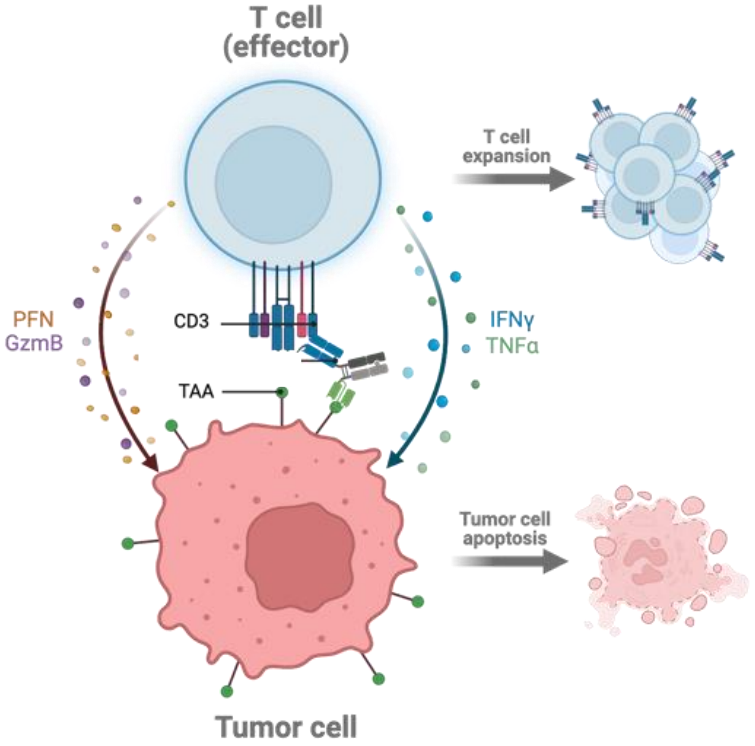
Advancing ZW209 Trispecific Design with In Vitro & In Vivo Models to Improve DLL3-Directed Responses

10th Tumor Models Summit San Francisco

T cell Engagers for Cancer Immunotherapy

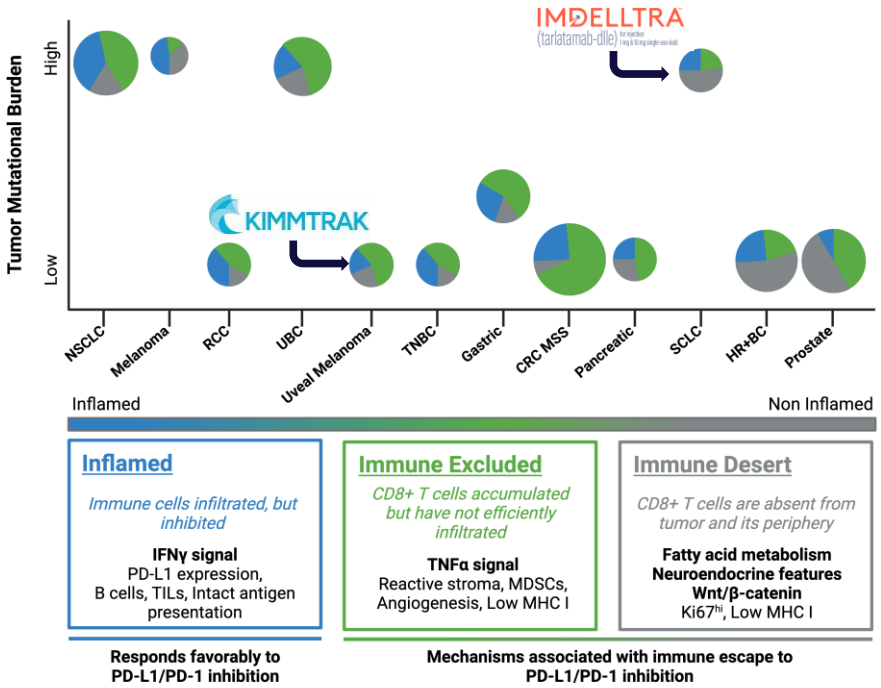


- T cell Engagers (TCEs) are classically designed as bi-specific antibody like proteins that simultaneously engage
  T cells and tumor cells leading to targeted tumor cell death

- Targeting of T cells through anti-CD3 arm leads to T cell recruitment and activation

- Targeting of tumor cells through anti-Tumor Associated Antigen (TAA) antibody increases specificity of killing

T Cell Engagers Active in Solid Tumors, but Unmet Need Remains



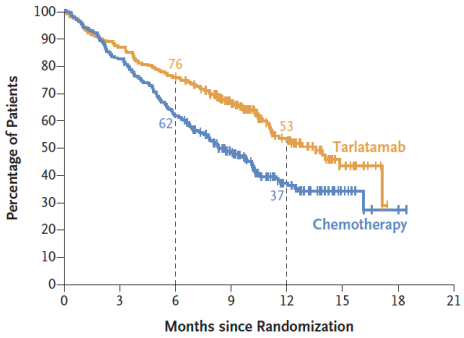
Adapted from Hedge and Chen 2020 Immunity 52

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Tarlatamab in Small-Cell Lung Cancer after Platinum-Based Chemotherapy

N ENGL J MED 393;4 NEJM.ORG JULY 24, 2025



Median Overall Survival (95% CI)

	mo
Tarlatamab (N=254)	13.6 (11.1–NR)
Chemotherapy (N=255)	8.3 (7.0–10.2)

Stratified hazard ratio for death, 0.60 (95% CI, 0.47–0.77)
P<0.001

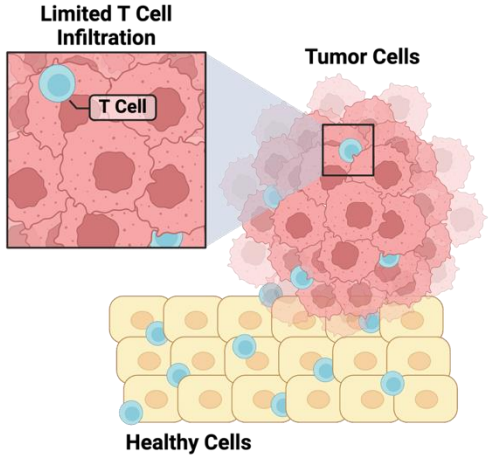
No. at Risk

Tarlatamab	254	220	192	131	60	17	0
Chemotherapy	255	210	156	97	42	9	2	0

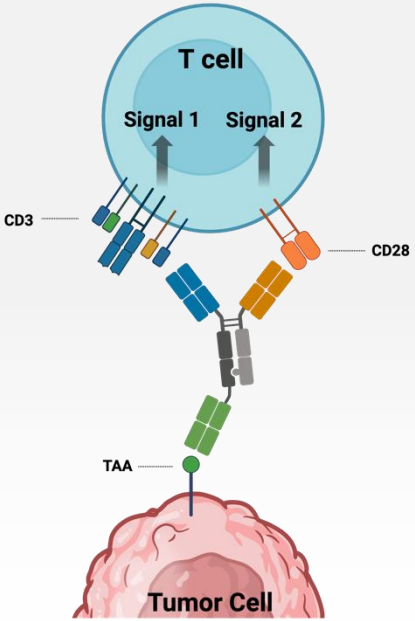
	ORR	PFS	OS	Gr3 AE
Tarlatamab	35%	4.2m	13.6m	54%
Chemotherapy	20%	3.7m	8.3m	80%

