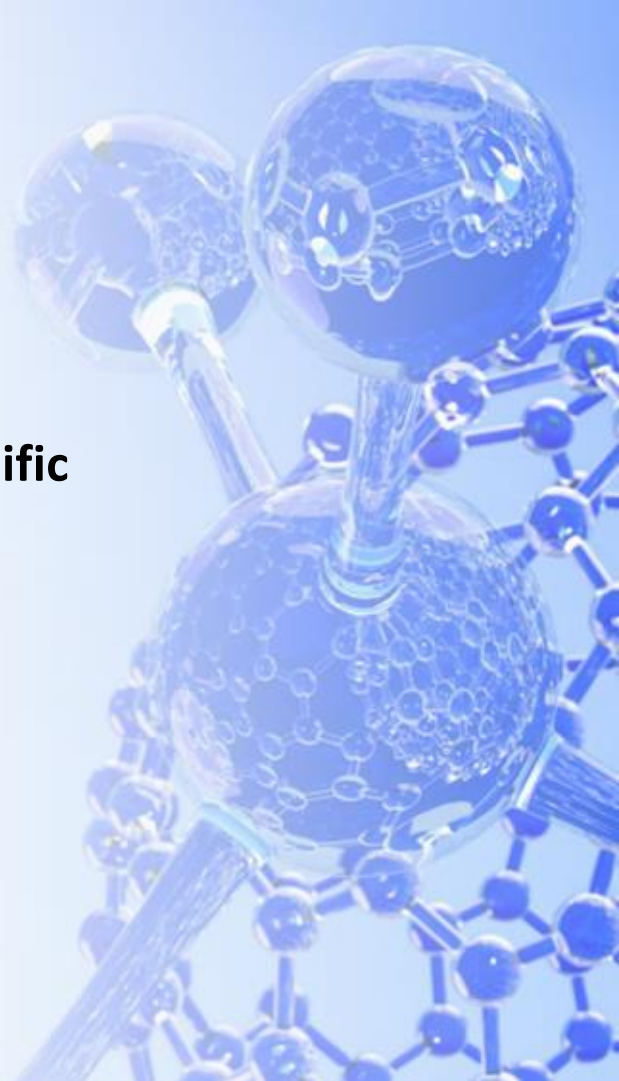
Engineering Next-Generation T Cell Engagers: A Trispecific Platform for Cancer Immunotherapy

PepTalk 2026

Desmond Lau, PhD
Senior Scientist, Protein Engineering



Multiple T cell engagers (TCE) approved in blood tumors, but only two T cell engagers approved in solid tumor

9 approved TCE in hematology

Drug Name	Target	Indications	First Approval and Country	Company
Blinatumomab	CD3/CD19	B-ALL	December 2014 (USA)	Amgen
Mosunetuzumab	CD3/CD20	FL	June 2022 (EU)	Roche/Chugai/Biogen
Glofitamab		DLBCL	March 2023 (Canada)	Roche/Chugai
Epcoritamab		DLBCL	May 2023 (USA)	AbbVie/Genmab
Teclistamab	CD3/BCMA	MM	August 2022 (EU)	Janssen
Elranatamab		MM	August 2023 (USA)	Pfizer
Talquetamab	CD3/GPRC5D	MM	August 2023 (USA)	Janssen
Odronextamab	CD3/CD20	FL	2024 (USA)	Regeneron
Linvoseltamab	CD3/BCMA	MM	2025 (USA)	Regeneron

B-ALL: B-cell acute lymphoblastic leukemia, FL: Follicular lymphoma, DLBCL: Diffuse large B-cell lymphoma, MM: Multiple myeloma

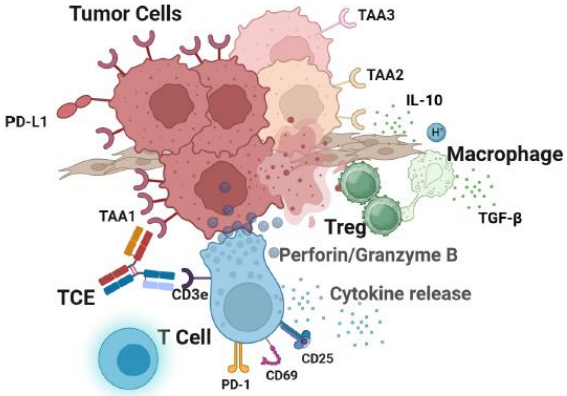
2 approved TCE in solid tumor

Drug Name	Target	Indications	First Approval and Country	Company
Tarlatamab	CD3/DLL3	SCLC	May 2024 (USA)	Amgen
Tebentafusp	CD3/TCR	UM	Jan 2022 (USA)	Immunocore

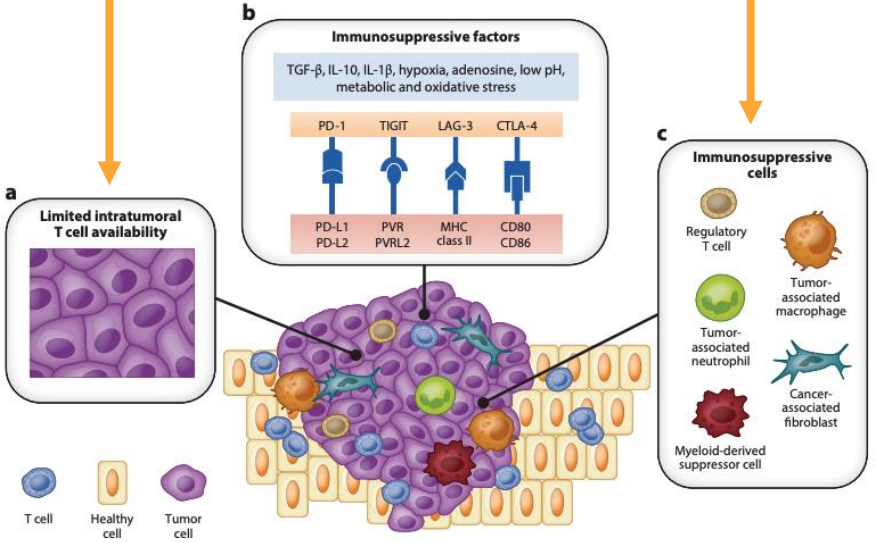
SCLC: Small cell lung cancer, UM: Uveal Melanoma

