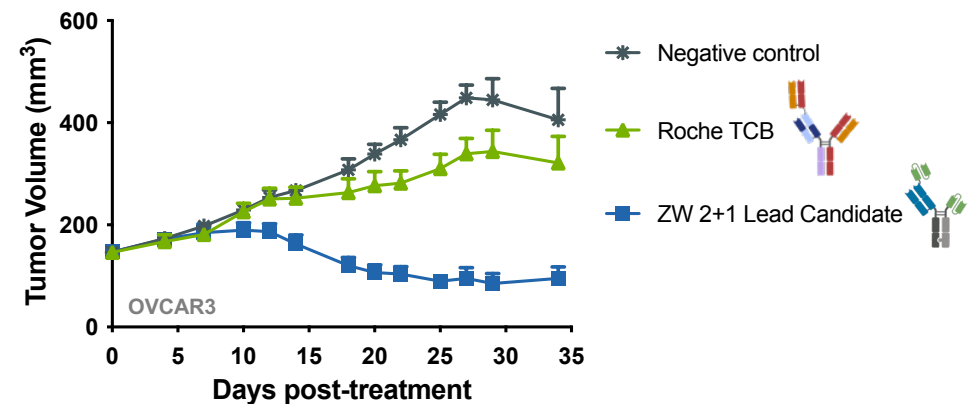
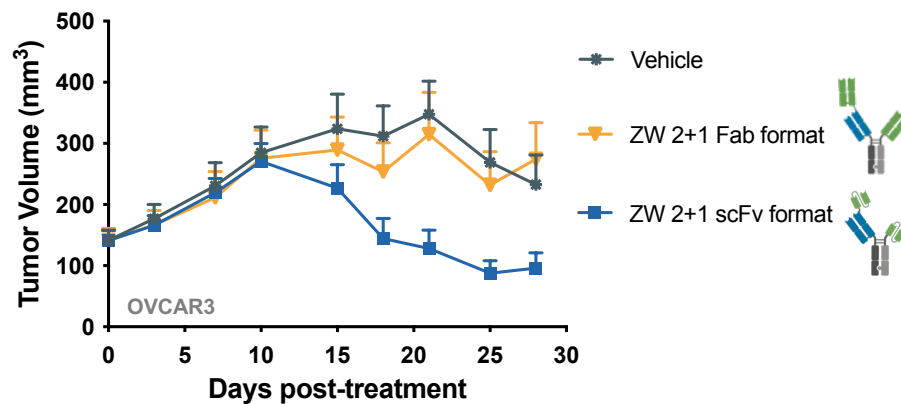


## Potent Cytotoxicity in MSLN+ Tumor Cells but not Normal Cells



## ZW171 Format is Critical for Activity and is Superior to Other 2+1 Formats

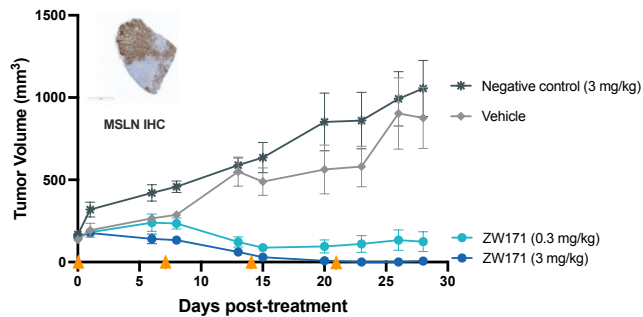
2+1 format consisting of 2 anti-MSLN scFvs and one anti-CD3 Fab is critical



**Ovarian Cancer Model:** OVCAR-3 tumor fragments were engrafted subcutaneously in NOG mice. After tumors reached 100-200 mm<sup>3</sup>, mice were humanized with donor PBMC (3 donors) then treated 2QW x4 with test article (vehicle: A5Su, Negative control: anti-MSLN MAb)

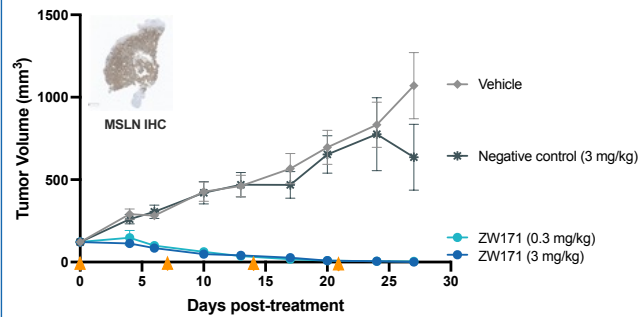
# ZW171: Mediates Strong Anti-Tumor Activity in Patient-derived Models

## Patient-derived NSCLC Humanized Mouse Model



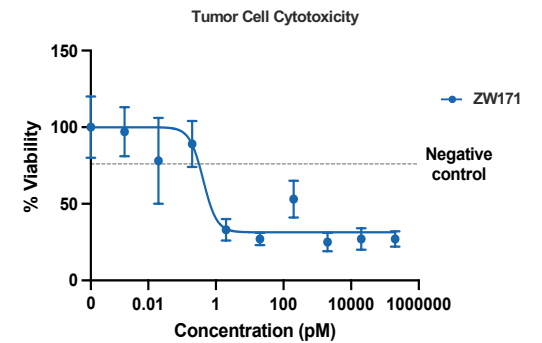
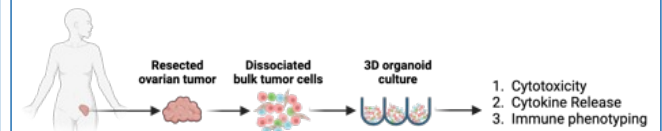
CD34 engrafted mice were engrafted with CTG-2579. When tumors reached 100-200 mm<sup>3</sup>, mice were dosed IV QW x4 with ZW171 at 3 or 0.3 mg/kg, the neg control (HAXCD3) at 3 mg/kg, or vehicle (H6Su).

## Patient-derived Pancreatic Cancer Humanized Mouse Model



CD34 engrafted mice were engrafted with CTG-1375. When tumors reached 100-200 mm<sup>3</sup>, mice were dosed IV QW x4 with ZW171 at 3 or 0.3 mg/kg, the neg control (HAXCD3) at 3 mg/kg, or vehicle (H6Su).

## Ovarian Cancer Model Leveraging Endogenous Tumor T cells



3D patient-derived ovarian carcinoma organoids were generated, and ZW171 activity assessed using Kiyatec proprietary technologies (Lassahn, 2023). Following incubation of organoids with ZW171 for 72hr, tumor cell viability was assessed using a CellTiter-Glo 3D (Promega) assay.

