



Engineering Next-Generation T Cell Engagers: A Trispecific Platform for Cancer Immunotherapy

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Festival of Biologics

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Differentiated Pipeline of Multifunctional Therapeutics

Program	Technology	Target	Indication	Discovery	Preclinical	Phase 1	Phase 2	Phase 3
Solid Tumor Oncology: Antibody-Drug Conjugates (ADC)								
ZW191 TopoII ADC DAR 8 Fc WT	ZD06519 Payload	FR α	Gynecological Thoracic	NCT06555744				
ZW251 TopoII ADC DAR 4 Fc WT	ZD06519 Payload	GPC3	Digestive System (HCC)	NCT07164313				
ZW220 TopoII ADC DAR 4 Fc Mut	ZD06519 Payload	NaPi2b	Gynecological Thoracic					
ZW327 TopoII ADC DAR 8 Fc Mut	ZD06519 Payload	Ly6E	Multiple indications					
Solid Tumor Oncology: Multispecific Antibody Therapeutics (MSAT)								
Zanidatamab Bispecific	Azymetric™	HER2	Multiple indications	Development partners: Jazz Pharmaceuticals and BeOne				
ZW209 Trispecific TCE Tri-TCE Costim	Azymetric™, Novel anti-CD3 Conditional CD28	DLL3 x CD3 x CD28	Thoracic	Anticipated IND in 2026				
ZW239 Trispecific TCE Tri-TCE Costim	Azymetric™, Novel anti-CD3 Conditional CD28	CLDN18.2 x CD3 x CD28	Digestive System					
Autoimmune & Inflammatory Diseases								
ZW1528 Dual Cytokine Blocker	Azymetric™ Hetero-Fab YTE	IL4R α x IL-33		Anticipated regulatory submission in 2026				
ZW1572 Dual Cytokine Blocker	Azymetric™ Hetero-Fab YTE	IL4R α x IL-31						

Azymetric™: Multispecific Antibody Platform Adaptable to Different Formats

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 (e.g. YTE). Compatible with linker/payload conjugation

High-throughput Screening

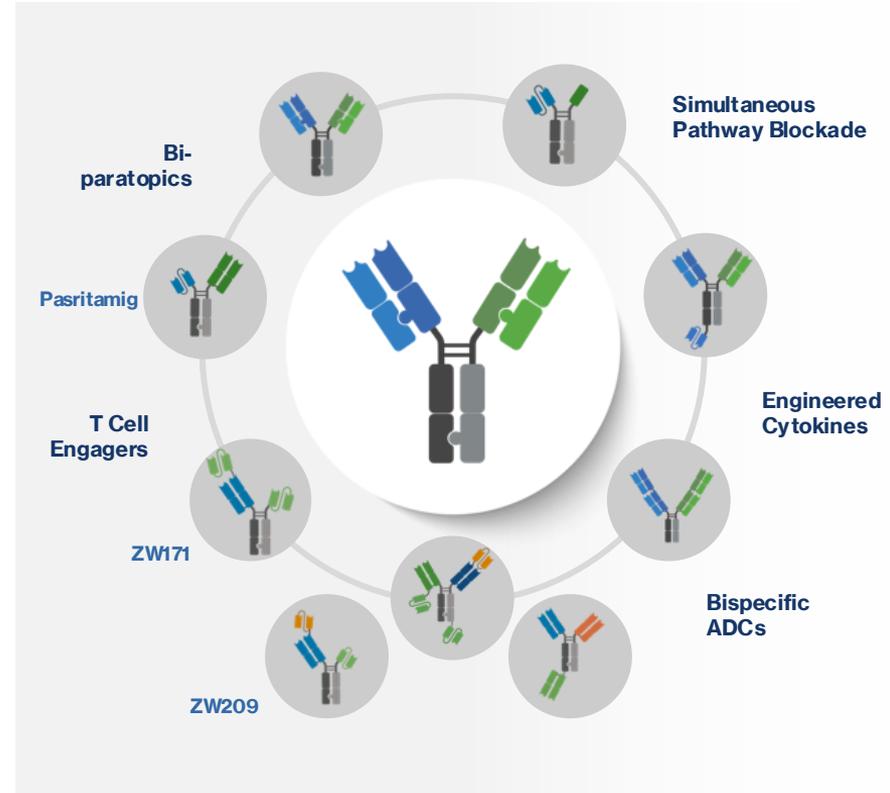
Best-in-class activity requires screening of alternative targets, epitopes, sequences, target engagement geometries, and mechanisms of action (blocking, lytic, ADC)

Highly Manufacturable

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

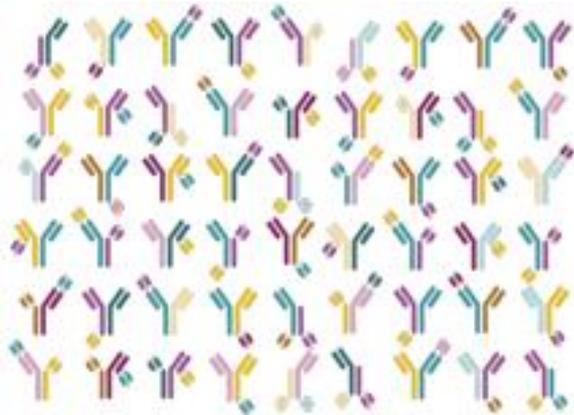


Flexible Formatting Enables Range of Applications



Format Matters! Unique Formats Drive Novel Biology

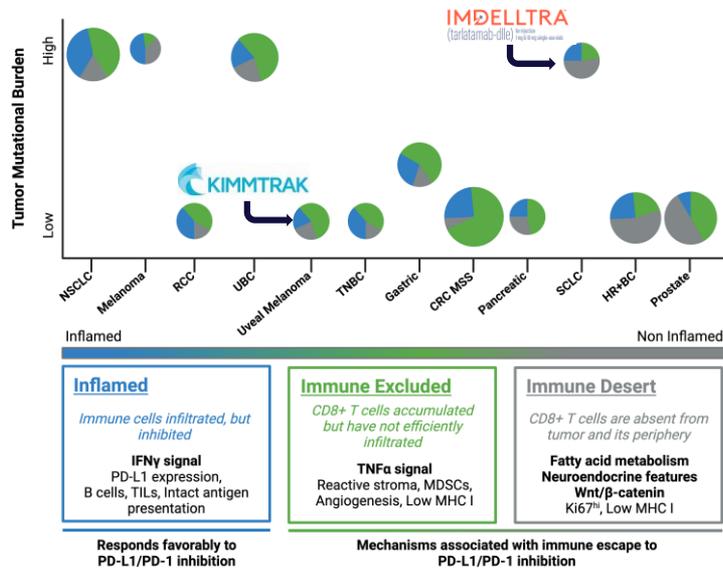
- Interrogating a broad geometric and format space is critical to identifying differentiated candidates
- Traditional multispecific antibody screens often limited in number of formats tested