Challenges Remain: Gen 1 TCE Limited by Narrow Therapeutic Window & Solid Tumors Present Obstacles not Found in Blood Cancers

Key Problem 1:
Tumor heterogeneity and limitations due to concomitant cytokine release



Key Problem 2:
Low T cell infiltration
T cell anergy



Key Problem 3:
Immunosuppressive tumor microenvironment

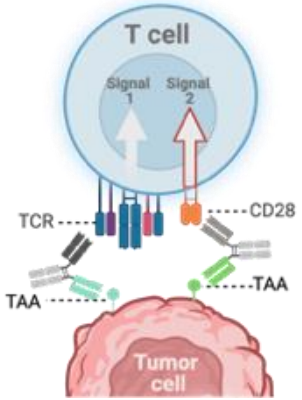
Cao L et al Front Immunol 2025

Arvedson T et al Ann Rev Cancer Biol 2022

Current Co-stimulatory T Cell Engager Approaches

Bispecific CD28 T cell Engagers (monotherapy or in combination)

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

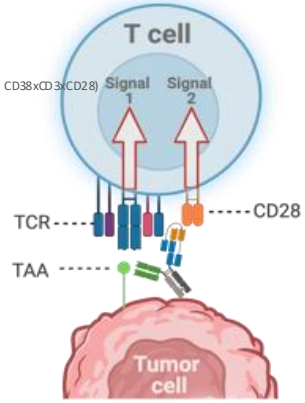


Limitations:

- 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

Sanofi (HER2xCD3xCD28; CD38xCD3xCD28)

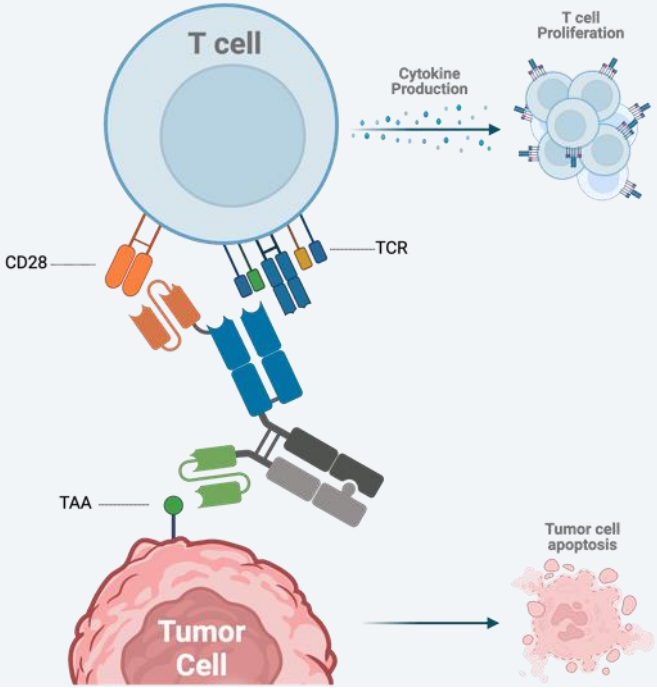


Limitations:

- First generation used 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-T cell** activation

[1] Stein et al., Journal Clinical Oncology (2023); [2] Seung et al., Nature (2022); [3] Promsote et al., Nature Communications (2023)

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



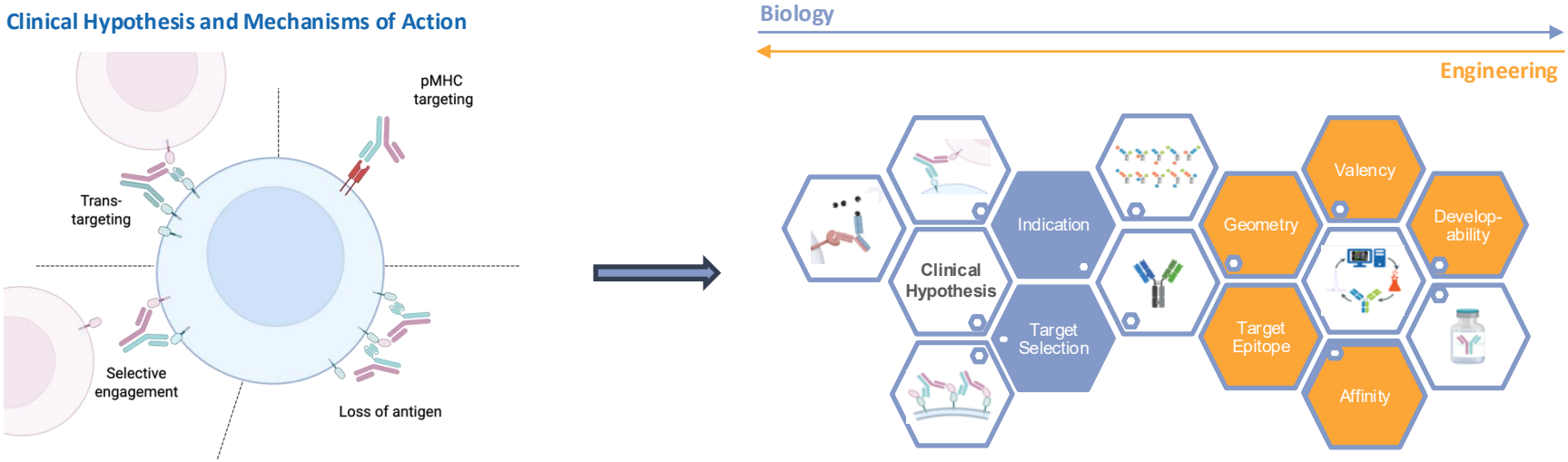
	Design Feature	Expected Benefit
1	**Balanced activation of CD3 and CD28**	Potential to provide more durable responses and activate T cell responses in 'cold' tumors with lower T cell infiltration
2	**Low affinity, Conditional CD28 engagement**	Requires co-engagement of CD3
3	**Obligate *cis*-T cell (CD3xCD28) 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