Zymeworks Trispecific Co-Stimulatory T Cell Engagers: Overcoming Lack of Efficacy and Durability of Responses in Solid Tumors by Optimization of Signal 1 and 2

Low T cell infiltration and primary or treatment related 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**

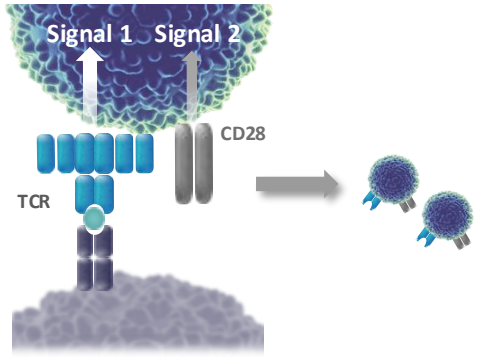
Engineered to balance signal 1 and 2 for optimized **target-dependent T cell activation** and expansion

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

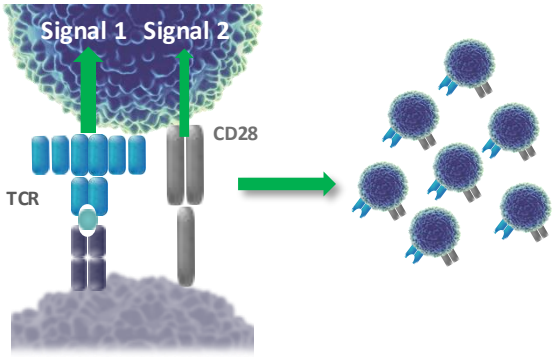
Arvedson T et al Ann Rev Cancer Biol 2022

Balance of T Cell Activation by Signal 1 and Signal 2 Critical to Achieve Optimal T Cell Activation And Prevent Severe Adverse Events

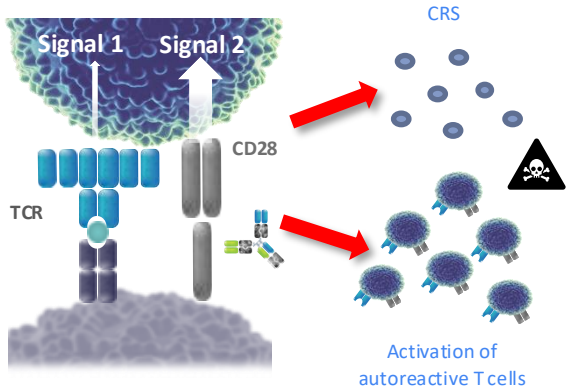
No signal 2
T cell anergy and limited T cell proliferation



Optimal Signal 1 and Signal 2
T cell activation and proliferation



Strong T cell (CD28) Activation
Induction of cytokine release syndrome (CRS) and/or immune-related adverse events (irAEs)



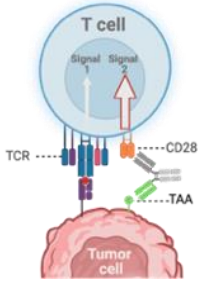
T cell anergy, reduced T cell activation and proliferation

Optimal signal strength for T cell activation

Toxicities associated with T cell overactivation

CD28 Co-stimulatory T Cell Engager Approaches

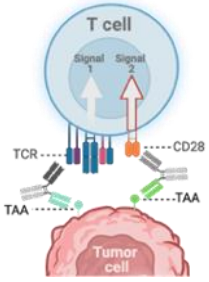
Bispecific CD28 T cell Engagers



CD28 x TAA +/- PD1

Limitations:

Initial clinical activity for CD28-TAA +PD1, but potential toxicity due to autoreactive T cells[1]

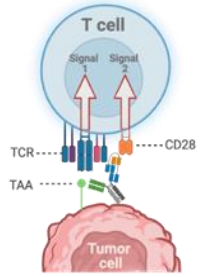


CD28 x TAA + CD3 x TAA

Limitations:

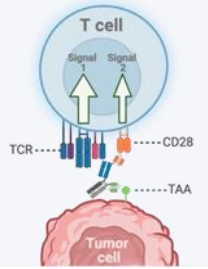
- Optimized for single agent activity and strong CD28 agonism, potential for similar toxicity to CD28-TAA and difficult to optimize by dose adjustment
- Exposure of two molecules at required dose levels potentially suboptimal

Trispecific CD28 T cell Engagers



First Generation:

- High affinity CD3 and CD28 superagonist paratopes[2,3]
- T cell binding, activation and TMDD observed in periphery[2,3]
- Target-independent activity and T cell activation



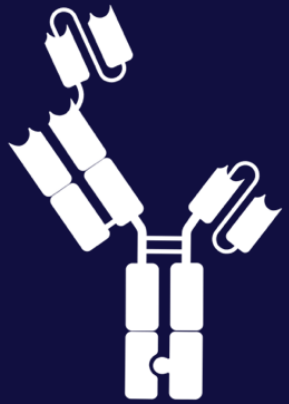
Zymeworks' Next Generation Solution:

- 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

[1]Stein et al., Journal Clinical Oncology (2023); [2]Seung et al., Nature (2022); [3]Promsote et al., Nature Communications (2023)
TAA: tumor-associated antigens, TMDD: Target-mediated drug disposition

ZW209

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



On track for IND submission in 2026

Optimized design

- Potential first-in-class costimulatory TriTCE targeting DLL3
- Optimized DLL3, CD3, CD28 binding affinities and coordinated binding geometry using Azymetric™ and EFECT™ platforms
- Leverages obligate cis-T cell binding and conditional CD28 engagement to prevent unintended T cell activation and fratricide

Differentiated profile

- Superior in vitro and in vivo pharmacology profile relative to DLL3 x CD3 bispecifics including tarlatamab
- Integrated co-stim drives long-term cytotoxicity at low effector to T cell ratios
- Increased T cell proliferation, survival, and anti-tumor activity upon prolonged exposure