Azymetric™ facilitates efficient heterodimeric antibody assembly

- Allows for HTP production and screening of multiple formats
- Allows for identification of geometries with novel biology

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



Adapted from Hedge and Chen 2020 Immunity 52

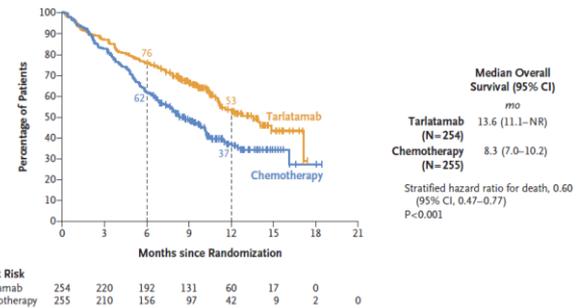
Additional targets to broaden responsive tumor types

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

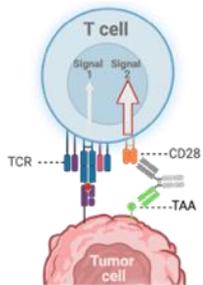


	ORR	PFS	OS	Gr3 AE
Tarlatamab	35%	4.2m	13.6m	54%
Chemotherapy	20%	3.7m	8.3m	80%

Complementary MoA to enhance responses rates

CD28 Co-stimulatory T Cell Engager Approaches

Bispecific CD28 T cell Engagers

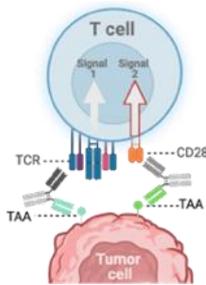


CD28 x TAA +/- PD1

Limitations:

Initial clinical activity for CD28-TAA +PD1, but potential toxicity due to autoreactive T cells¹

Regeneron
(PSMAxCD28)



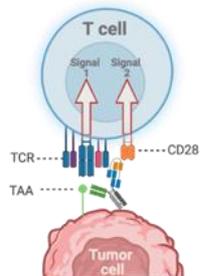
CD28 x TAA + CD3 x TAA

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

Regeneron (MUC16xCD3 + MUC16xCD28, CD20xCD3 + CD22xCD28, PSMAxCD3 + PSMAxCD28)
Janssen (PSMAxCD28 + KLK2xCD3)
Roche (CD20xCD3 + CD19xCD28)

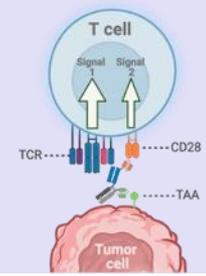
Trispecific CD28 T cell Engagers



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

Sanofi
(HER2xCD3xCD28; CD38xCD3xCD28)

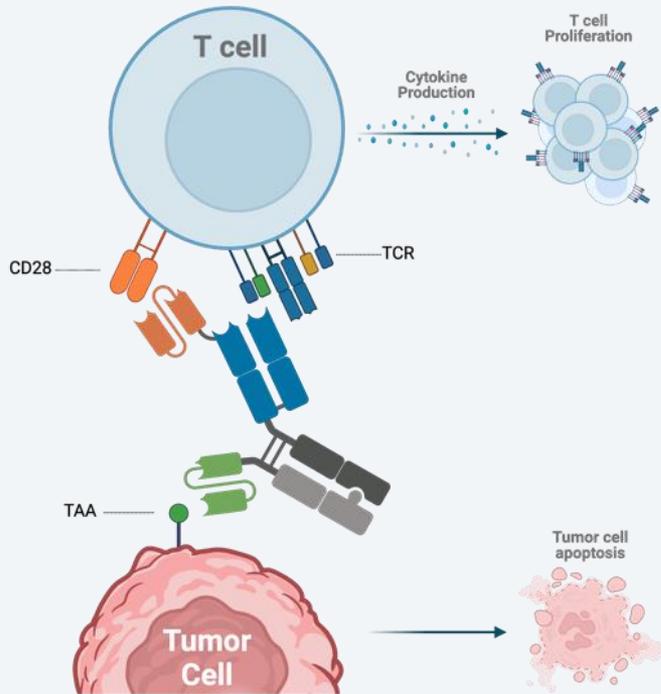


Zymeworks' Next Generation Solution:

- Balanced CD3 and CD28 affinity 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

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

Zymeworks' Solution: TriTCE Co-stim, Designed to Optimize T cell Activation and Anti-Tumor Activity, and Maximize Safety



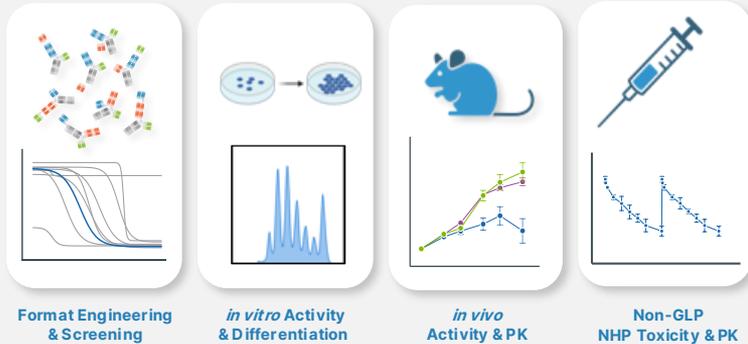
	Design Feature	Expected Benefit
1	Balanced activation of CD3 and CD28	Potential to provide more durable responses and activate T cell responses in 'cold' tumors with lower T cell infiltration
2	Low affinity, Conditional CD28 engagement	Requires co-engagement of CD3
3	Obligate <i>cis</i>-T cell (CD3xCD28) binding	No T cell-to-T cell bridging or T cell fratricide
4	Target-dependent activity	Low T cell binding and no T cell activation in absence of tumor target