# ZW171 Global Phase 1 Study in MSLN-Expressing Solid Tumors (NCT06523803)

USA

**USA**

FDA IND Approval  
Sites Activated

UK

**United Kingdom**

MHRA CTA Approval  
Sites Activated

DEU

**Germany**

EU CTA Approval

SK

**South Korea**

MFDS CTA Approval  
Sites Activated

## Open-label, FIH, dose-escalation study

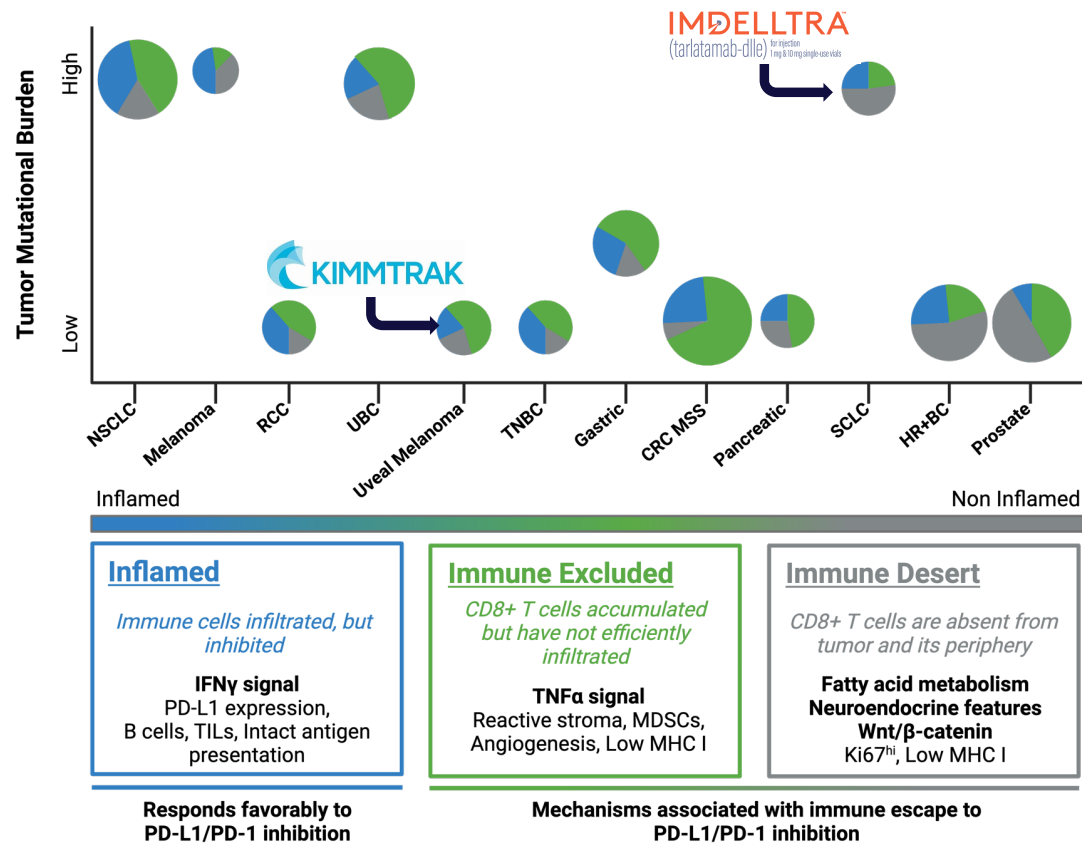
### Part 1: Dose Escalation

N= ~160

### Part 2: Dose Optimization and Expansion



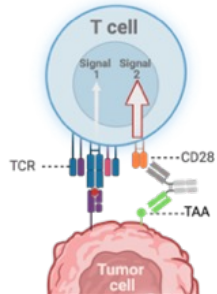
# T Cell Engagers Exhibit Activity in Solid Tumors, but Unmet Need Remains



Adapted from Hedge and Chen 2020 Immunity 52

# CD28 Co-stimulatory T Cell Engager Approaches

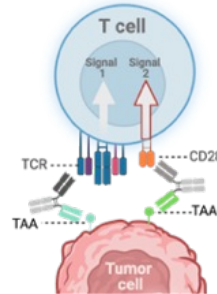
## Bispecific CD28 T cell Engagers



### CD28 x TAA +/- PD1

#### Limitations:

Initial clinical activity for CD28-TAA +PD1, but potential toxicity due to autoreactive T cells<sup>1</sup>

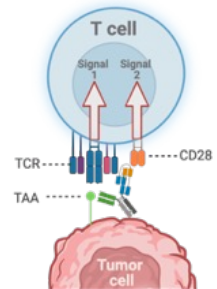


### CD28 x TAA + CD3 x TAA

#### Limitations:

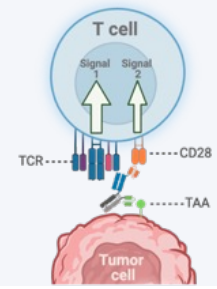
- Optimized for single agent activity and strong CD28 agonism, potential for similar toxicity to CD28-TAA and difficult to optimize by dose adjustment
- Exposure of two molecules at required dose levels potentially suboptimal

## Trispecific CD28 T cell Engagers



### First Generation:

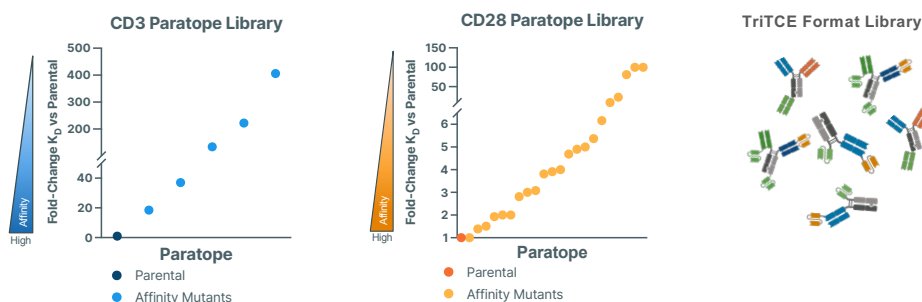
- High affinity CD3 and CD28 superagonist paratopes<sup>2,3</sup>
- T cell binding, activation and TMDD observed in periphery<sup>2,3</sup>
- Target-independent activity and T cell activation



### Zymeworks' Next Generation Solution:

- Balanced low affinity CD3 and CD28 engagement
- Conditional CD28 binding that only binds in cis with CD3 engagement
- Strict target-dependent activity and T cell activation
- Identified via Azymetric™ screening of various antibody geometries and CD3 and CD28 paratope affinities

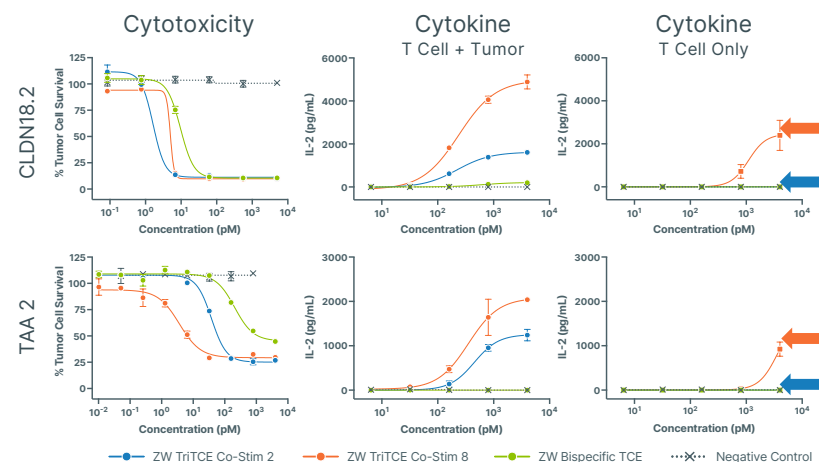
# Lead TriTCE Format Selected Following Extensive, Parallel Paratope and Format Screening, Exhibits TAA-dependent Cytotoxicity and Cytokine Release



Antibody Format	1	2	3	4	5	6	7	8	9	10
Target-Dependent?	✓	✓	✓	✗	✓	✗	✗	✗	✗	✗
Potency ( $IC_{50}$ ; pM)										