Significant patient need

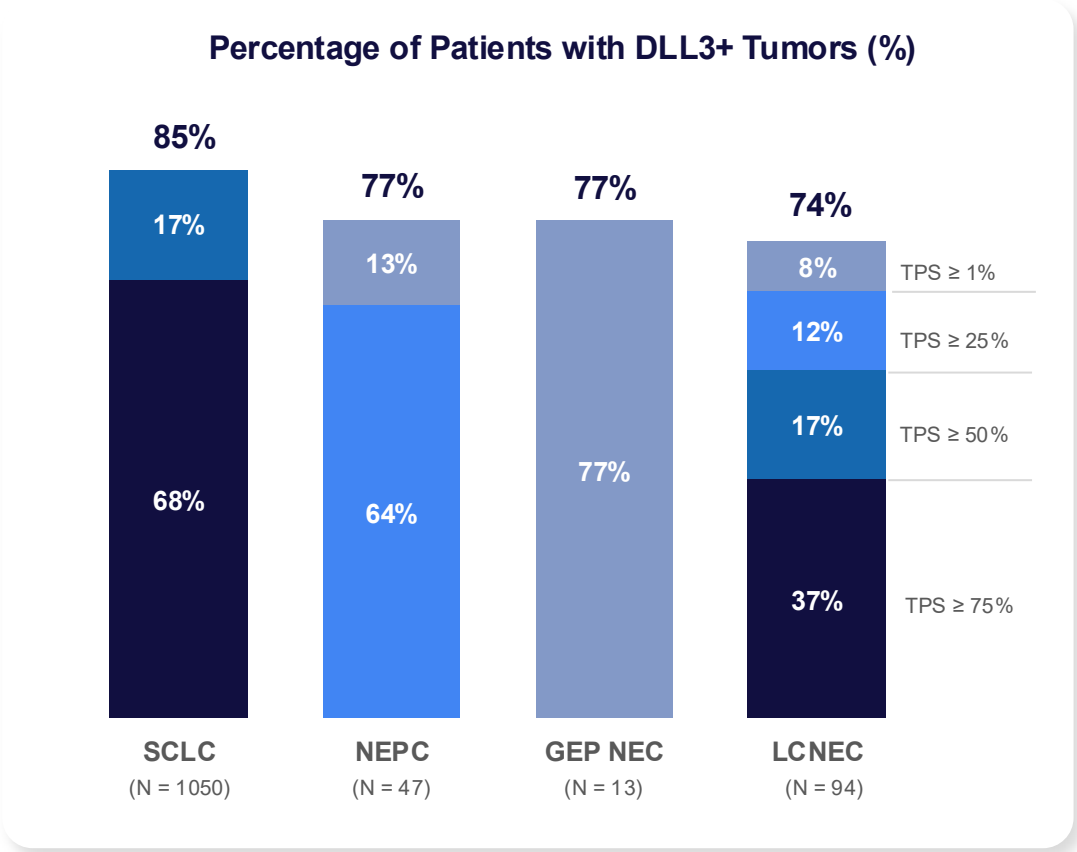
- DLL3 is expressed on the surface of SCLC and other neuroendocrine tumors but rarely on the surface of normal cells
- SCLC accounts for about 15% of all lung cancer diagnoses in the U.S. each year[1]

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 TCell Engager.

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

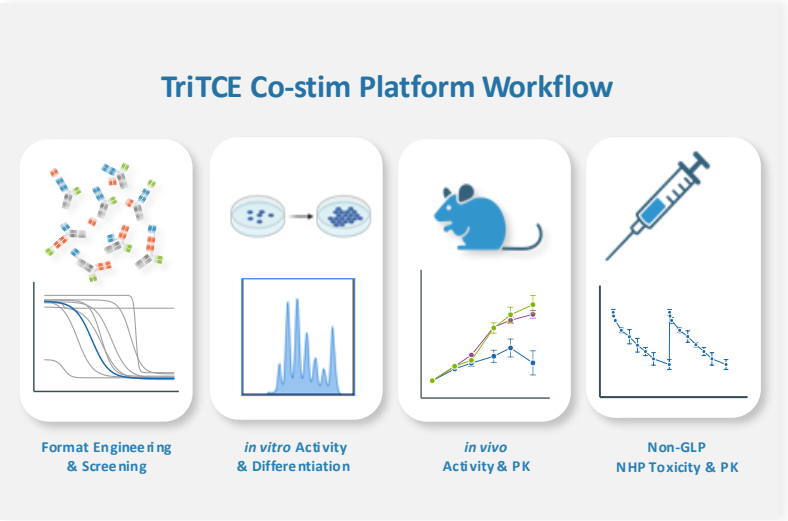
- Responsiveness of DLL3-expressing tumors to TCE modality validated with Imdelltra™ and other DLL3 bispecific TCEs; however, opportunity for improved responses remains
- DLL3 is expressed on the surface of SCLC and other neuroendocrine tumors but rarely on the surface of normal cells
- Clean expression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



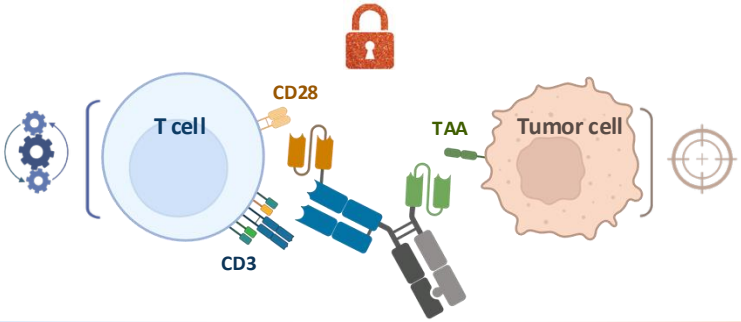
Adapted from: Rojo F et al. Lung Cancer 2020. International real-world study of DLL3 expression in patients with small cell lung cancer. Puca L et al. Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. Sci Transl Med. 2019. 11: eaav0891. Liverani C et al. Endocrine Pathol 2021. Diagnostic and Predictive Role of DLL3 Expression in Gastroenteropancreatic Neuroendocrine Neoplasms. 32:309-27. Hermans BCM et al. DLL3 expression in large cell neuroendocrine carcinoma (LCNEC) and association with molecular subtypes and neuroendocrine profile. Lung Cancer 2019. 138:102-8. DLL3: Delta-like Ligand 3; GEP NEC: Gastroenteropancreatic Neuroendocrine Cancer; LCNEC: Large Cell Neuroendocrine Cancer; NEPC: Neuroendocrine Prostate Cancer; SCLC: Small Cell Lung Cancer TCE, T cell engager; TPS: Tumor Proportion Score.

TriTCE Co-stim: A Differentiated TCE Platform with conditional *cis* CD28 co-stimulation and transferability to diverse targeting strategies

Established workflow, transferable format
Validated on multiple TAAs, including CLDN18.2, DLL3



Locked CD3/CD28 geometry:
optimized for efficient conditional *cis* CD28 co-stimulation and strict TAA dependence



Fine-tune CD3 and CD28 affinity:

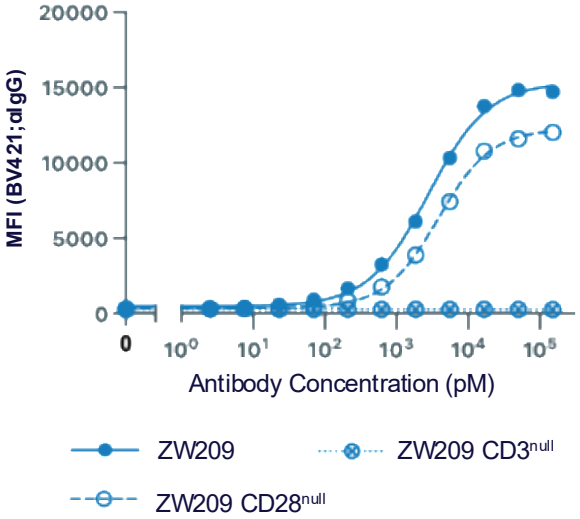
- Cytotoxic potency
- T cell activation
- Cytokine production
- T cell proliferation