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

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

TriTCE Co-stim Platform Workflow

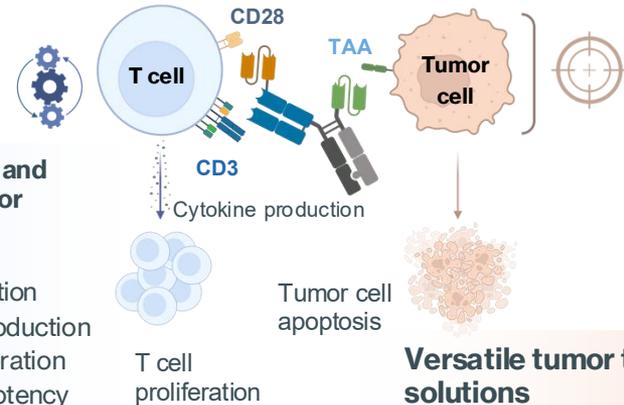


Fine-tune CD3 and CD28 affinity for optimal TAA-dependent:

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

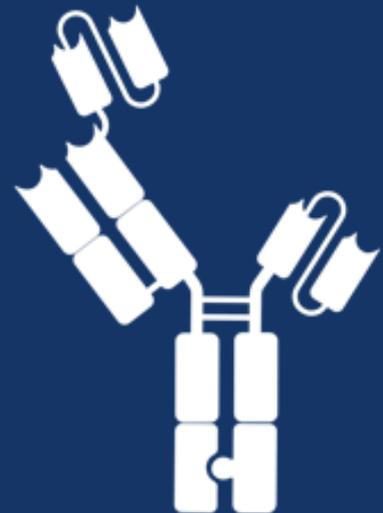


Locked CD3/CD28 geometry in TriTCE Co-stim platform optimized for efficacy and tolerability



Versatile tumor targeting solutions

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



ZW209

DLL3 x CD3 x CD28
TriTCE

On track for Ph1 initiation in 1H 2026

Optimized Design

- ✓ Potential first-in-class costimulatory TriTCE targeting DLL3
- ✓ TriTCE with optimized DLL3, CD3, CD28 binding affinities and geometry
- ✓ *cis*-T cell binding and conditional CD28 engagement to prevent inappropriate T cell activation

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

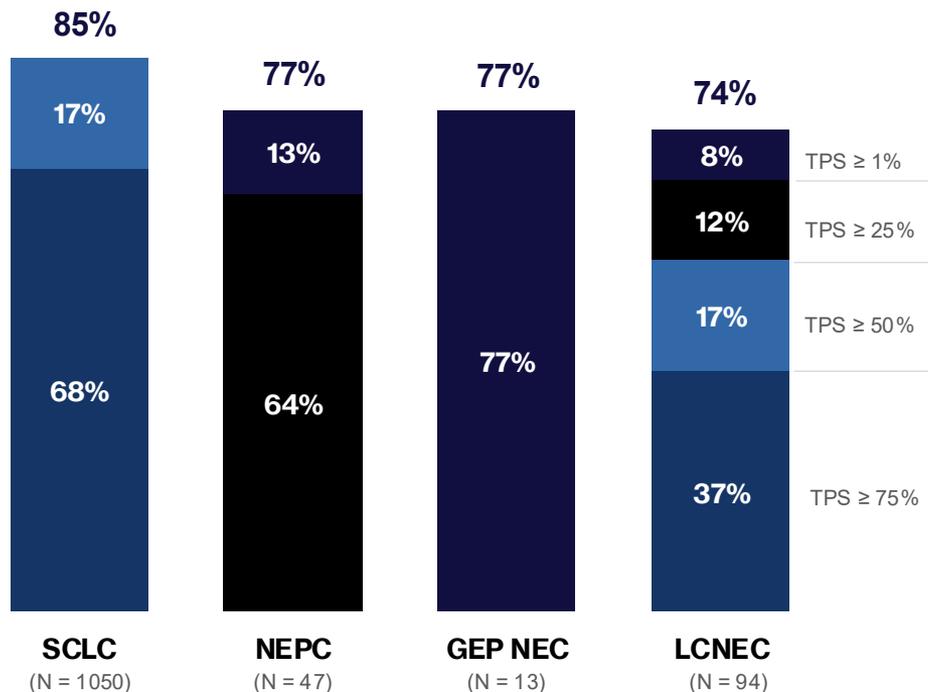
Safety and Developability

- ✓ No T cell activation or cytokine activation in absence of DLL3 target cells
- ✓ No significant toxicity observed at 30mg/kg in GLP NHP tox
- ✓ Antibody like PK profile supportive of IV or SC dosing
- ✓ GLP manufacturing complete, clinical supply available end of Q1 2026

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

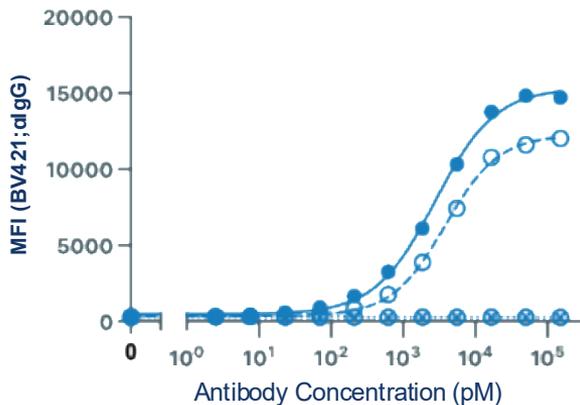
Percentage of Patients with DLL3+ Tumors (%)



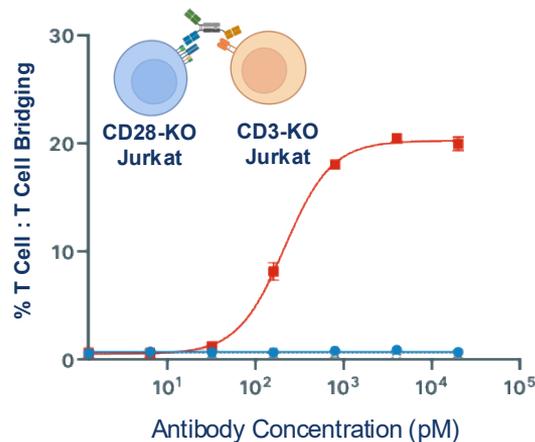
Adapted from: Rojo F et al. Lung Cancer 2020. International real-world study of DLL3 expression in patients with small cell lung cancer. Puca L et al. Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. Sci Transl Med. 2019. 11: eaav0891. Liverani C et al. Endocrine 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. 138:1028. 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.