TAA-Dependent

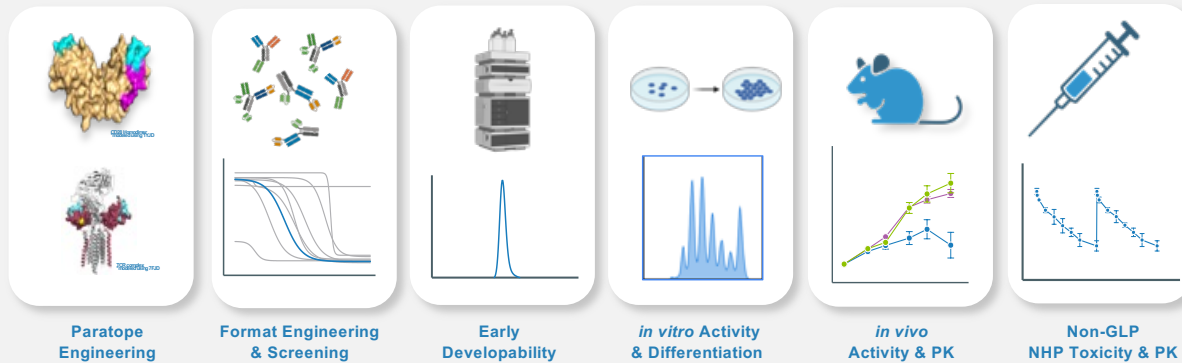
TAA-Independent



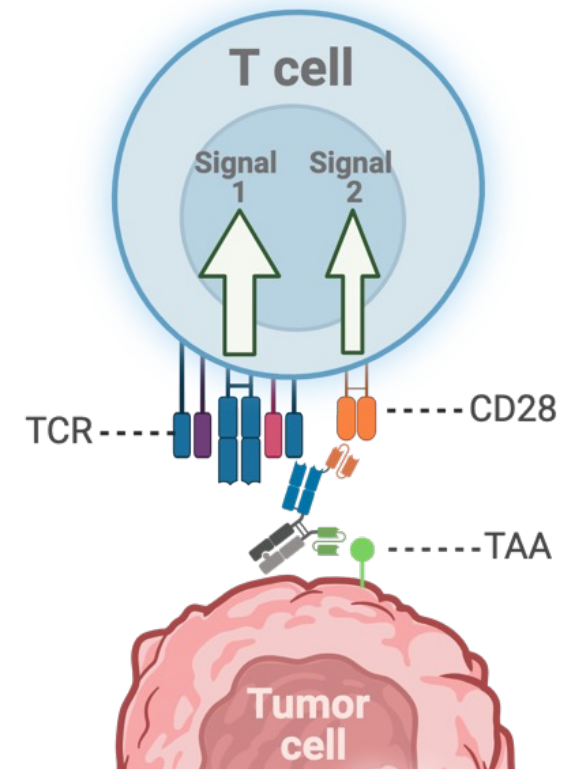


# TriTCE Co-stim: A Next Generation Trispecific T Cell Engager Platform

## TriTCE Co-stim Platform Workflow

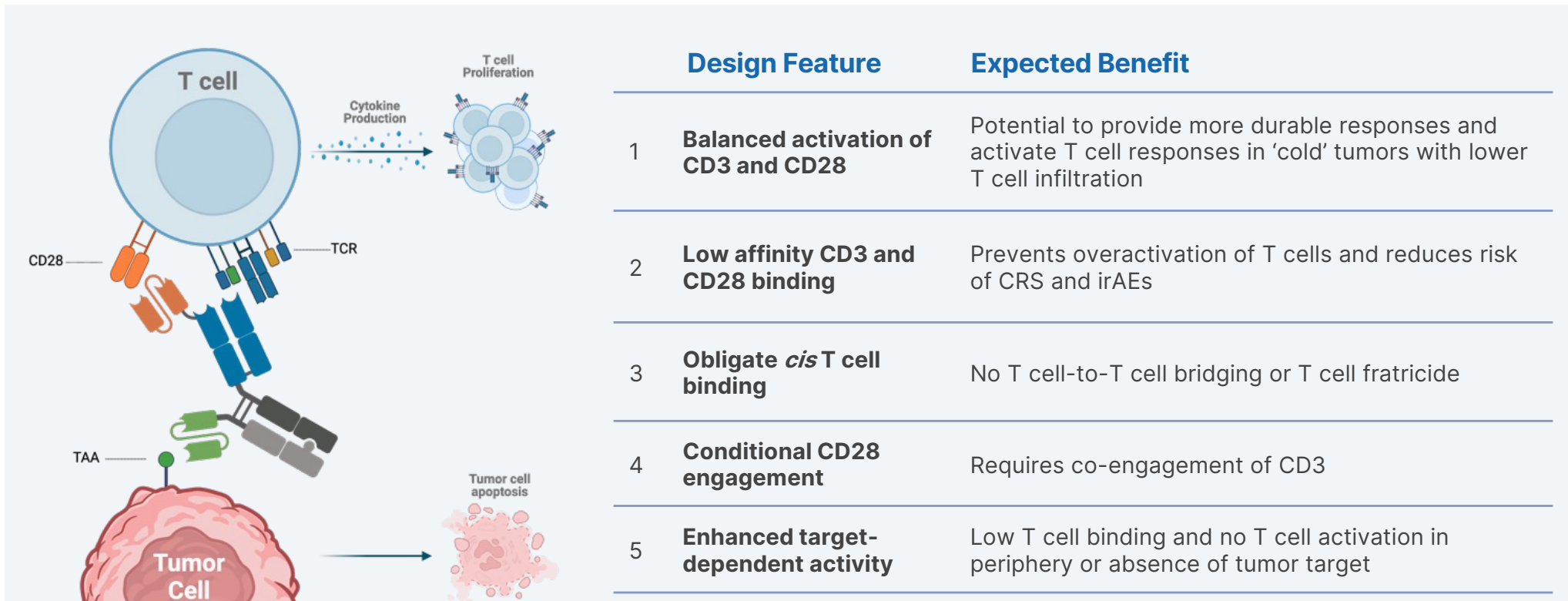


**Established workflow, transferable format  
Validated on multiple TAAs**

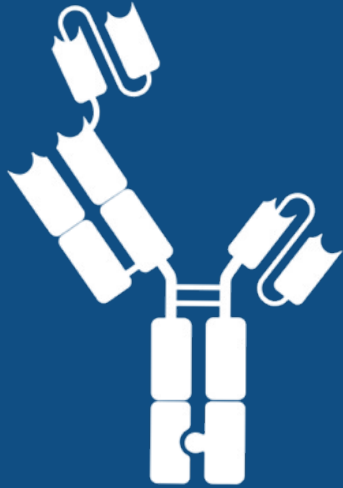


# TriTCE Co-stim Designed to Optimize T cell Binding, Activation and Anti-Tumor Activity

Conditional CD28 Co-stimulation and Obligate *cis* T cell Binding



CRS: Cytokine release syndrome; irAEs: immune-related adverse events



## ZW209

Trispecific T cell engager  
(TriTCE) Designed to Target  
DLL3-expressing Solid Tumors

On track for IND submission 1H 2026

### Optimized Design

- Potential first-in-class TriTCE that targets DLL3-expressing tumor cells, and CD3 and CD28 on T cells.
- TriTCE with potentially optimized TAA, CD3, CD28 binding affinity and geometry using Azymetric™ and EFECT™ platforms.
- Leverages obligate cis-T cell binding and conditional CD28 engagement to prevent unintended T cell activation, while enabling tumor-targeted cytotoxicity.

### Differentiated Profile

- Clean expression profile and absence of on-target, off-tumor side effects observed for DLL3 x CD3 bispecifics provides ideal TriTCE Co-stim target profile.
- Long term cytotoxicity at low effector to T cell ratios, increased T cell proliferation, survival, and anti-tumor activity with reduced cytokine release.
- Validated responsiveness of DLL3-expressing tumors to TCE modality.

### Significant Patient Need

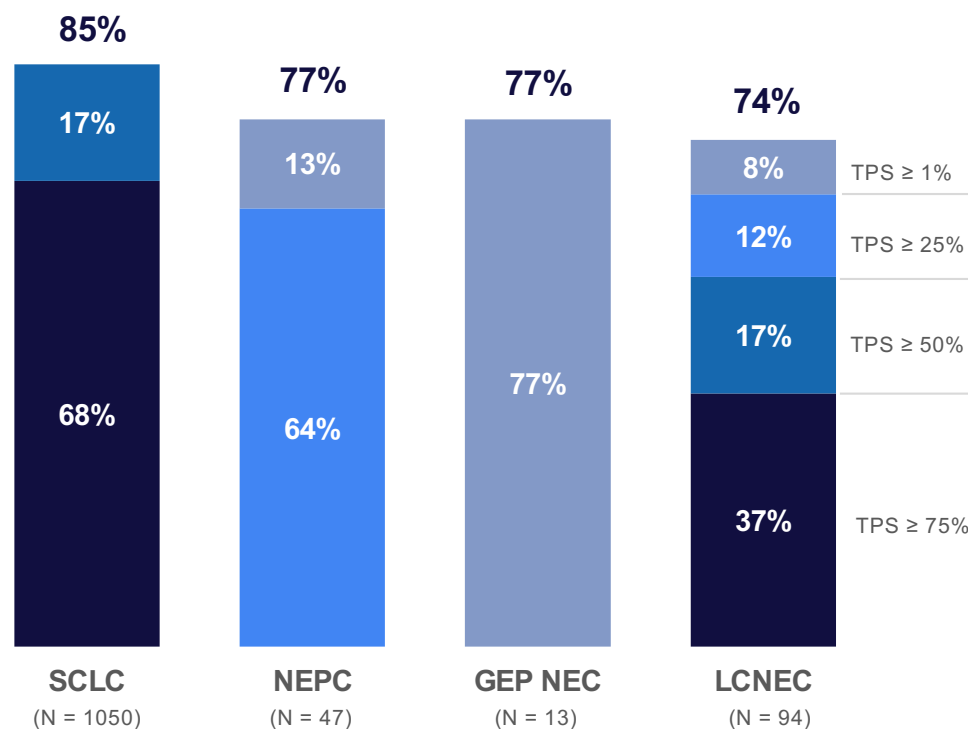
- DLL3 is expressed on the surface of SCLC and other neuroendocrine tumors but rarely on the surface of normal cells.
- SCLC accounts for about 15% of all lung cancer diagnoses in the U.S. each year.<sup>1</sup>

1. <https://www.yalemedicine.org/conditions/small-cell-lung-cancer#:~:text=There%20are%20two%20primary%20forms,and%20improving%20quality%20of%20life.>  
DLL3: Delta-like ligand 3; SCLC: Small Cell Lung Cancer; TAA: tumor-associated antigen; TriTCE: Tri-specific T Cell Engager.