Versatile tumor targeting solutions

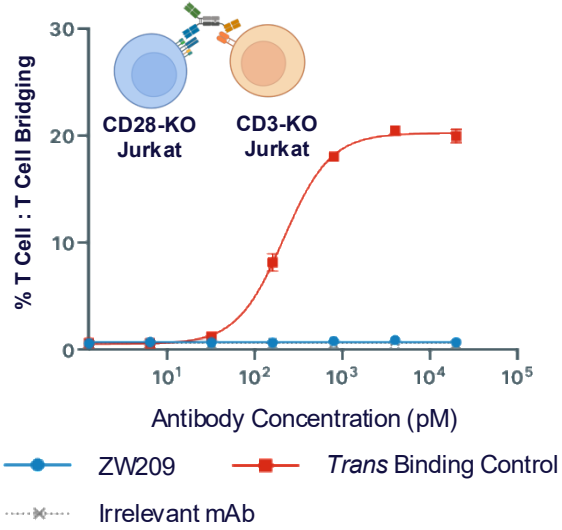
- Monovalent/bivalent Fab, scFv, VHH
- Multi-TAA logic-gated designs
- pMHC targeting

ZW209 Design Facilitates Desirable T Cell Engagement

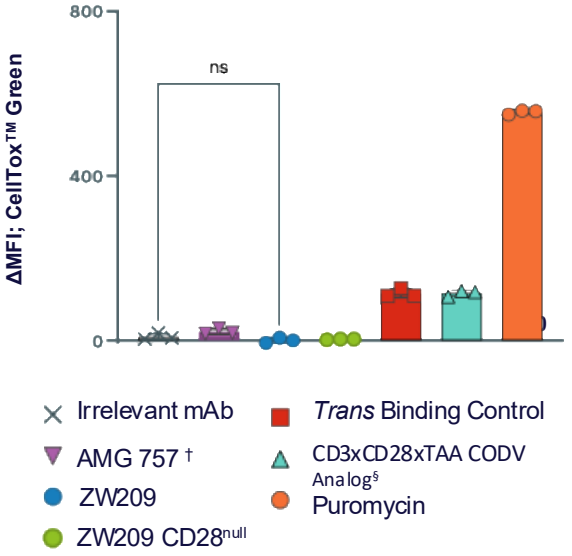
Conditional binding of CD28, requiring co-engagement of CD3



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

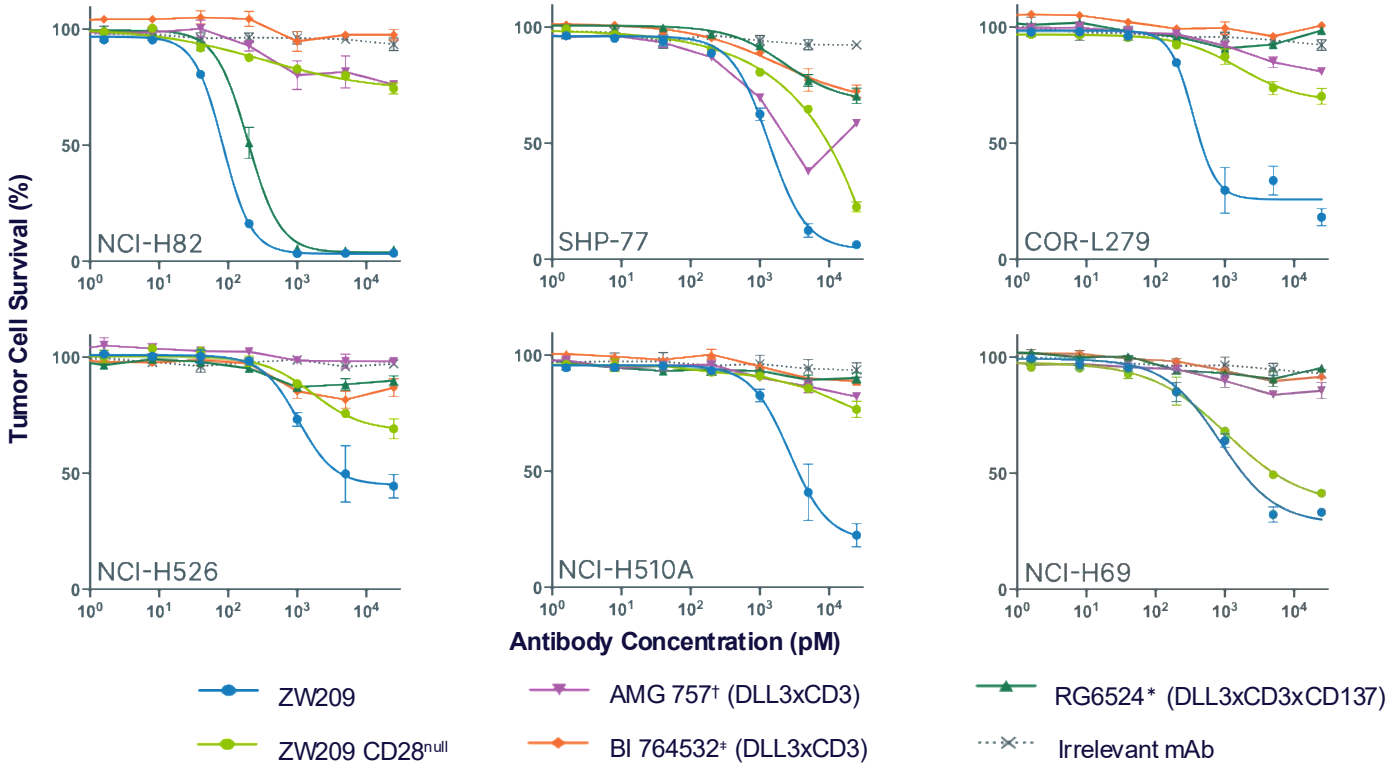


ZW209 does not mediate T cell lysis



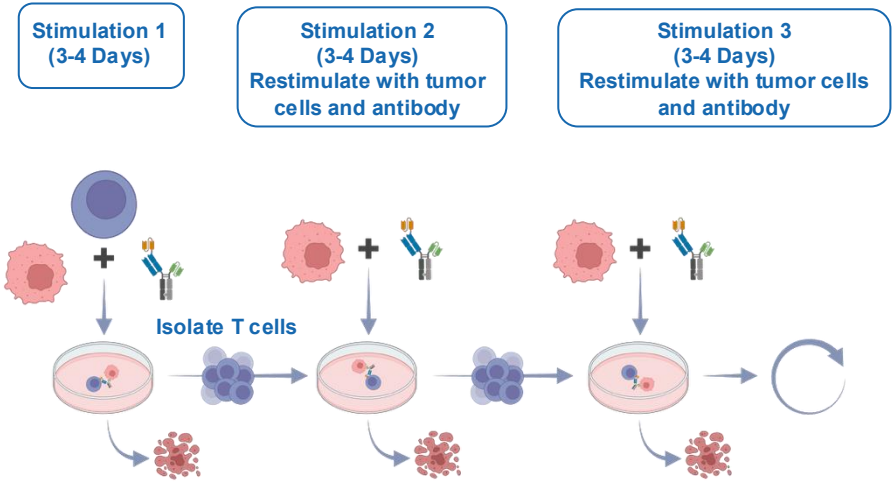
LEFT On cell binding of ZW209, ZW209 CD3^{null} and ZW209 CD28^{null} to human pan T cells assessed by flow cytometry. **MID** Ability of trispecific antibodies to cross-link of CD3-KO and CD28-KO Jurkat cells measured by flow cytometry. Representative schematic of cell bridging (**inset**). **RIGHT**Antibody 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. Lau *et al.* AACR 2025