Multispecific Antibody Development Requires Optimization of Multiple Parameters Specific to Desired MOA

Understanding the interplay of antibody geometry with optimal paratope affinity, valency, and target epitope is critical to identifying best-in-class multispecific antibody therapeutics

Zymeworks' Azymetric™ platform enables the development of multispecific antibody with high flexibility and manufacturability

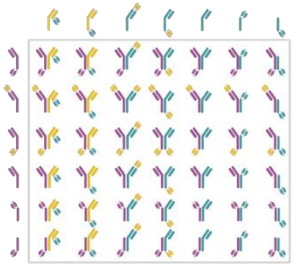


High Throughput TriTCE Co-stim Screening Workflow Allows Interrogation of Multiple Formats in Parallel and Rapid Hit-to-Lead Progression

1. TriTCE Panel Design

Screening of TriTCE formats, affinities and paratopes

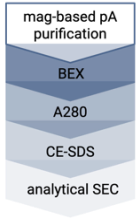
- ~200-400 variants in parallel



2. Cloning and Production

Automated 96-well plate expression and 1-step purification

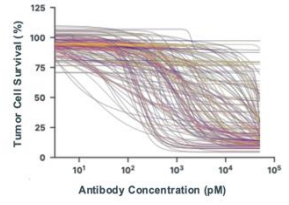
- Azymetric™ facilitates efficient heterodimeric antibody assembly
- Enables 1-step purification across variable formats

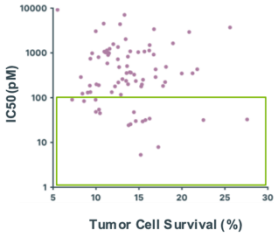


3. Functional Screen

HTP *in vitro* assessment of >150 TCEs

- Automated HTP TDCC assay for functional screening

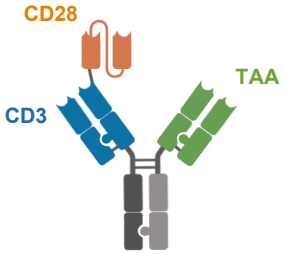




4. Lead Selection

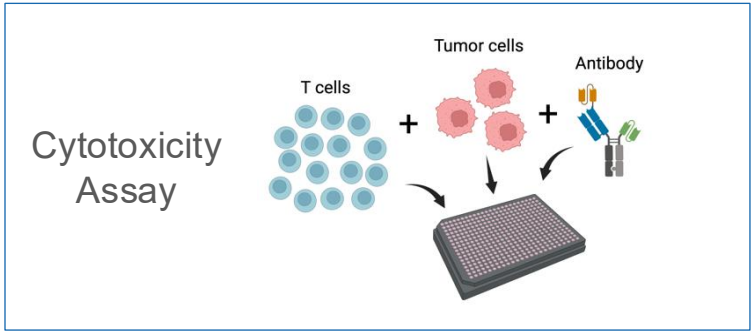
Lead selection

- Optimal TriTCE format
- Optimized CD3 and CD28 affinities

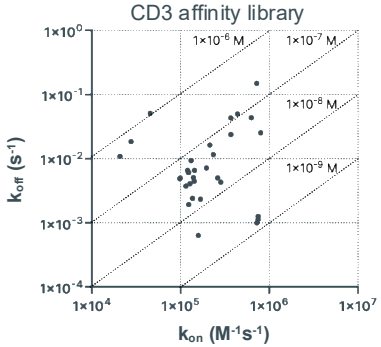


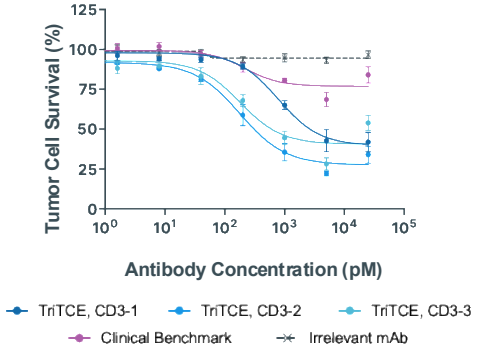
*representative format

Integrated Screening of Multivalent Geometries and Affinities to Select Differentiated Trispecific T cell Engagers with Optimized CD3 and CD28 Activity