## DLL3 is an Ideal Target to Evaluate TriTCE Co-stim Platform, with Opportunities in Multiple Cancers

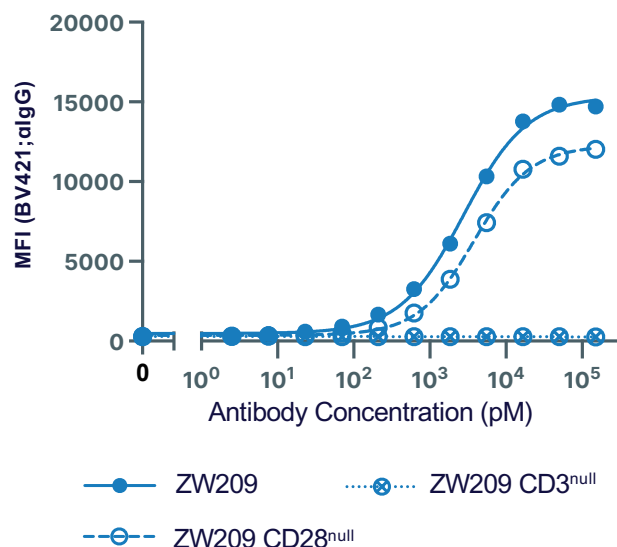
- Responsiveness of DLL3-expressing tumors to TCE modality validated with Imdelltra™ and other DLL3 bispecific TCEs; however, opportunity for improved responses remains
- DLL3 is expressed on the surface of SCLC and other neuroendocrine tumors but rarely on the surface of normal cells
- Clean expression profile and absence of on-target, off-tumor side effects observed for DLL3 x CD3 bispecifics provides ideal TriTCE Co-Stim target profile

Percentage of Patients with DLL3+ Tumors (%)

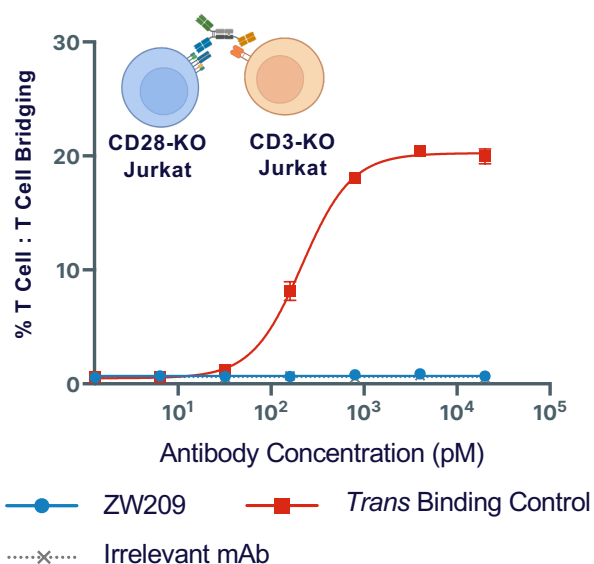


# ZW209 Design Facilitates Desirable T Cell Engagement

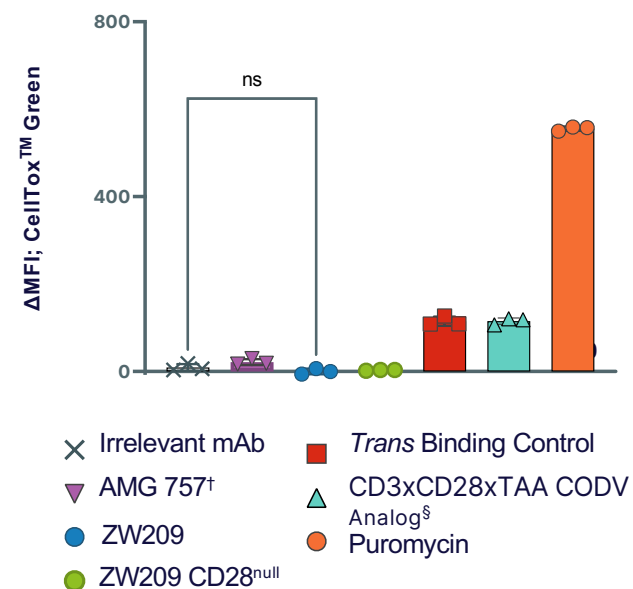
Conditional binding of CD28, requiring co-engagement of CD3



ZW209 does not bridge T cells via *trans* binding of CD3 and CD28.