ZW209 Design Facilitates Desirable T Cell Engagement

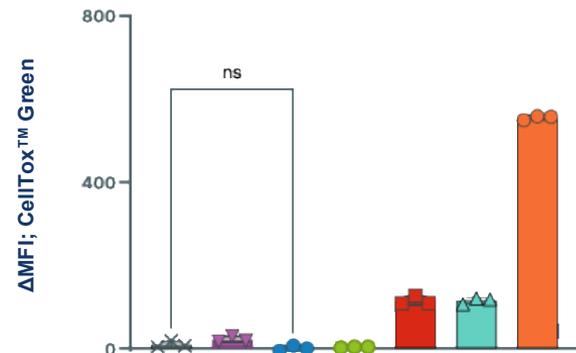
Conditional binding of CD28, requiring co-engagement of CD3



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

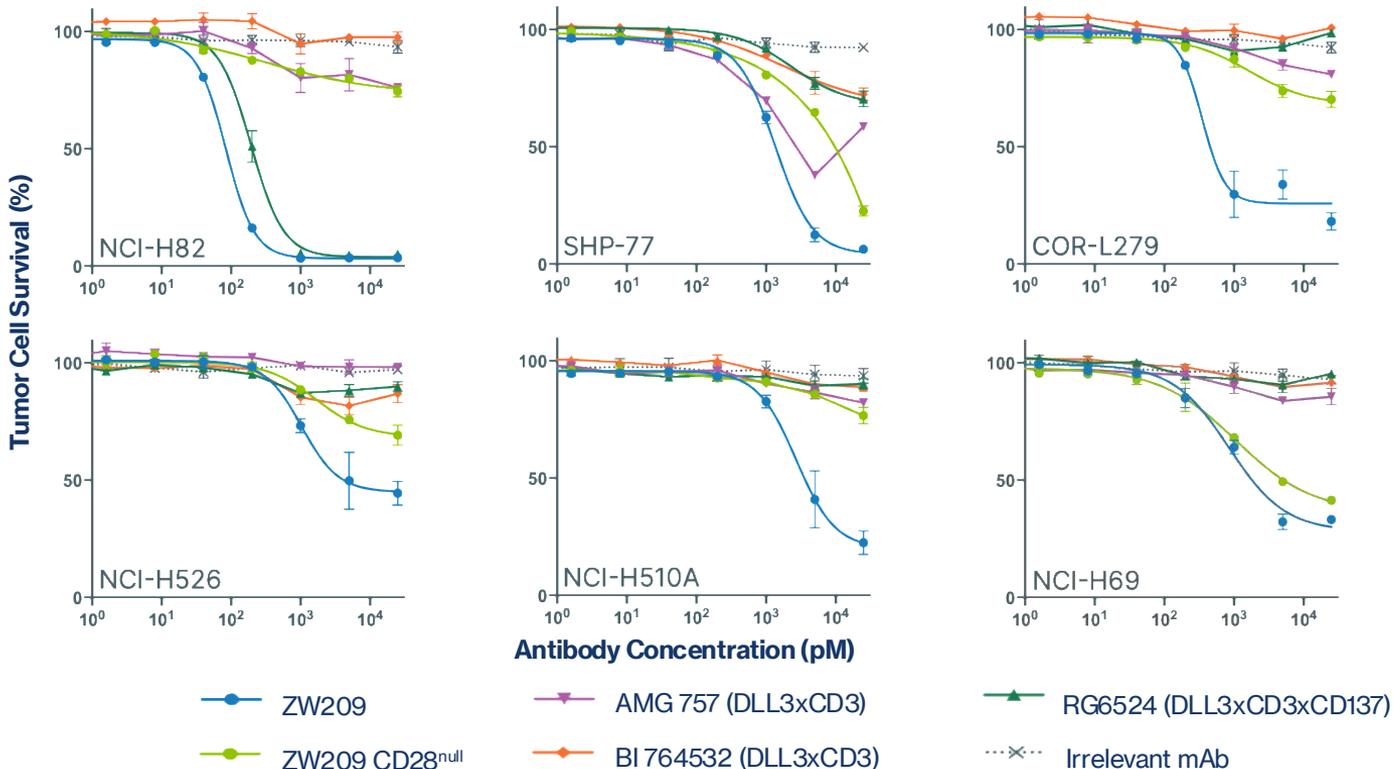


ZW209 does not mediate T cell lysis



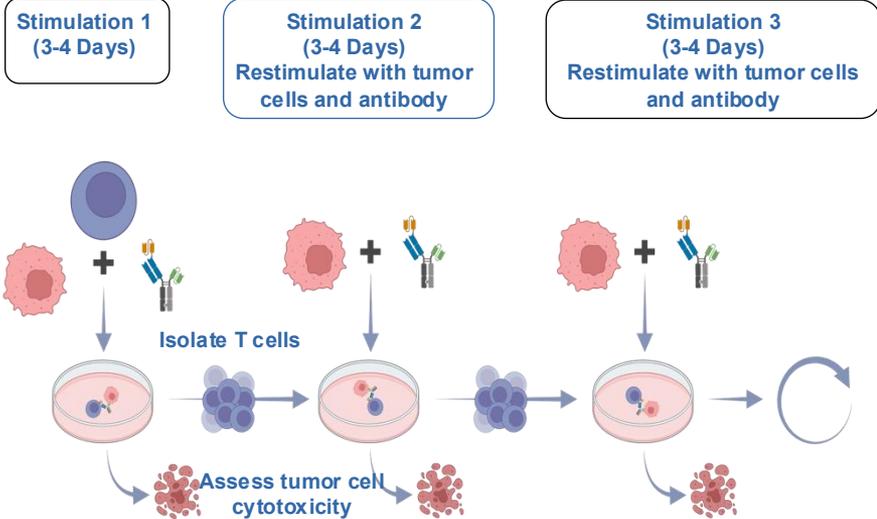
LEFT On cell binding of ZW209, ZW209 CD3^{null} and ZW209 CD28^{null} to human pan T cells assessed by flow cytometry. MID Ability of trispecific antibodies to cross-link of CD3-KO and CD28-KO Jurkat cells measured by flow cytometry. Representative schematic of cell bridging (inset). RIGHT Antibody mediated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

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

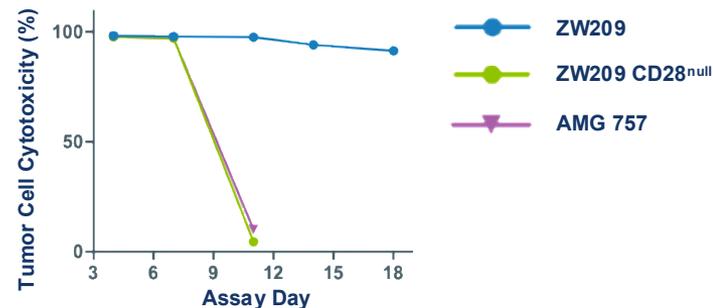


Test articles were incubated with T cells co-cultured with DLL3-expressing SCLC tumor cell lines at low E:T ratio for 7 days and evaluated for cytotoxicity.