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

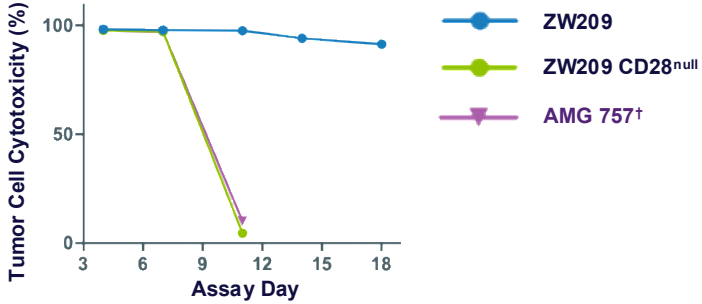


Test articles were incubated with T cells co-cultured with DLL3-expressing SCLC tumor cell lines at low E:T ratio for 7 days and evaluated for cytotoxicity. Lau *et al.* AACR 2025

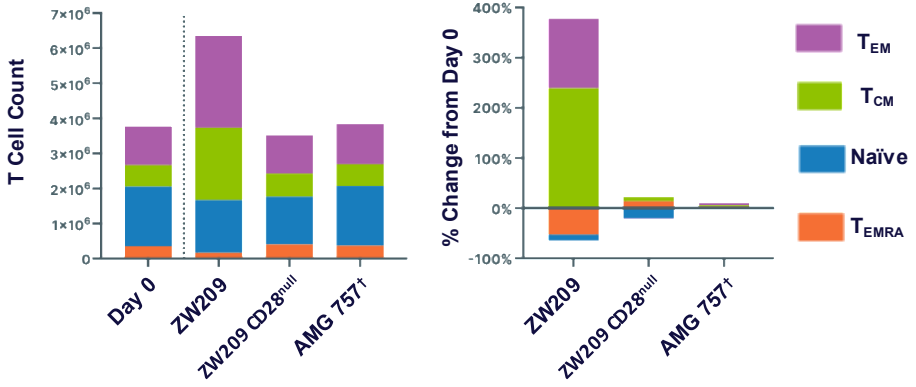
ZW209 Mediates Sustained T Cell-Mediated Cytotoxicity Over Repeated Stimulations



Sustained cytotoxicity relative to bispecific TCEs



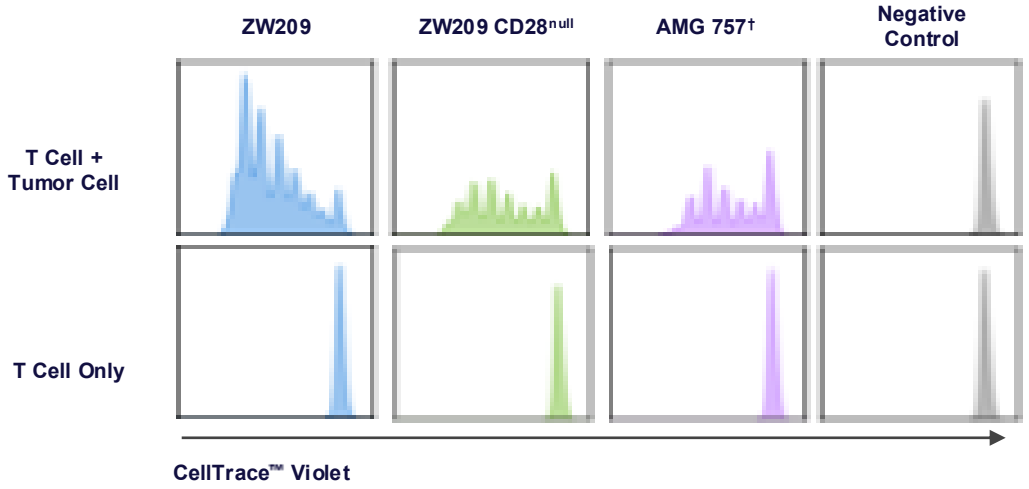
Expanded effector memory (T_{EM}) and central memory (T_{CM}) T cell populations after 2nd stimulation (Day 7)



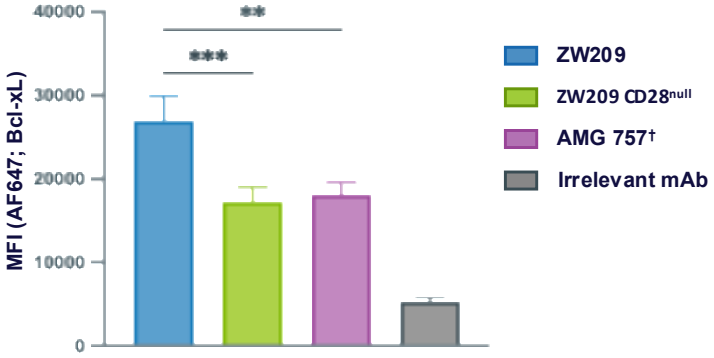
T cells were incubated with DLL3+ NCI-H82 cells and test article. For each subsequent round of stimulation, T cells were collected, counted, and re-stimulated with fresh NCI-H82 target cells and test article. Schematic of T cell restimulation. Following each round of stimulation, co-cultures were assessed for tumor cell cytotoxicity. Following 3rd stimulation, ZW209 CD28null and AMG 757† showed no anti-tumor activity. 3 days after 2nd 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. Lau *et al* AACR 2025