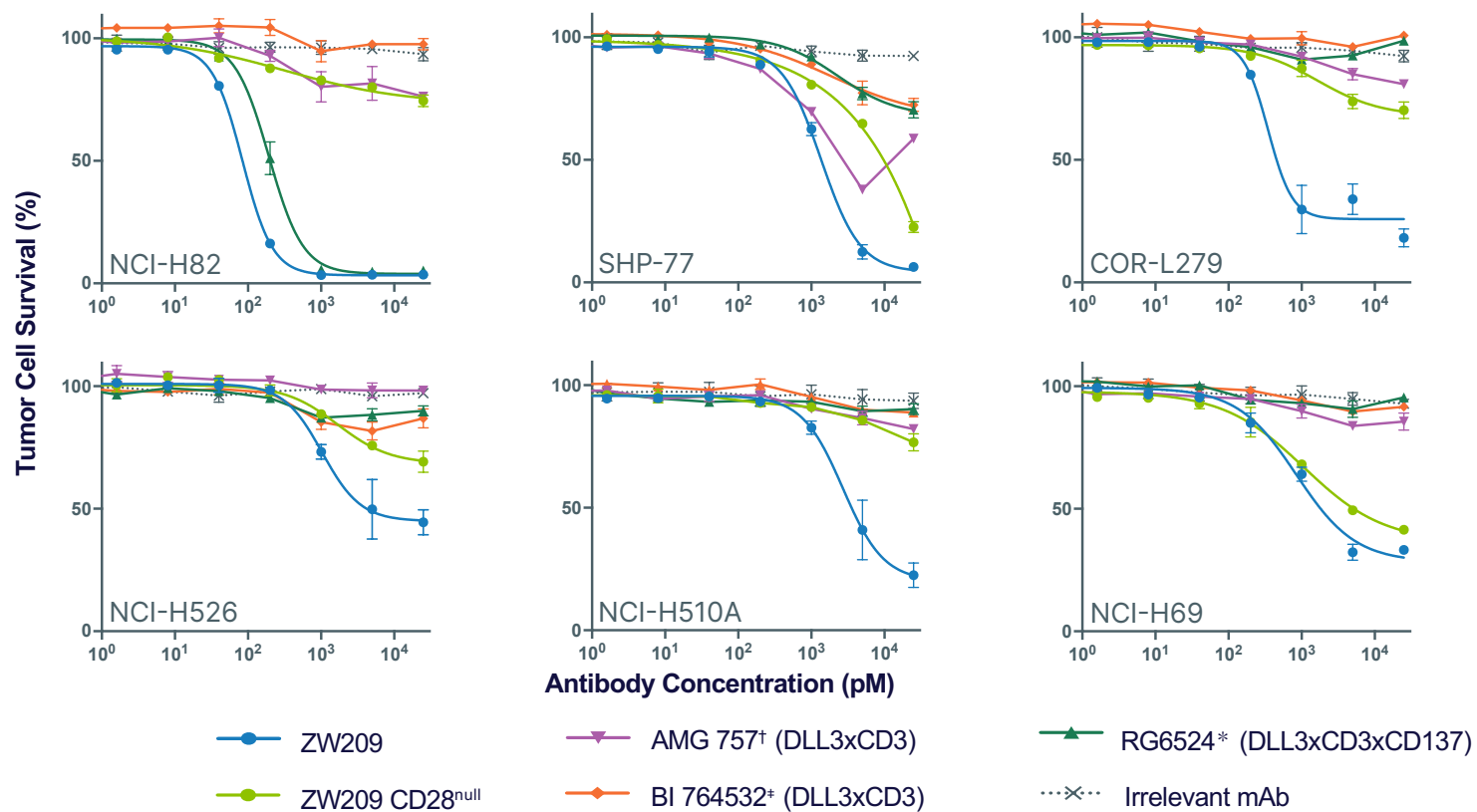


ZW209 does not mediate T cell lysis



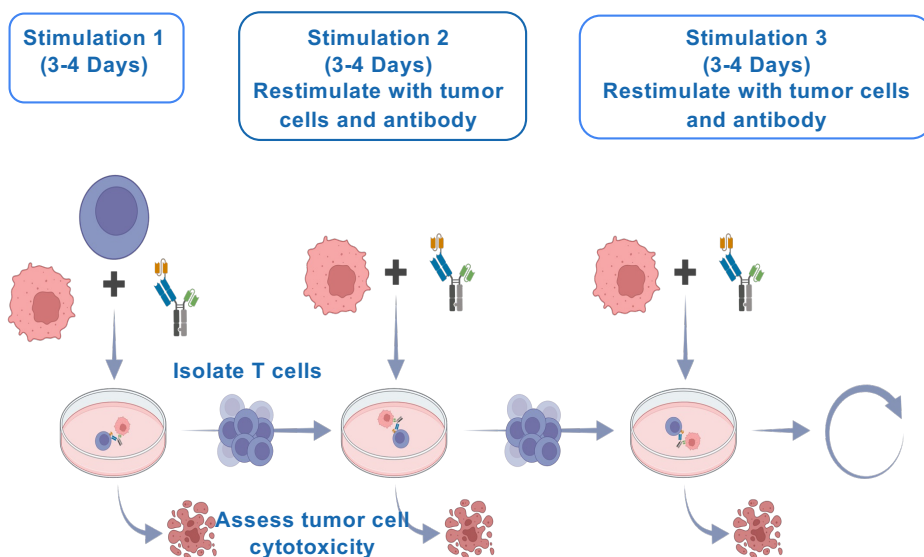
LEFT On cell binding of ZW209, ZW209 CD3<sup>null</sup> and ZW209 CD28<sup>null</sup> to human pan T cells assessed by flow cytometry. MID Ability of trispecific antibodies to cross-link of CD3-KO and CD28-KO Jurkat cells measured by flow cytometry. Representative schematic of cell bridging (inset). RIGHT Antibody mediated T cell lysis in a monocultures of T cells was assessed using CellTox™ Green. The positive control trispecific antibody and CODV Analog are CD3xCD28xTAA trispecific antibody formats are positive controls that exhibit *trans* binding of T cells via CD3 and CD28.

## ZW209 Exhibits Improved Potency Relative to Bispecific and Trispecific Clinical TCE Benchmarks at Low Effector: Target Ratios



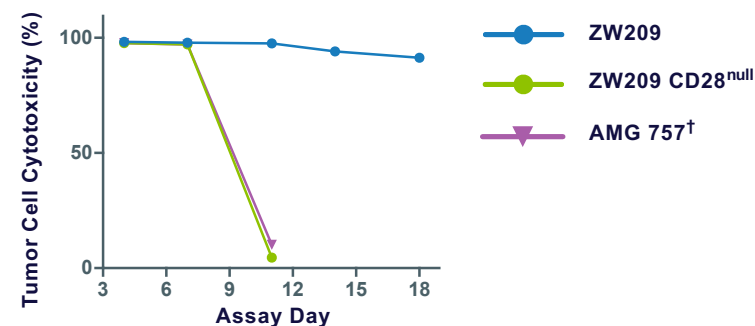
Test articles were incubated with T cells co-cultured with DLL3-expressing SCLC tumor cell lines at low E:T ratio for 7 days and evaluated for cytotoxicity.

# ZW209 Mediates Sustained T Cell-Mediated Cytotoxicity Over Repeated Stimulations

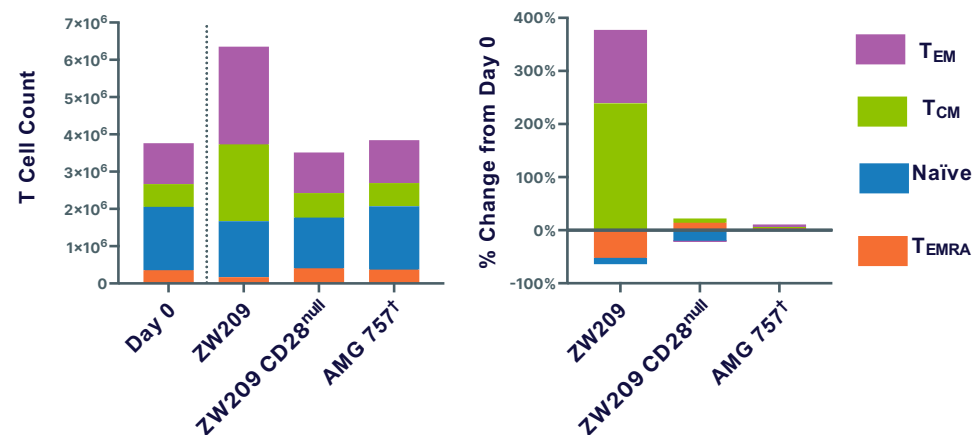


T cells were incubated with DLL3+ NCI-H82 cells and test article. For each subsequent round of stimulation, T cells were collected, counted, and re-stimulated with fresh NCI-H82 target cells and test article. Schematic of T cell restimulation. Following each round of stimulation, co-cultures were assessed for tumor cell cytotoxicity. Following 3<sup>rd</sup> stimulation, ZW209 CD28<sup>null</sup> and AMG 757<sup>†</sup> showed no anti-tumor activity. 3 days after 2<sup>nd</sup> stimulation (day 7), T cell memory populations were assessed by flow cytometry staining for CD45RO and CCR7 expression. T cells stimulated by ZW209 displayed an increased number of effector and central memory T cells relative to bispecific TCEs.

## Sustained cytotoxicity relative to bispecific TCEs



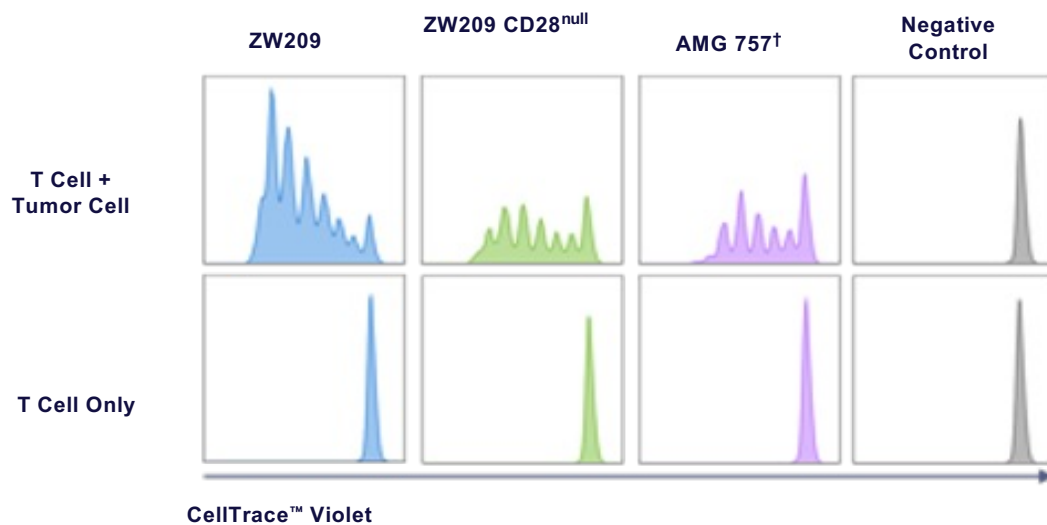
## Expanded effector memory (T<sub>EM</sub>) and central memory (T<sub>CM</sub>) T cell populations after 2<sup>nd</sup> stimulation (Day 7)