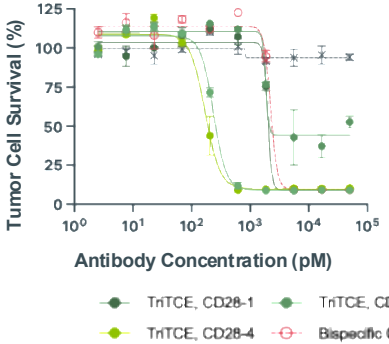


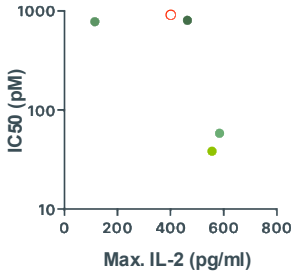
Fine-tuning CD3 kinetics



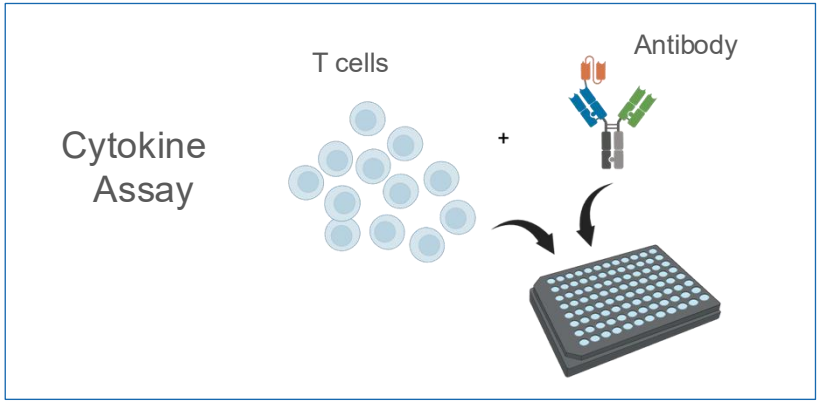


Fine-tuning CD28 co-stimulation

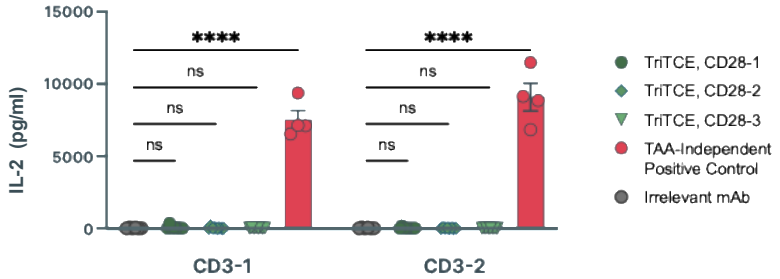




Trispecific T cell Engagers with Optimized CD3 and CD28 Activity Maintain Safety Profile

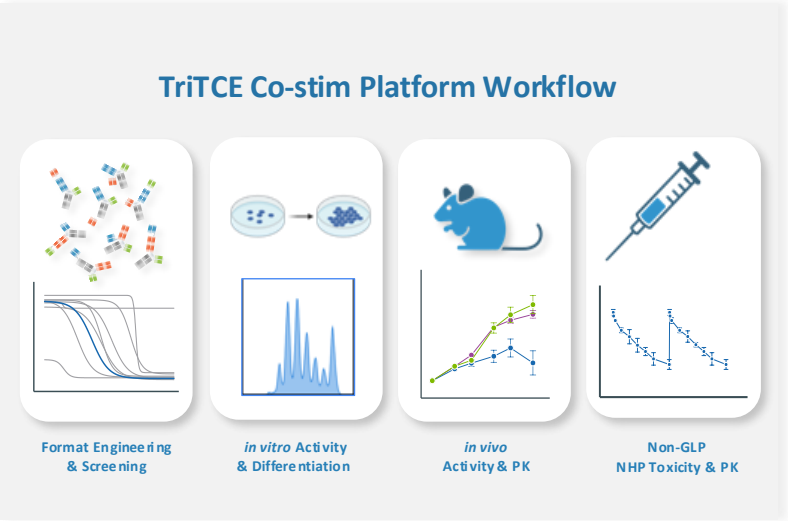


Safety profile maintained for all CD3/CD28 paratopes

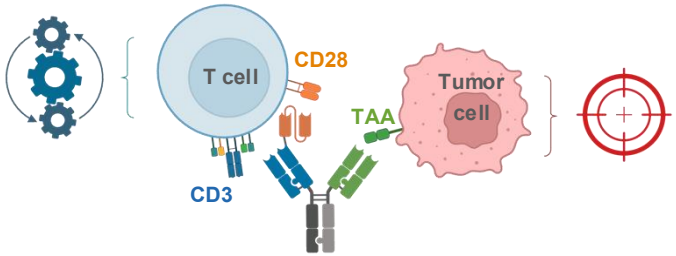


TriTCE Co-stim: A Differentiated TCE Platform with conditional *cis* CD28 co-stimulation and transferability to diverse targeting strategies

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



Optimized CD3/CD28 geometry:
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

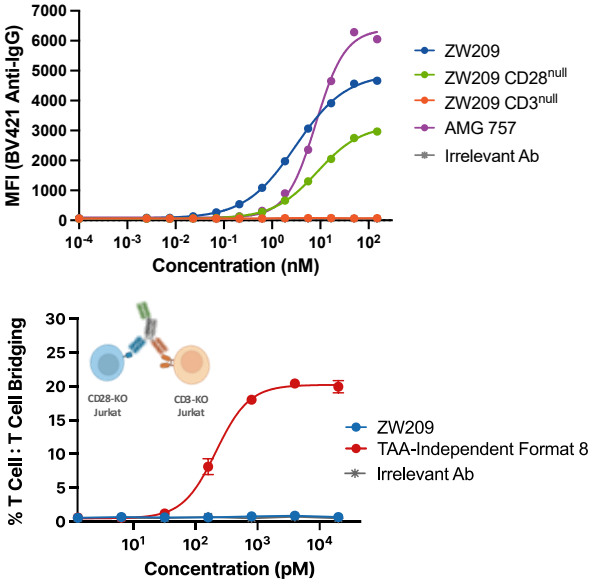
- Monovalent/bivalent Fab, scFv, VHH
- Multi-TAA logic-gated designs
- pMHC targeting

ZW209: DLL3 x CD3 x CD28 Trispecific T Cell Engager Designed For Treatment of Small Cell Lung Cancer

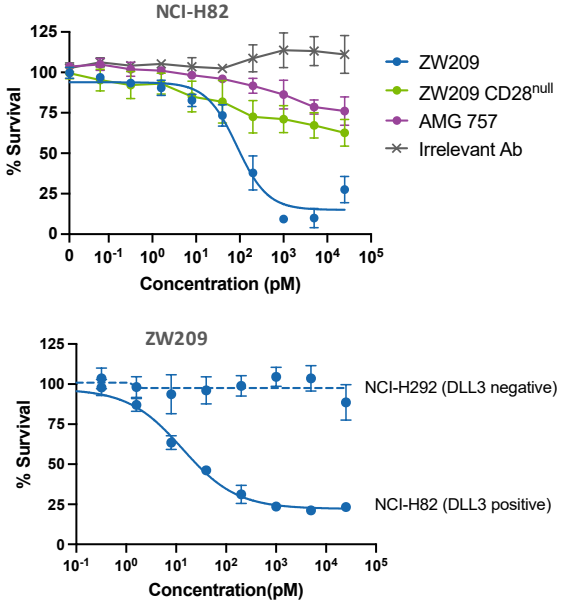
ZW209 design facilitates desirable T cell engagement