ZW209 Mediates Enhanced DLL3-dependent T Cell Proliferation and Survival

Target-Dependent T Cell Proliferation



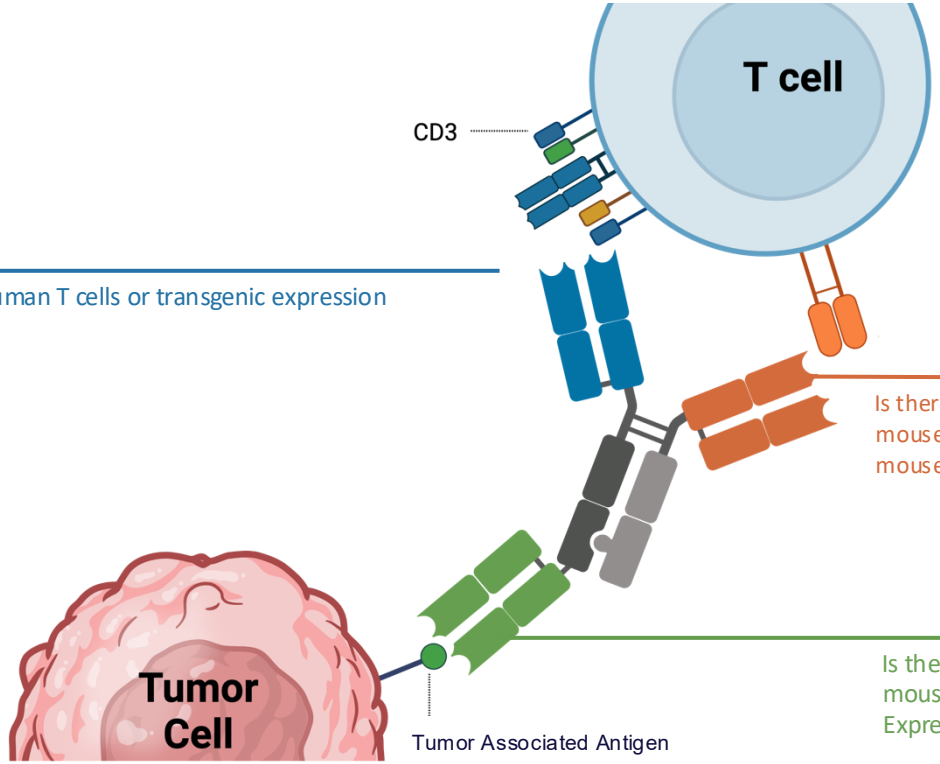
T Cell Survival



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. Lau *et al* AACR 2025

How about preclinical *in vivo* models?

Engineering of TCEs Increases Complexity of *in vivo* Modeling



αCD3 Paratope

Requires presence of human T cells or transgenic expression of human CD3

Co-stimulation Paratope

Is there cross-reactivity to mouse proteins or is a transgenic mouse required? Is your signaling pathway intact? Does the mouse biology reflect the human biology?

αTAA Paratope

Is there cross-reactivity to mouse protein or is a transgenic mouse required? Cell line expression of human protein? Expression patterns.

PBMC Humanized Models: Admix vs Established

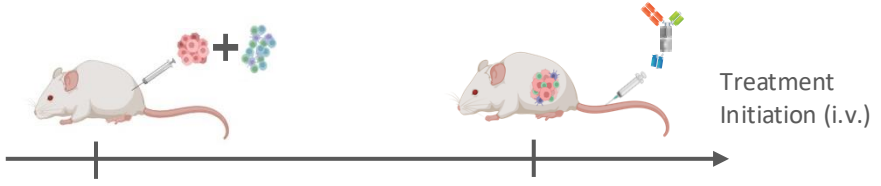
Admix

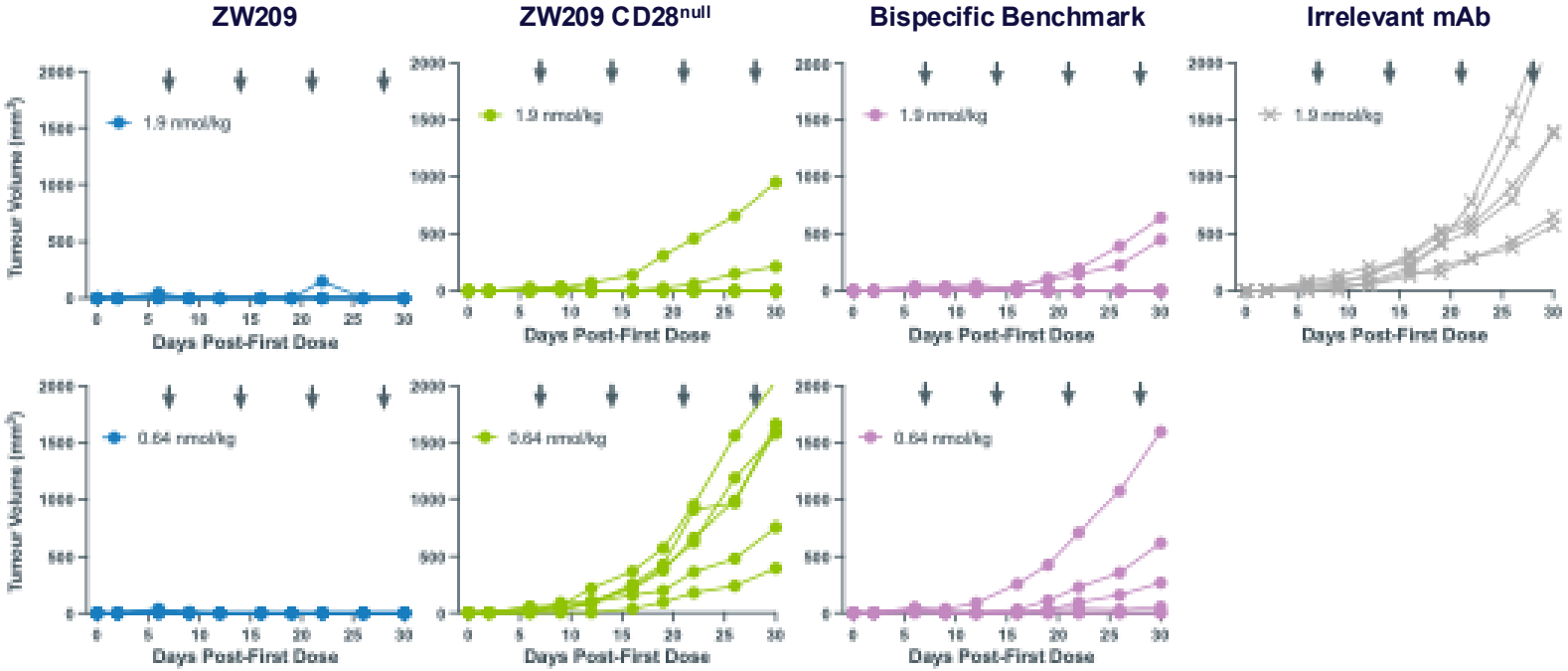
- Controlled tumor/T cell ratio
- Fast and cost-effective
- Ideal for rapid initial screening of multiple constructs

Established

- More physiologic T cell priming and engagement
- Allows control over experimental window (immune reconstitution vs tumor growth)
- Allows assessment of target specificity and donor variability effects