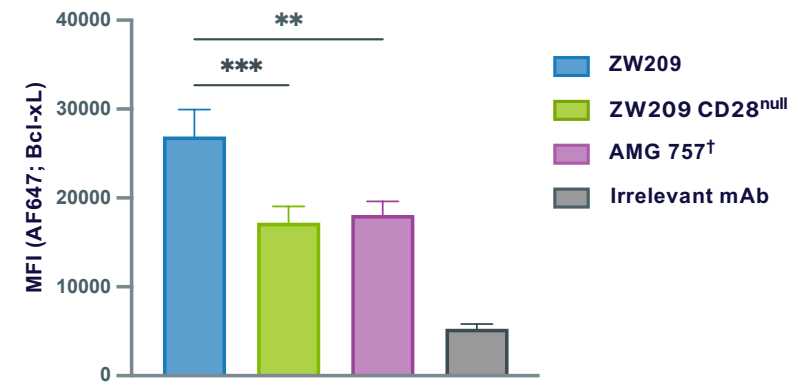


# ZW209 Mediates Enhanced DLL3-dependent T Cell Proliferation and Survival

## Target-Dependent T Cell Proliferation

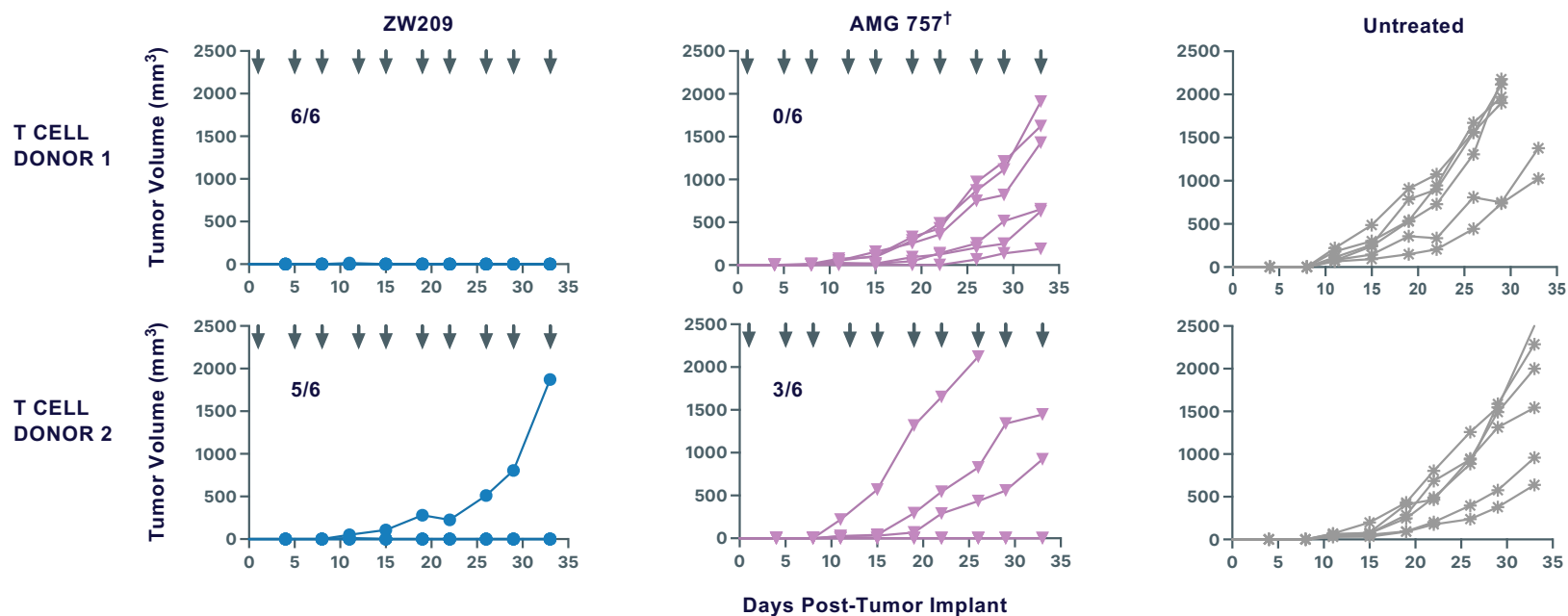


## T Cell Survival

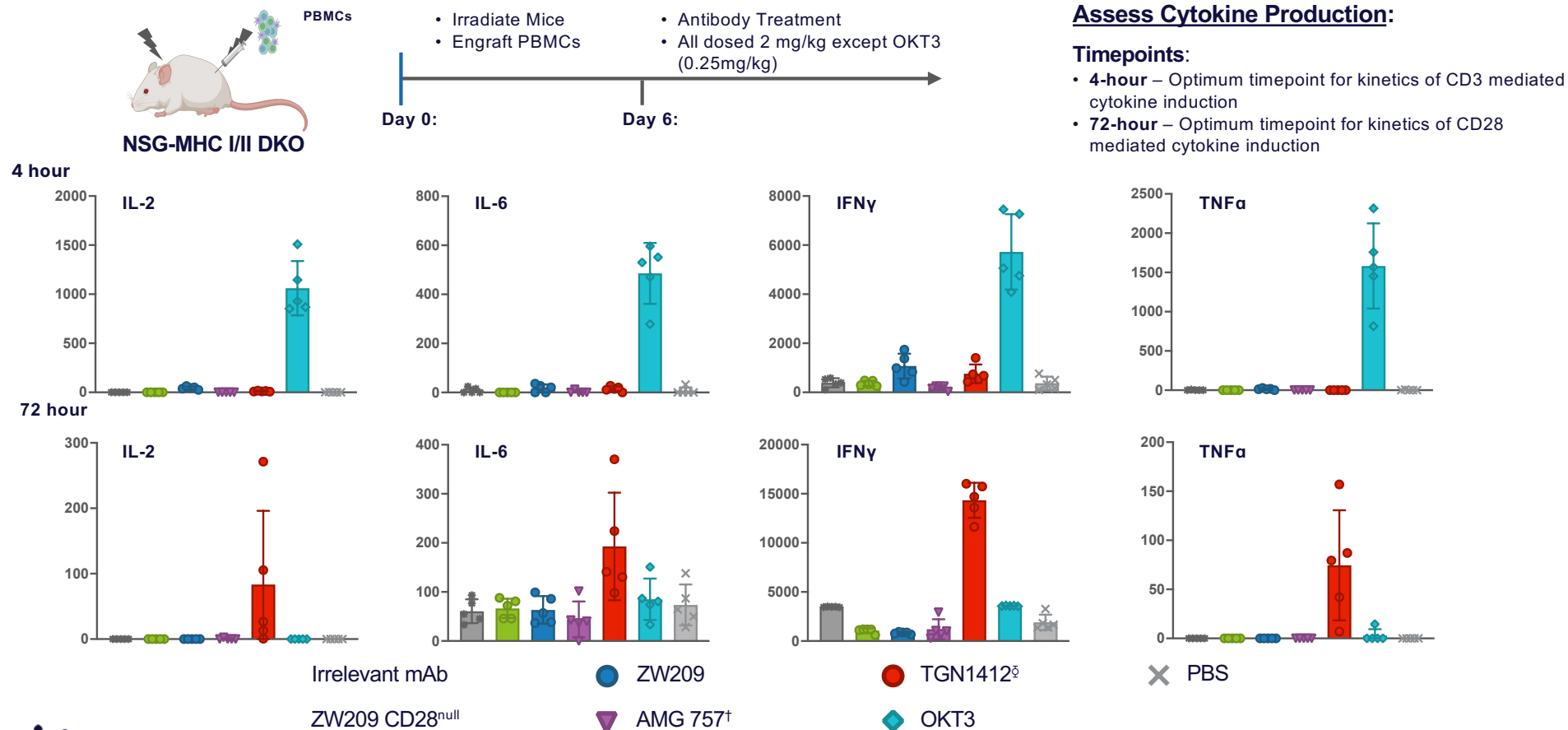


Test articles (5 nM) were incubated with CellTrace Violet™ labeled T cells alone or co-cultured with NCI-H82 cells for 5 days and assessed by flow cytometry. Right Test articles (5 nM) were incubated with T cells co-cultured with NCI-H82 cells for 48 hours and evaluated for Bcl-xL expression by flow cytometry. \*\* p<0.01, \*\*\* p<0.001