Design Feature
☑ Balanced activation of CD3 and CD28
☑ Low affinity, Conditional CD28 engagement
☑ Obligate *cis*-T cell (CD3xCD28) binding
☑ Target-dependent activity

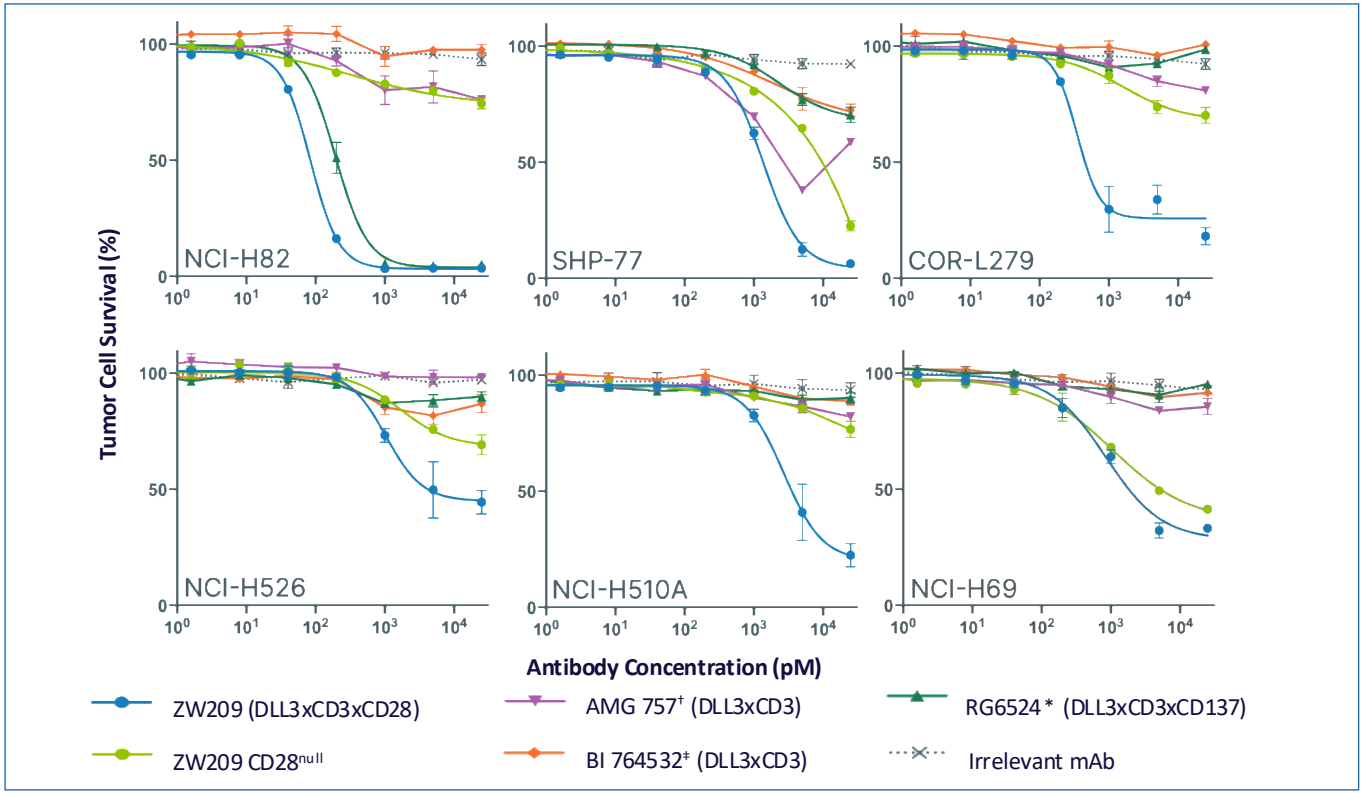
Conditional Binding of CD28, Requiring Co-engagement of CD3; Obligate Cis Binding



Improved Cytotoxicity Over Bispecifics in Low E:T Conditions

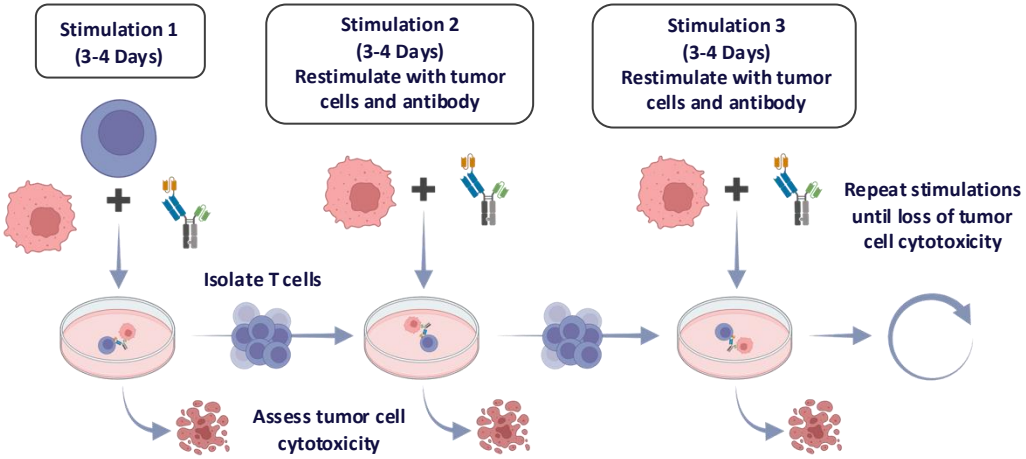


ZW209 Exhibits Improved *in vitro* Potency Relative to Bispecific and Trispecific Clinical TCE Benchmarks at Low Effector: Target Ratios

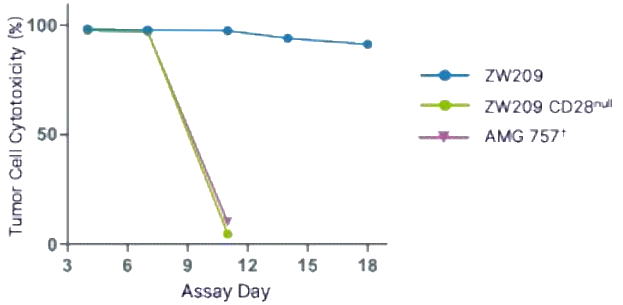


Test articles were incubated with T cells co-cultured with DLL3-expressing SCLC tumor cell lines at low E:T ratio for 7 days and evaluated for cytotoxicity.