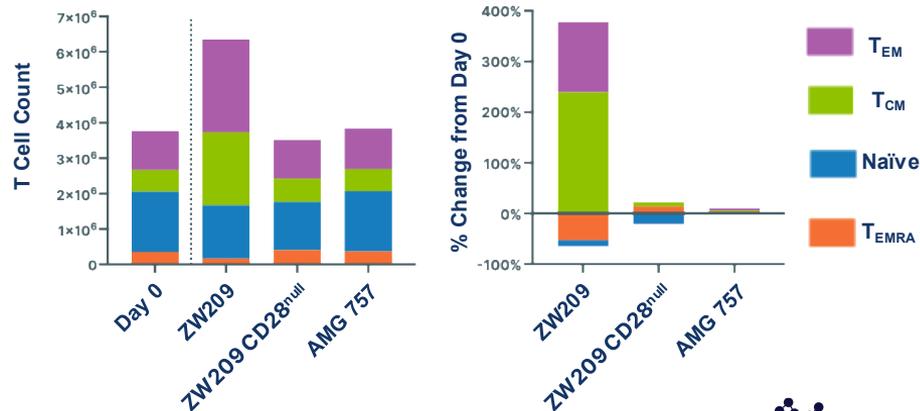
ZW209 Mediates Sustained T Cell-Mediated Cytotoxicity Over Repeated Stimulations



Sustained cytotoxicity relative to bispecific TCEs



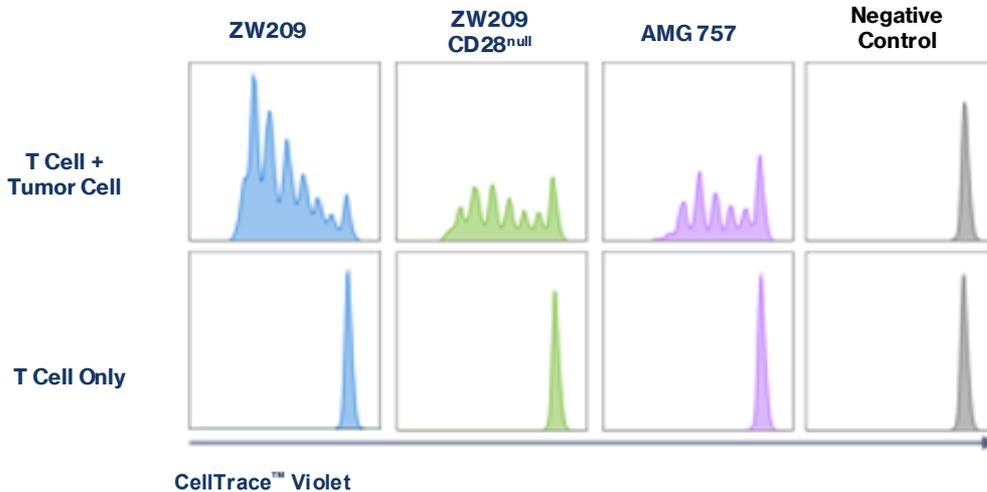
Expanded effector memory (T_{EM}) and central memory (T_{CM}) T cell populations after 2nd stimulation (Day 7)



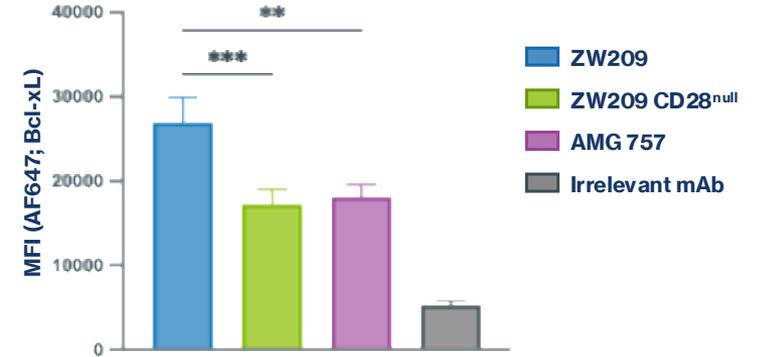
T cells were incubated with DLL3+ NCI-H82 cells and test article. For each subsequent round of stimulation, T cells were collected, counted, and re-stimulated with fresh NCI-H82 target cells and test article. Schematic of T cell restimulation. Following each round of stimulation, co-cultures were assessed for tumor cell cytotoxicity. Following 3rd stimulation, ZW209 CD28^{null} 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.