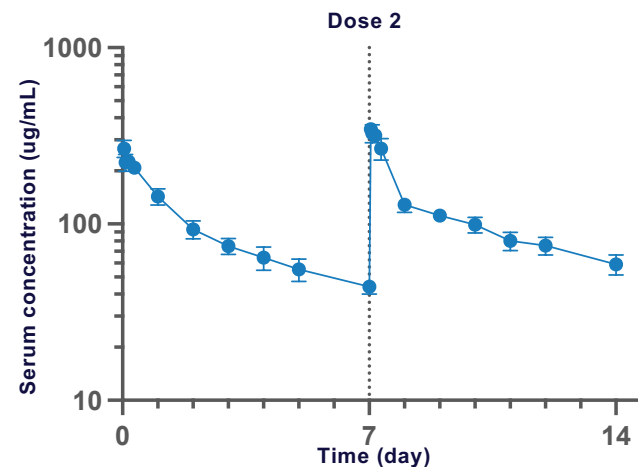
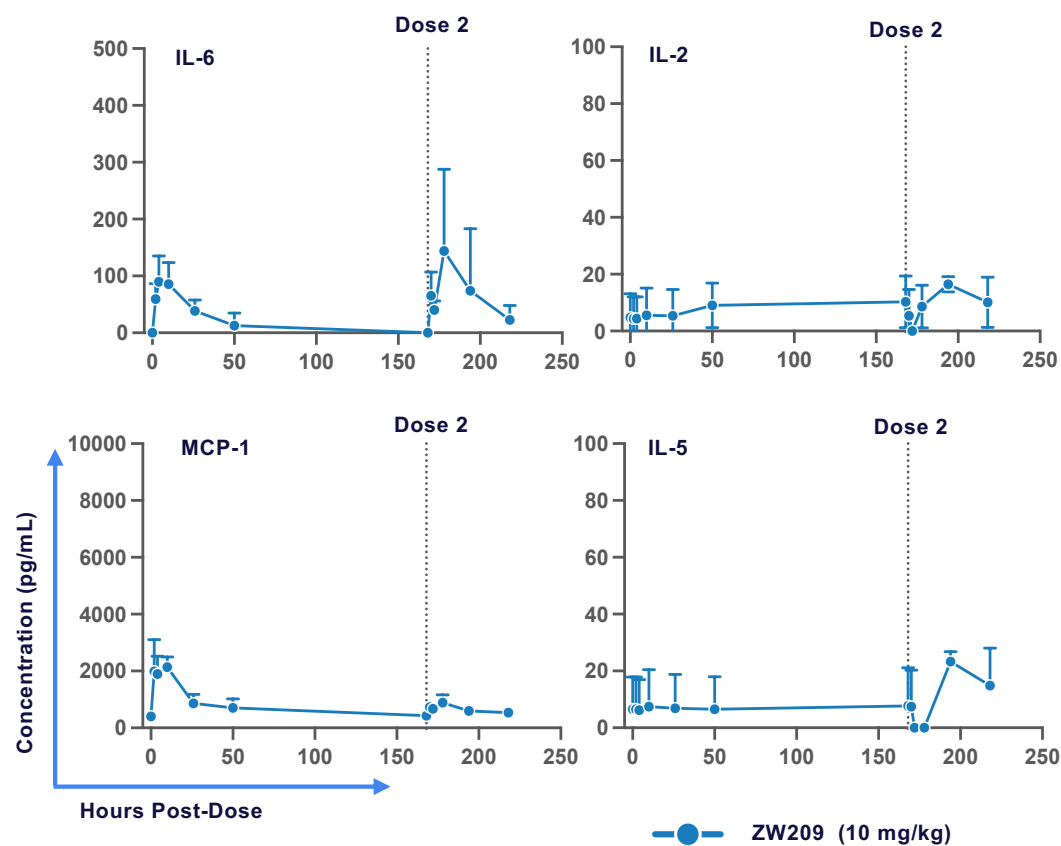
# ZW209 Mediates Enhanced Anti-tumor Activity in an Admixture Xenograft Model



# ZW209 Displays Favorable In vivo Safety Profile: No Systemic Cytokine Induction Observed in an *in vivo* Cytokine Release Model



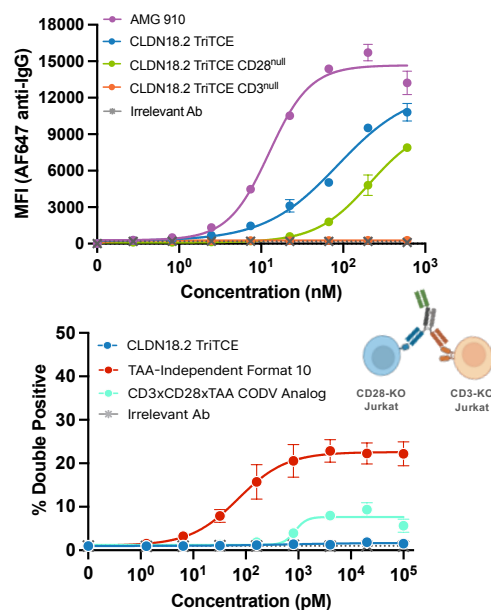
## ZW209 is Well-tolerated in Cynomolgus Monkeys



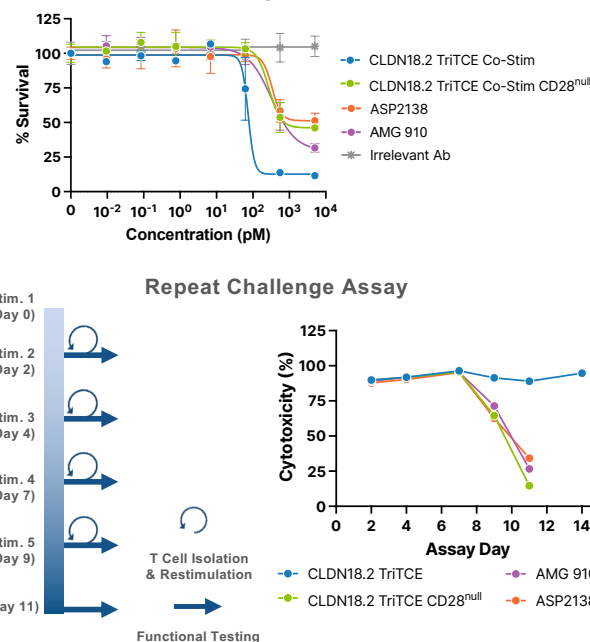
**ZW209 exhibits transient, mild increases in serum cytokine expected of TCEs, and an antibody-like PK profile in non-GLP NHP**

# ZW239: CLDN18.2-Targeted TriTCE Co-Stim

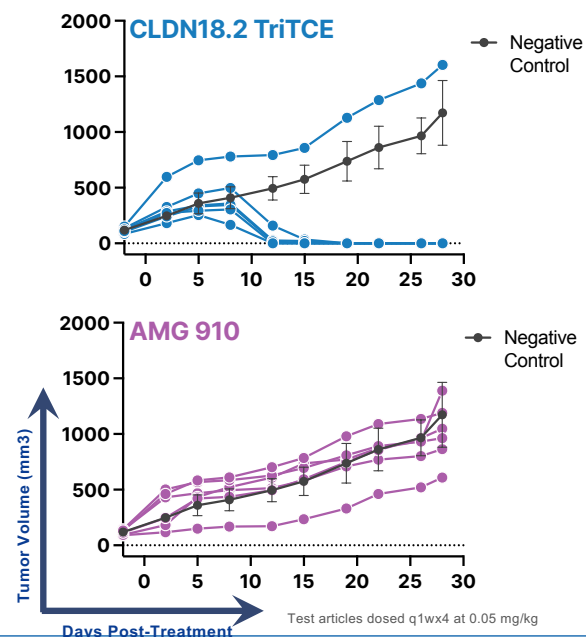
## CD3-dependent CD28 Binding, Obligate *cis* CD3-CD28 Binding