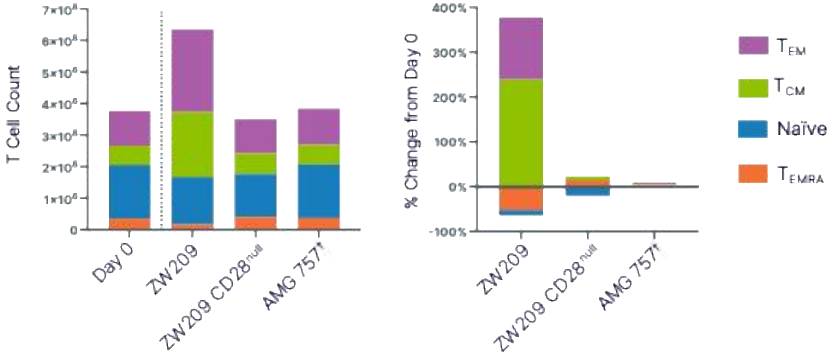
ZW209 Demonstrates Prolonged T cell Cytotoxicity in Repeat Challenge Assay



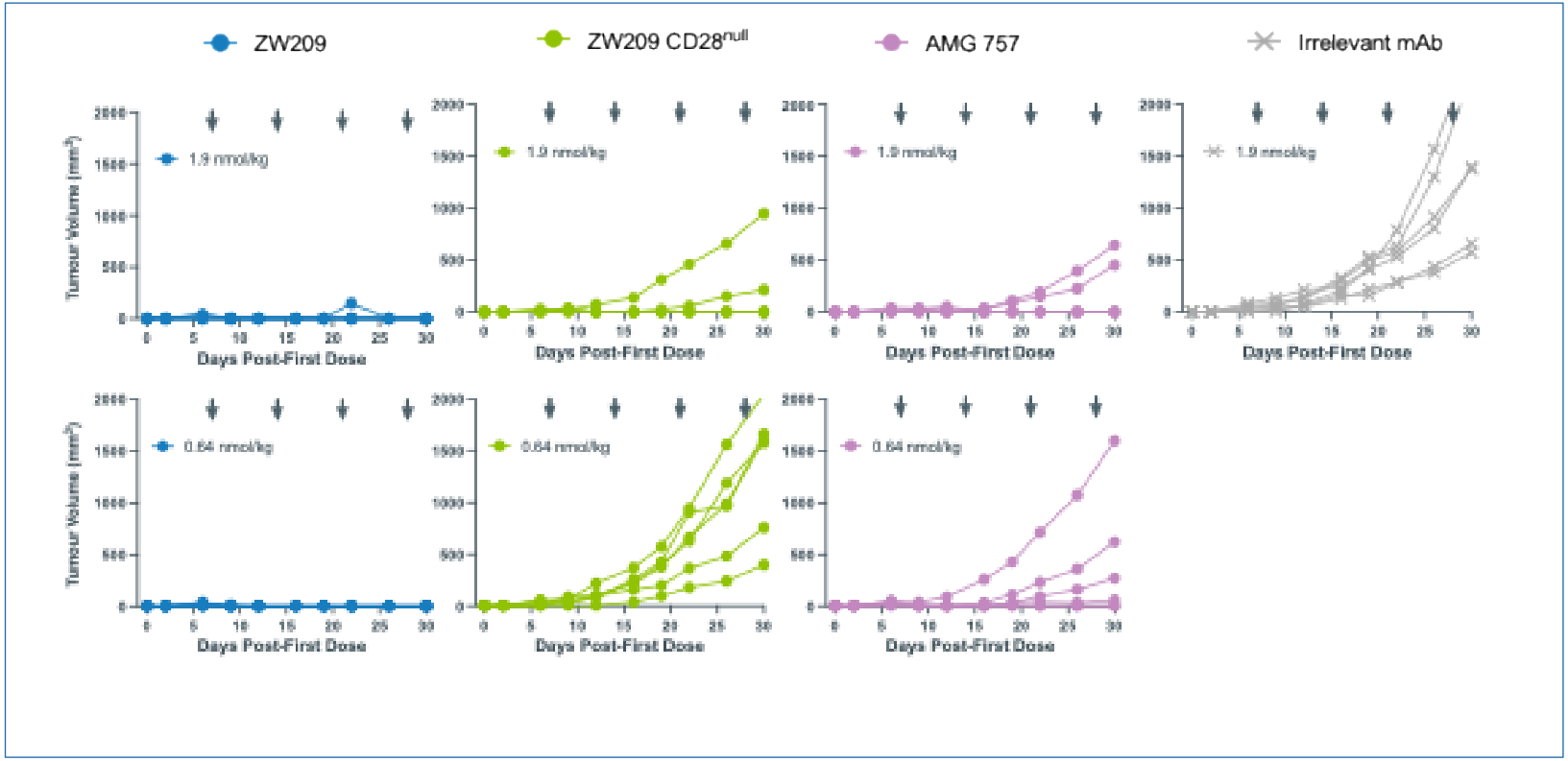
Sustained cytotoxicity relative to bispecific TCEs



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

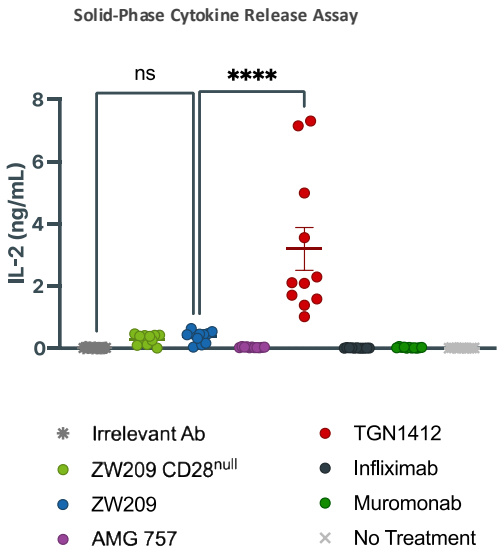


ZW209 Mediates Enhanced Antitumor Activity Compared to Bispecific and AMG 757 in NCI-H82 Admixture Xenograft Model