ZW209 Mediates Enhanced DLL3-dependent T Cell Proliferation and Survival

Target-Dependent T Cell Proliferation

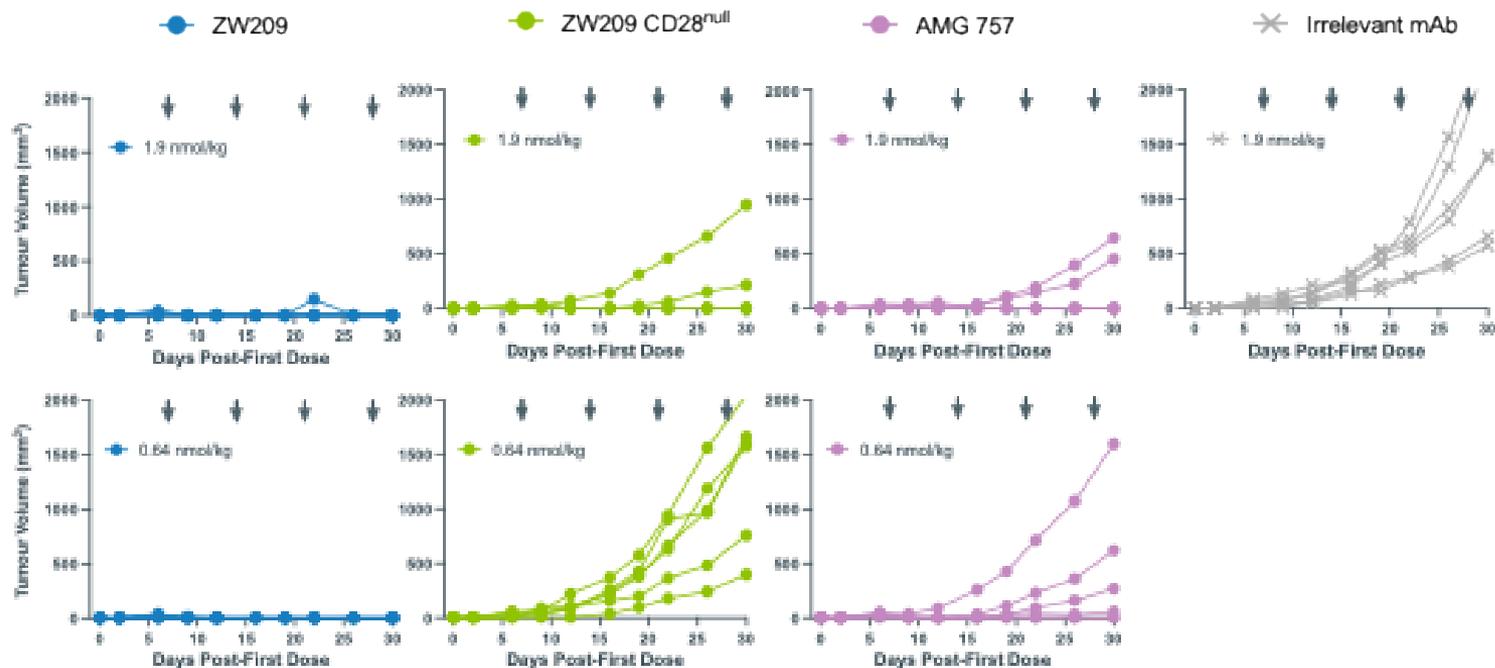


T Cell Survival



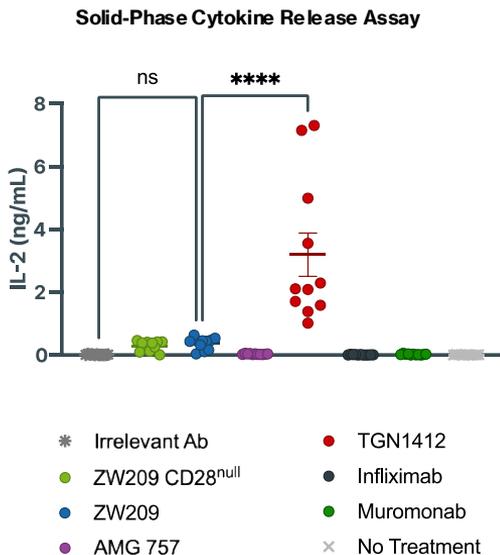
Test articles (5 nM) were incubated with CellTrace Violet™ labeled T cells alone or co-cultured with NCI-H82 cells for 5 days and assessed by flow cytometry. Right Test articles (5 nM) were incubated with T cells co-cultured with NCI-H82 cells for 48 hours and evaluated for Bcl-xL expression by flow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

ZW209 Mediates Enhanced Antitumor Activity Compared to Bispecific and AMG 757 in NCI-H82 Admixture Xenograft Model

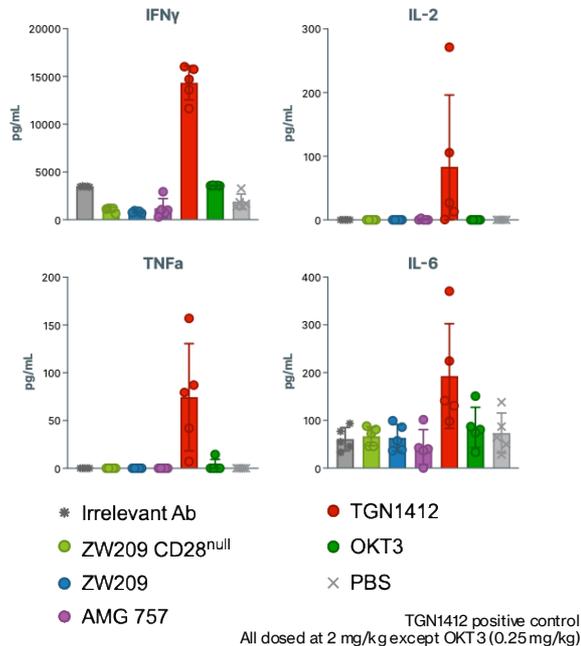


ZW209 Has a Favorable Safety Profile *In Vitro* and in Animal Studies

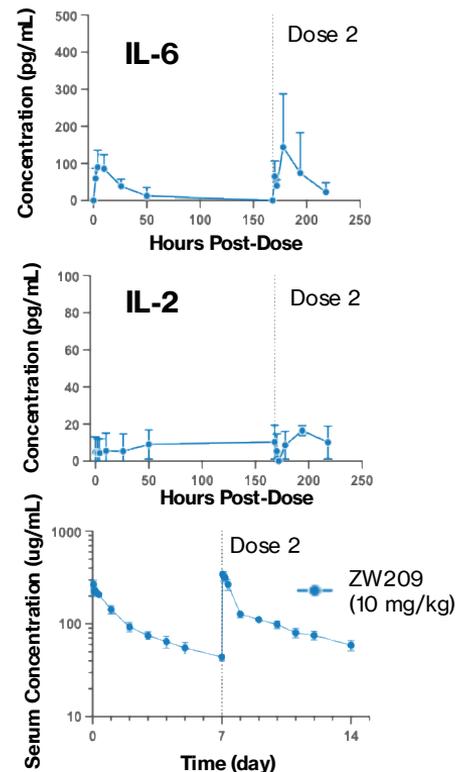
No Cytokine Activation with PBMCs Alone



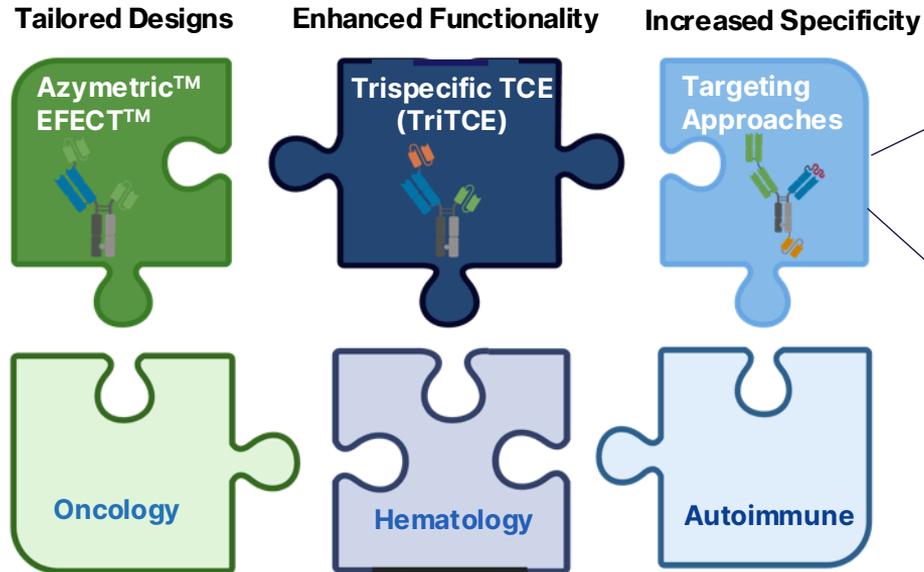
Induces Minimal Systemic Cytokine in Humanized Mouse CRS Model