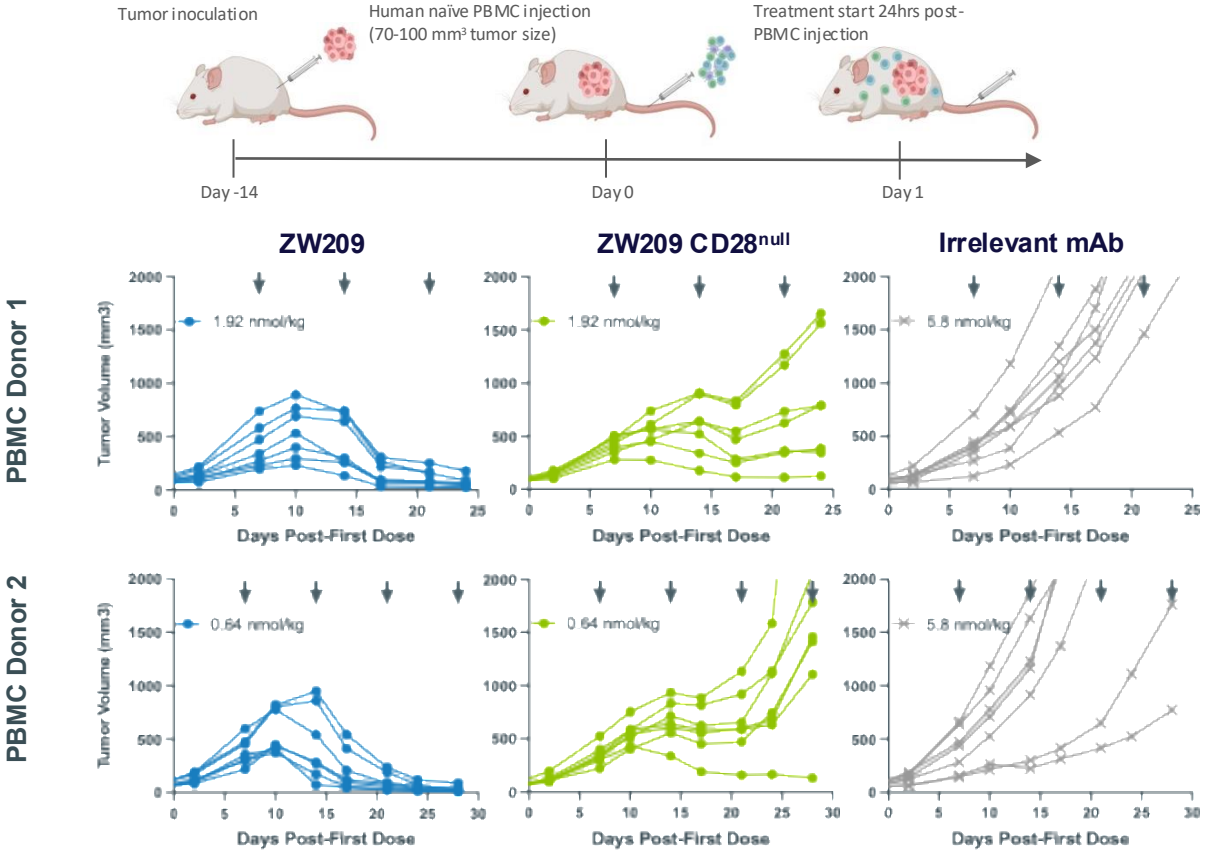
ZW209 Mediates Enhanced Anti-tumor Activity in an Admixture Xenograft Model





Tumor volume over time of mice treated IV with ZW209, ZW209 CD28^{null} or AMG 757 at 1.9 or 0.64 nmol/kg, q.w. x 5 (arrows indicate dosing days). Lau *et al* AACR 2025

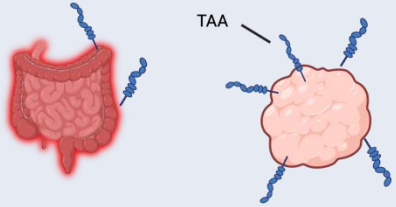
ZW209 Mediates Enhanced Anti-tumor Activity in an Established Xenograft Model



Tumor volume over time of mice treated IV with ZW209 or ZW209^{null} at 1.92 or 0.64 nmol/kg, q.w. x 4 (arrows indicate dosing days). Lau *et al* AACR 2025

Tolerability Concerns for TCEs

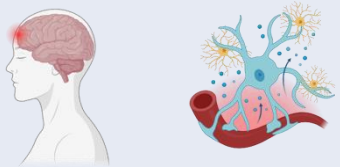
On Target Off Tumor Activity



Low TAA on Organ

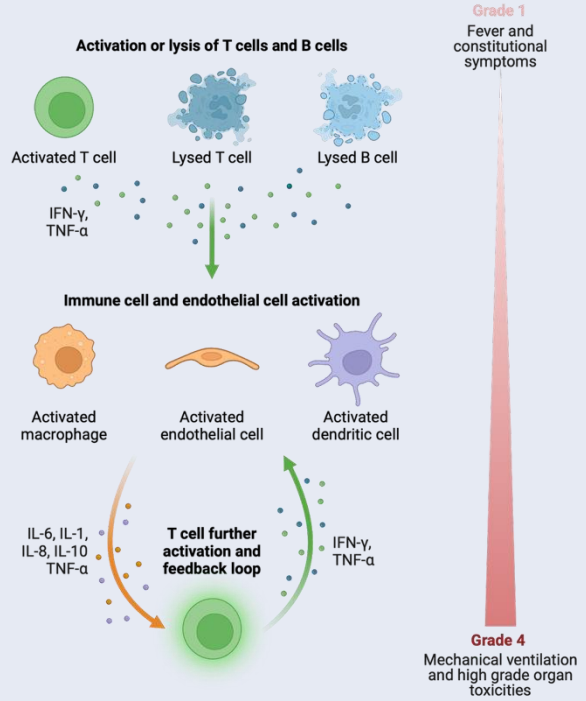
High TAA on tumor

Neurotoxicity

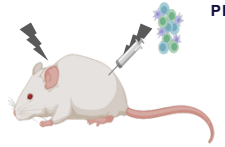


Immune cell-associated Neurotoxicity Syndrome (ICANS)

Cytokine Release Syndrome



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



NSG-MHC I/II DKO

- Irradiate Mice
- Engraft PBMCs

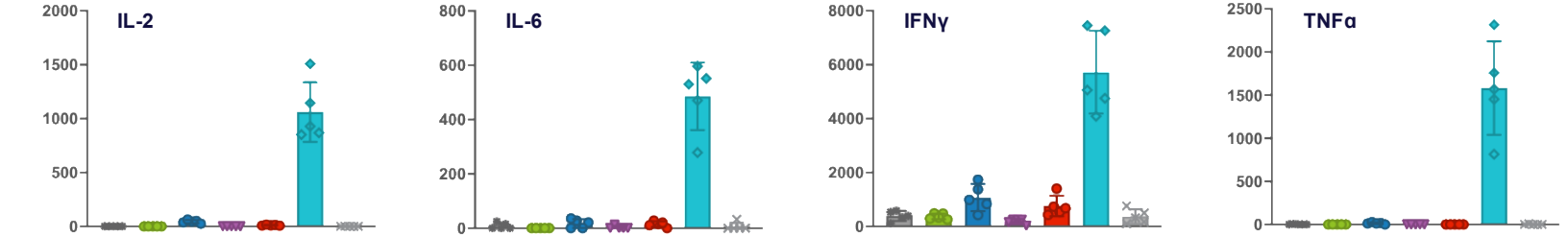
- Antibody Treatment
- All dosed 2 mg/kg except OKT3 (0.25mg/kg)

Day 0: Day 6:

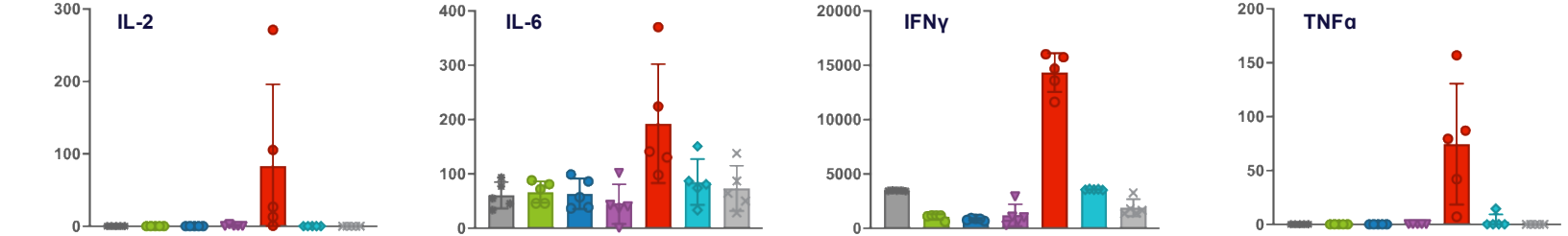
Assess Cytokine Production:

Timepoints:
- **4-hour** – Optimum timepoint for kinetics of CD3 mediated cytokine induction
- **72-hour** – Optimum timepoint for kinetics of CD28 mediated cytokine induction

4 hour

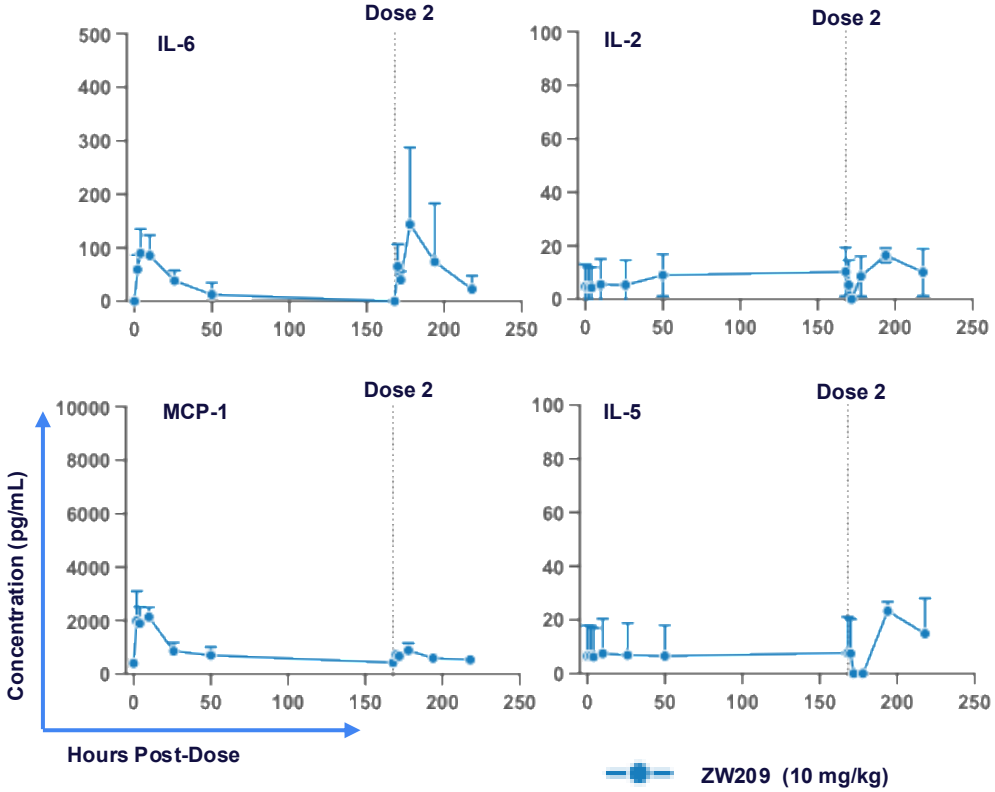


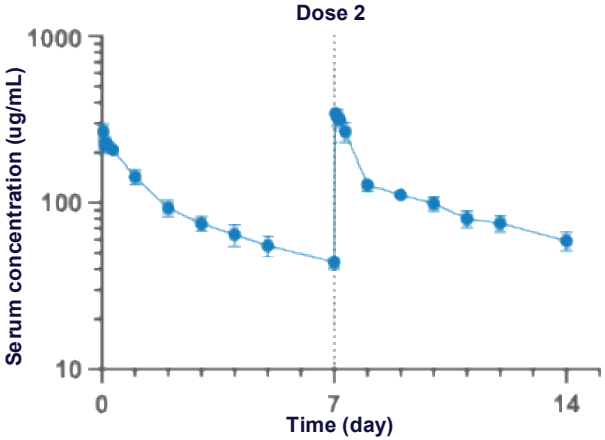
72 hour



Irrelevant mAb
ZW209
TGN1412δ
PBS
ZW209 CD28null
AMG 757†
OKT3

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

Cynomolgus monkeys (n=3) were given a repeat dose of 10 mg/kg ZW209 on day 0 and day 7. Toxicology findings were mild with transient, minor increased in serum cytokines observed and no histopathological changes. ZW209 displayed antibody-like pharmacokinetics with exposure confirmed upon repeat dosing.

Summary

- Zymeworks TriTCE Co-Stim provides balanced CD3 and CD28 activation to prevent overactivation of T cells
- Enhanced tumor target-dependent activity associated with sustained T cell viability and cytotoxicity resulting in improved anti-tumor activity in preclinical models compared to bispecific TCEs
- No CD28 binding in absence of CD3 engagement, lowering the risk of CD28-mediated immune related adverse events, well tolerated in in vivo systemic cytokine release (SCR) models
- ZW209 nominated for development with a planned IND submission in H1-2026

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. Weisser, Thomas Spreter von Kreudenstein

SITC 2025: **TriTCE Co-stim: A differentiated T cell engager platform with conditional *cis* CD28 co-stimulation and transferability to diverse targeting strategies**
Meghan Verstraete, Diego Perez Escanda, Peter Repenning, Desmond Lau, Alec Robinson, Yun Peng, Matteo Zago-Schmitt, Aditi Deshmukh , Diana Canals Hernaez, Fisal Elstone, Polly Shao, Dnyandeo Amberkar, Andrew Sharon, Harpreet Bamra, Larissa Patlan Ramirez, Nan Nan Liu, Caitlin Low, Aaron Tieu, Stella Kauryzhka, Akshay Kamath, Kurt Stahl, Catherine Wu, Richard Kunze, Cindy Park, Nichole Escalante, John Zhang, Hamed Shirvani, Begonia Silva Moreno, Gursev Anmole, Anna Von Rossum, Liz Halvorsen, David Kroeger, Lisa Newhook, Purva Bhojane, Chayne L. Piscitelli, Genevieve Desjardins, Nicole Afacan, Paul A. Moore, Thomas Spreter von Kreudenstein, Nina E. Weisser

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. Weisser

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 Zwierzchowski, Alec Robinson, Lauren Clifford, Harsh Pratap, David Douda, Chayne Piscitelli, Nicole Afacan, Thomas Spreter von Kreudenstein, Nina Weisser

Zymeworks' Multispecific Antibody Therapeutics Team