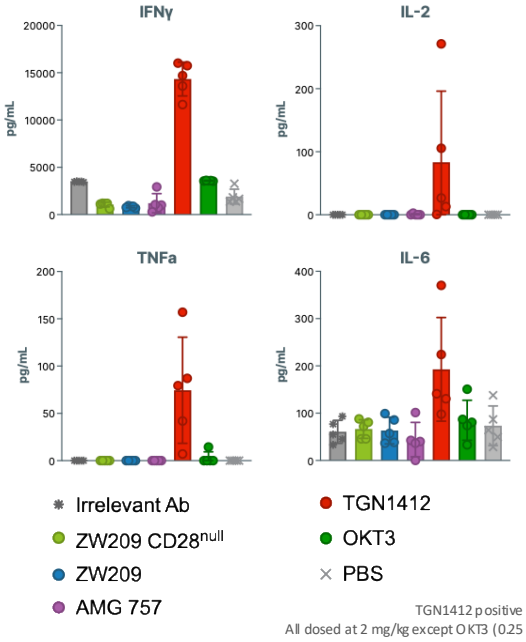


ZW209 Has a Favorable Safety Profile *In Vitro* and in Animal Studies

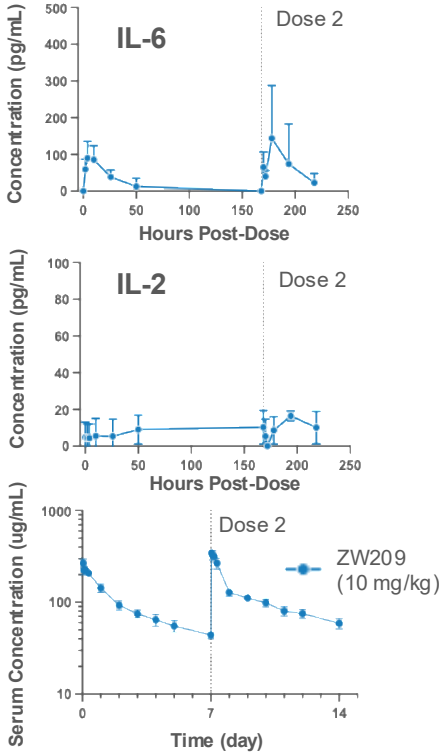
No Cytokine Activation with PBMCs Alone



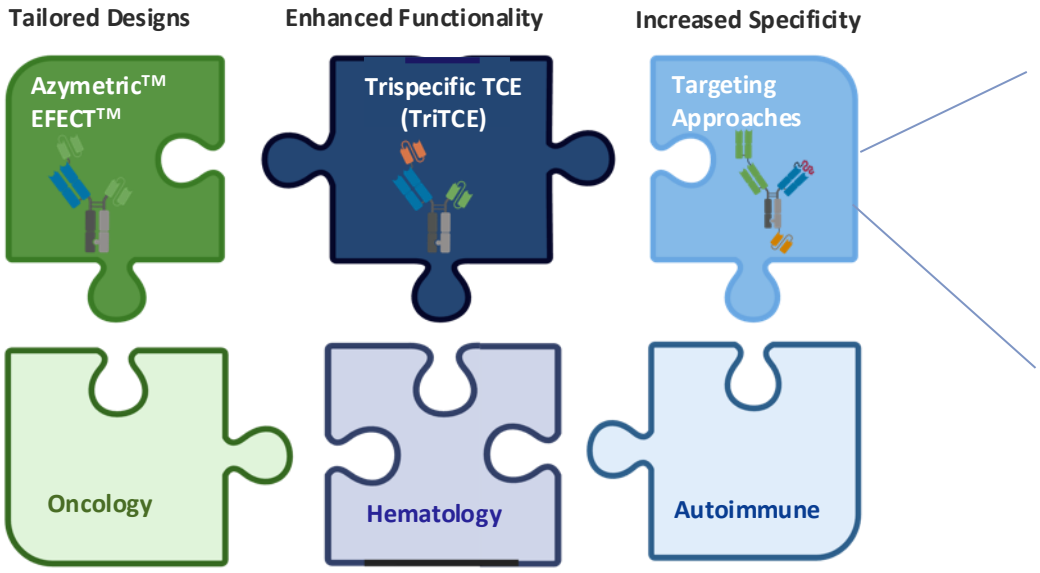
Induces Minimal Systemic Cytokine in Humanized Mouse CRS Model