Well-tolerated in Cynomolgus Monkeys



TriTCE Co-Stim is Transferable Across Diverse Targeting Strategies



Targeting Selectivity

- 2+1+1 formats
- Novel targets (proteomics)
- Multi-antigen targeting
- Intracellular targets

Conditional Activation

- Conditional masking with proprietary cleavage sequence

Versatility of TriTCE Co-Stim Tumor-targeting Domain Allows For Broad Therapeutic Applications Across Diverse Modalities

Solid tumor

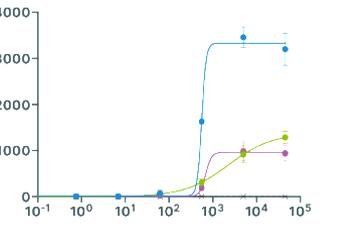
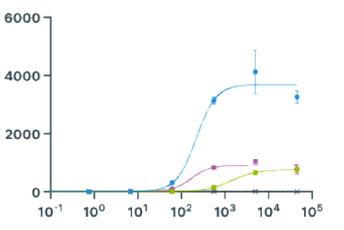
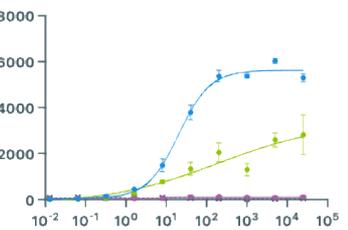
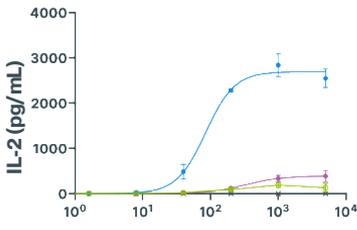
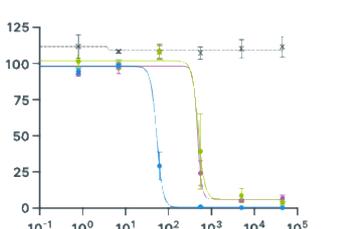
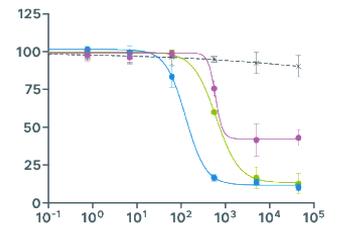
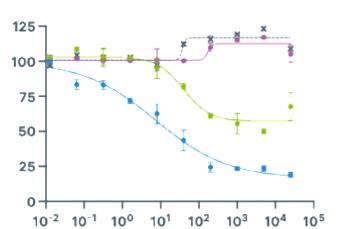
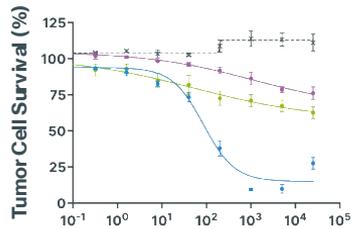
Hematology

1+1+1 (DLL3) Small cell lung cancer

2+1+1 (TAA #2) Colorectal cancer

scFv (TAA #3) Gastric cancer

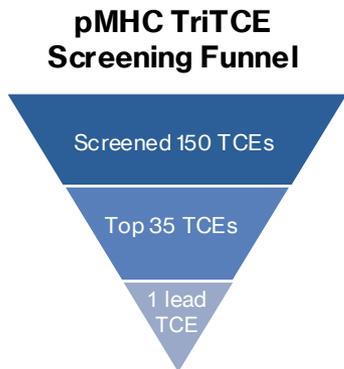
VHH (TAA #4) Multiple myeloma



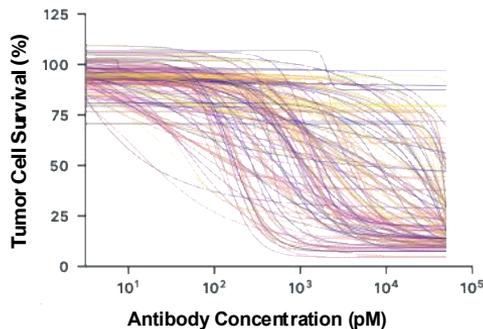
Antibody Concentration (pM)

● TriTCE Co-stim ● Bispecific Control ● Clinical Benchmark -x-x- Irrelevant mAb

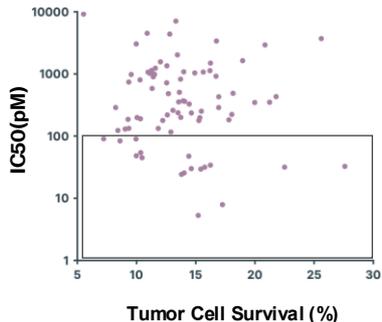
Targeting Peptide-MHC Using TriTCE Co-Stim Platform



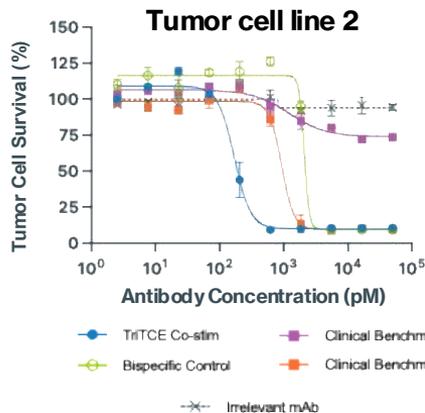
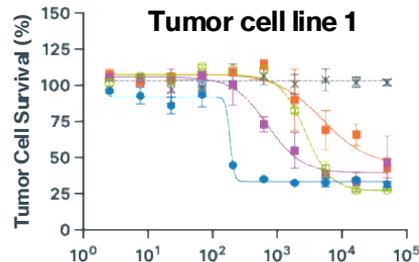
HTP *in vitro* assessment of 150 TCEs