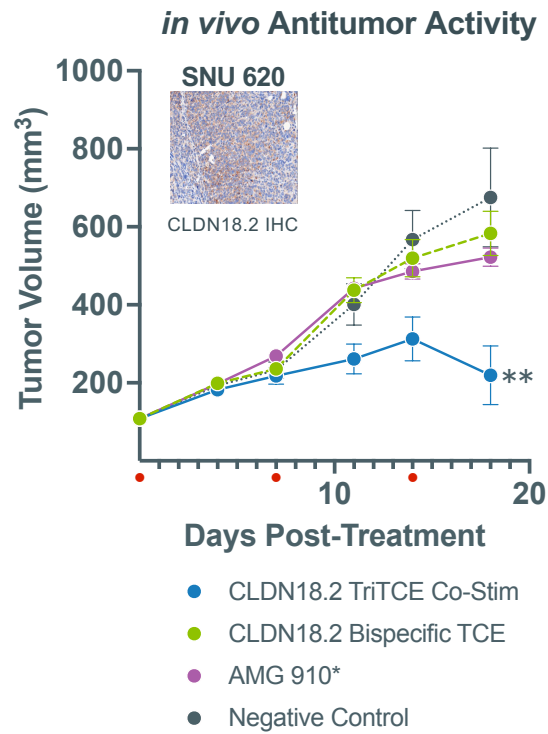
## Improved and Sustained Cytotoxicity over Bispecific TCE



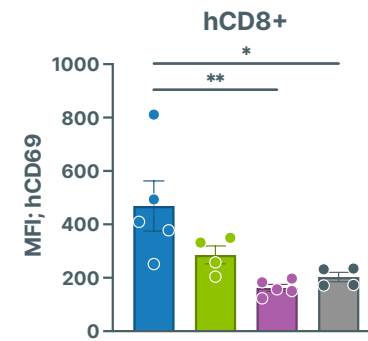
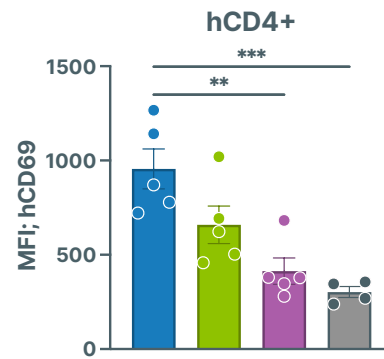
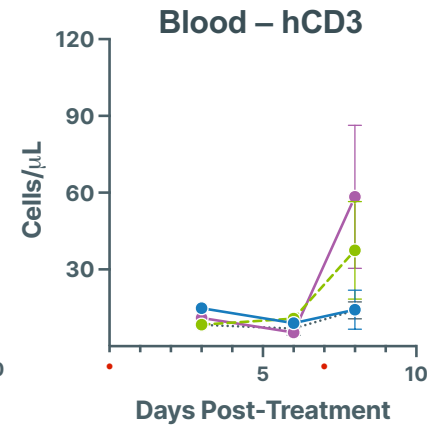
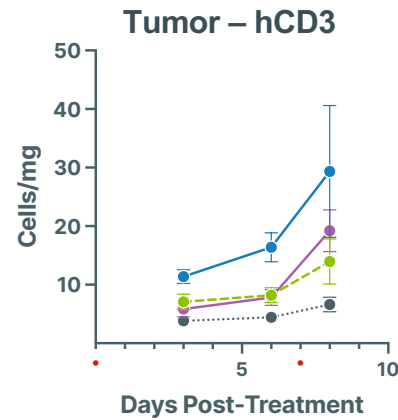
## Superior Anti-tumor Activity in vivo



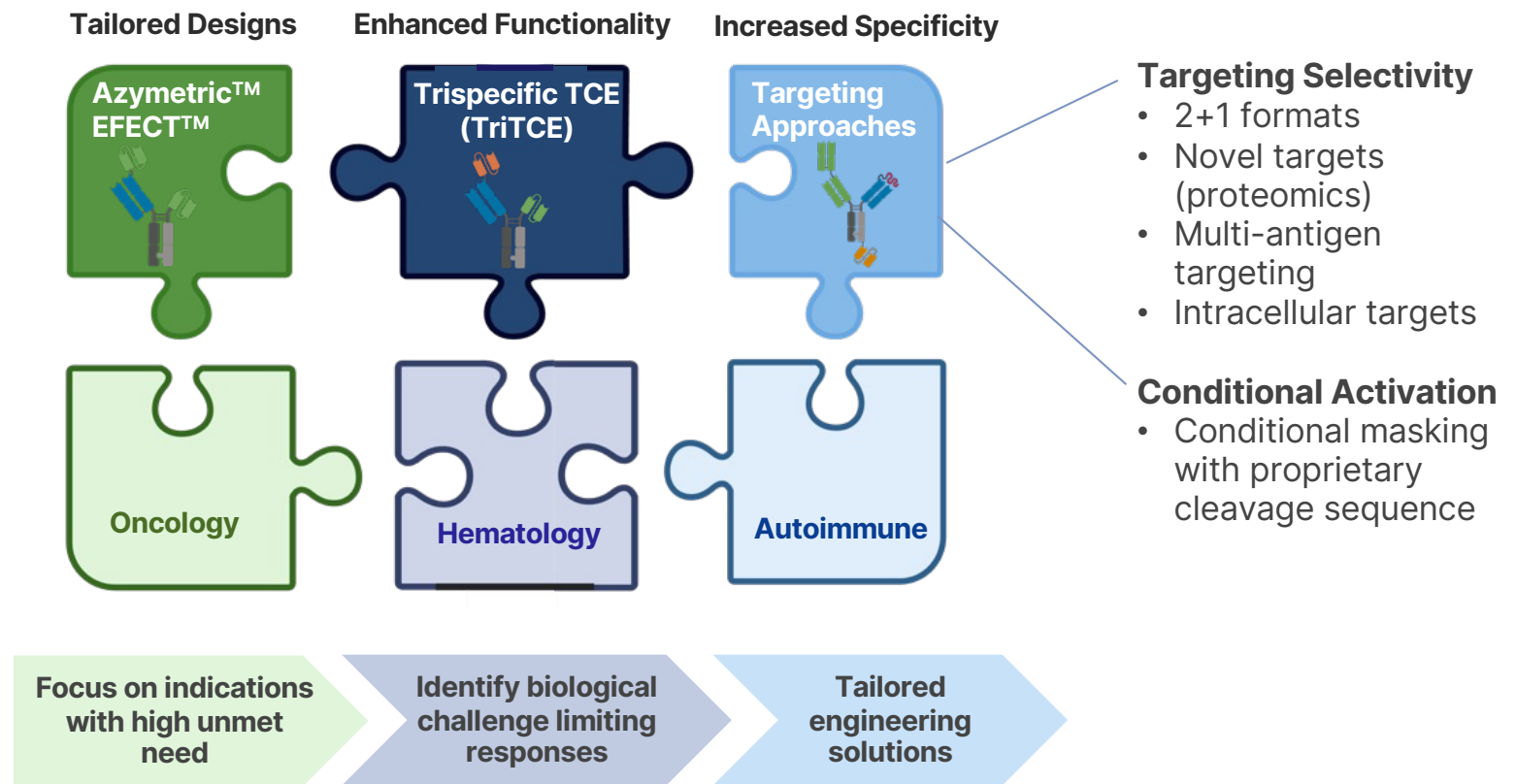
# CLDN18.2 TriTCE Co-Stim Mediates Enhanced Antitumor Activity and Increases Activated Intratumoral T cells *in vivo*



Test articles dosed at 0.01 mg/kg



# Enhancing Functionality and Specificity to Help Improve Responses Across Diverse Therapeutic Areas





## Acknowledgements...A Global Team Effort

<https://www.zymeworks.com/publications/>

### **AACR 2025: ZW209, a DLL3 targeted trispecific T cell engager with integrated CD28 costimulation, demonstrates safety and potent preclinical efficacy in models of small cell lung cancer**

Desmond Lau, Peter Repenning, Diana Canals Hernaez, Alec Robinson, Diego Perez Escanda, John Zhang, Hamed Shirvani, Catherine Wu, Kurt Stahl, Aditi Deshmukh, Nichole Escalante, Mariana Rocha, Begonia Silva Moreno, Lisa Newhook, Purva Bhojane, Paul A. Moore, Nina E. Weissner, Thomas Spreter von Kreudenstein

### **AACR 2024: 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. Weissner, Thomas Spreter von Kreudenstein

### **AACR 2024: 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. Weissner

### **AACR 2023: Next-generation co-stimulatory trispecific T cell engagers (TriTCEs) for the treatment of solid tumors**

Lisa Newhook, Purva Bhojane, Peter Repenning, Diego Perez, Nichole Escalante, Patricia Zwierchowski, Alec Robinson, Lauren Clifford, Harsh Pratap, David Douda, Chayne Piscitelli, Nicole Afacan, Thomas Spreter von Kreudenstein, Nina Weissner



**Zymeworks' Multispecific Antibody Therapeutics Team**