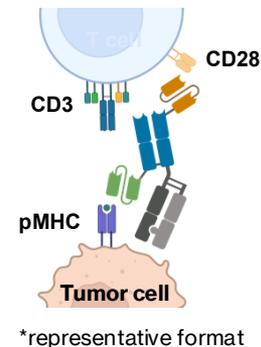
Selection based on cytotoxicity and potency



Lead TCE selection



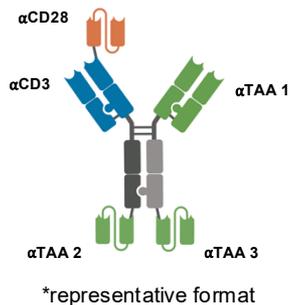
Identification of Lead α pMHC TriTCE format*



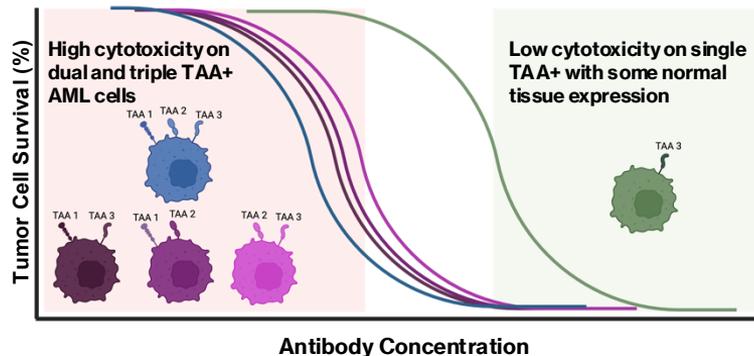
TriTCE Co-Stim Platform Can Be Designed to Overcome the Challenge of Antigen Escape in Acute Myeloid Leukemia

Biological Challenge	Limitation of Mono-antigen Targeted Therapies
Heterogeneous intertumoral antigen expression	Antigen escape
Lack of a clean single target between AML blast, LSCs and healthy cells	Narrow therapeutic window

Screened logic-gated TriTCE antibody formats for selective tumor cytotoxicity in the presence of two or three TAAs

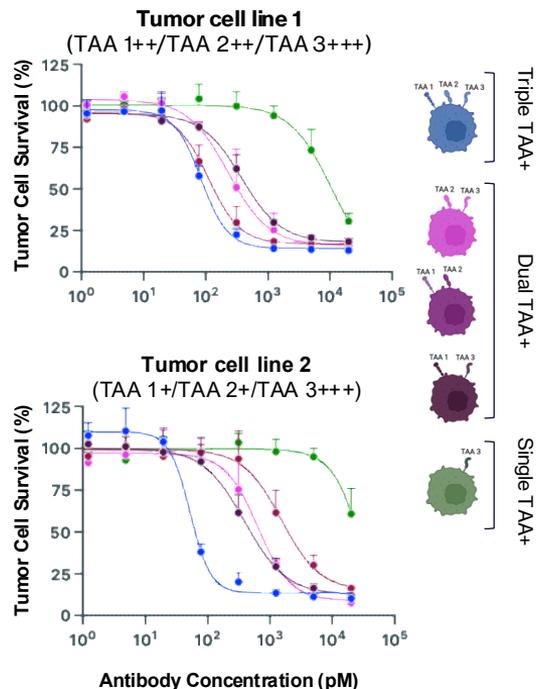


TAA expression profile	TAA	Cytotoxicity
Triple positive	TAA 1 & TAA 2 & TAA 3	✓
Dual positive	TAA 1 & TAA 2 OR TAA 2 & TAA 3 OR TAA 1 & TAA 3	✓
Single positive	TAA 3	✗

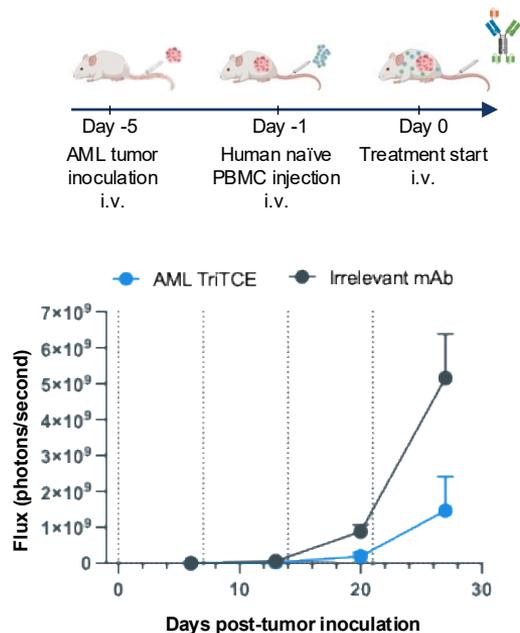


Logic-gated TriTCE Exhibits TAA-selective *In-Vitro* Cytotoxicity and *In-Vivo* Activity in AML Xenograft Model

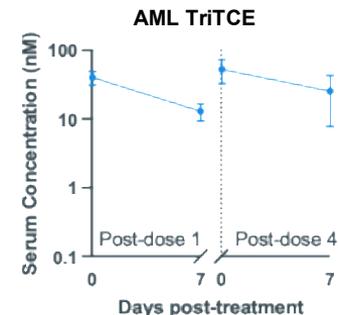
Lead logic-gated TriTCE demonstrates desired TAA-selective cytotoxicity



In vivo anti-tumor activity of AML TriTCE in disseminated AML xenograft model (TAA 1+/TAA 2++/TAA 3+++)



Antibody-like serum PK of AML TriTCE



Summary



Design

TriTCE Co-stim platform optimized for strict TAA dependent T cell activation with efficient conditional *cis* CD28 co-stimulation



Advanced Protein Engineering Solutions

Flexibility of Azymetric™ enables high throughput screening of multispecific formats to identify novel therapeutics with desired biology



Address Indications with High Unmet Need

Plug and play platform facilitates design of next generation T cell engagers to overcome complex biological challenges

Acknowledgements

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Patricia Zwierzchowski
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Janessa Li
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Peter Repenning
Diego Perez
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Matteo Zago
Nichole Escalante
Maya Poffenberger
Anna von Rossum
Kesha Patel
Alexandra Livernois
Madeline Fung
Prajwal Raghunatha
Maggie Ling
Daniela Garcia
Begonia Silva Moreno
Yun Peng
Andrew Sharon

Catherine Wu
Marylou Vallejo
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