Well-tolerated in Cynomolgus Monkeys



TriTCE Co-Stim is Transferable Across Diverse Targeting Strategies



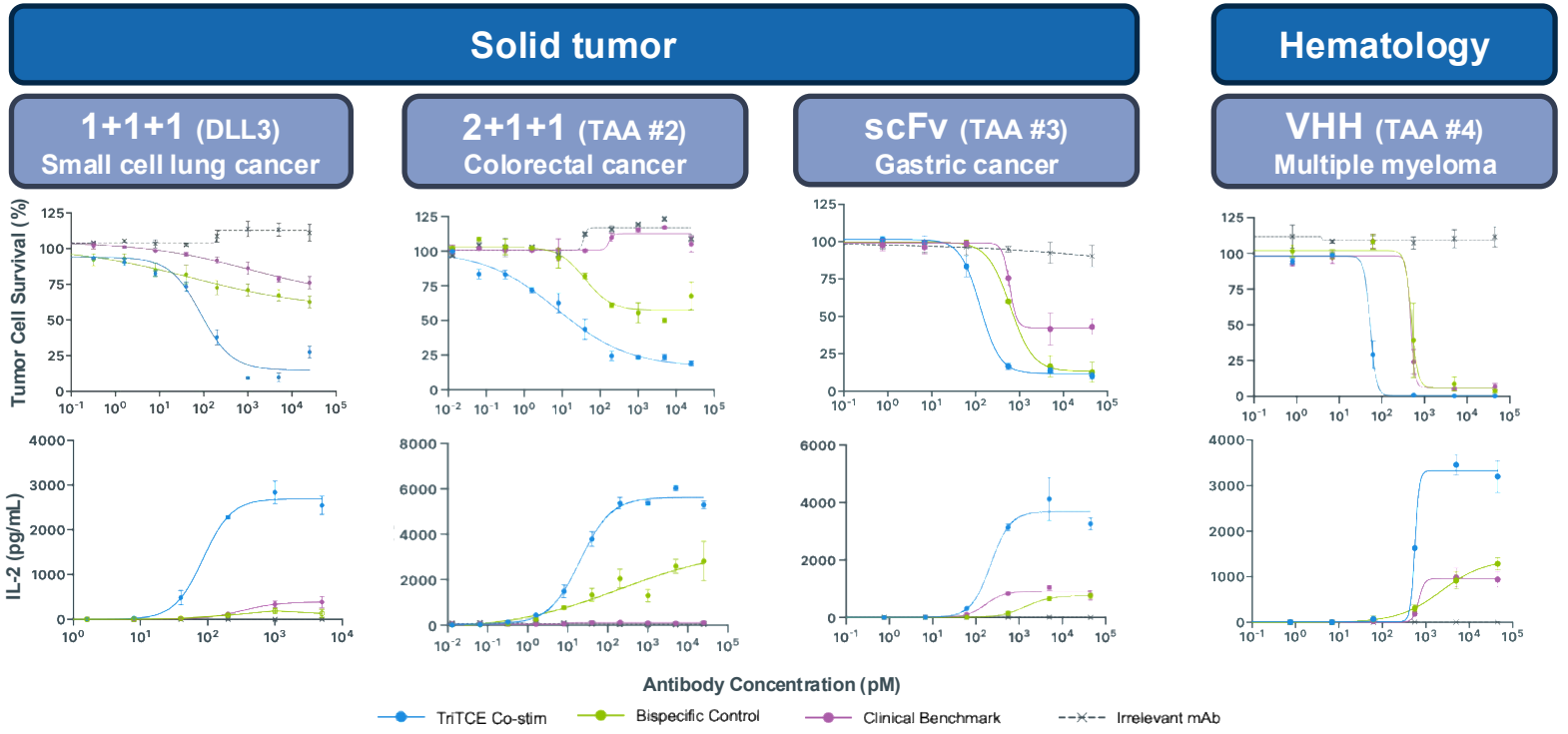
Targeting Selectivity

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

Conditional Activation

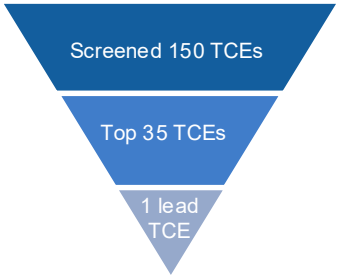
- Conditional masking with proprietary cleavage sequence

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

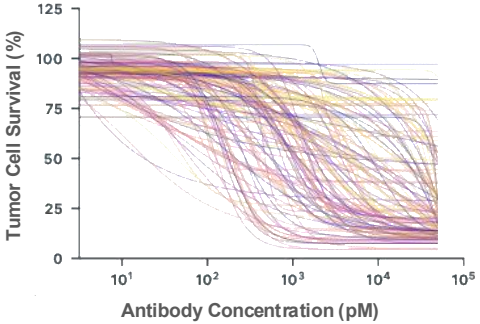


Targeting Peptide-MHC Using TriTCE Co-Stim Platform

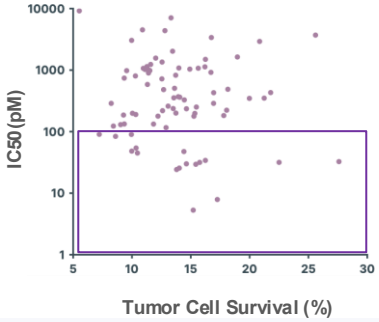
pMHC TriTCE Screening Funnel



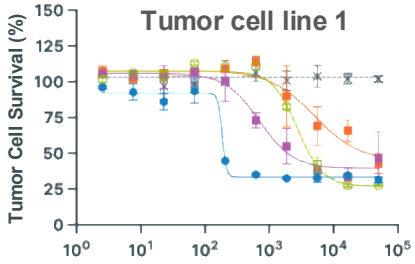
HTP *in vitro* assessment of 150 TCEs

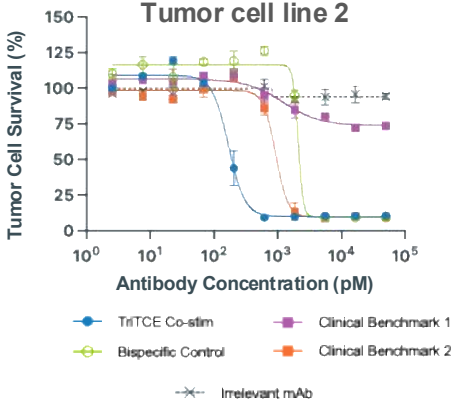


Selection based on cytotoxicity and potency

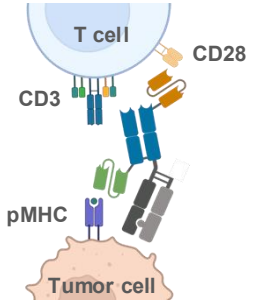


Lead TCE selection





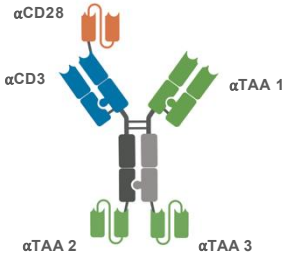
Identification of Lead αpMHC TriTCE format*



*representative format

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

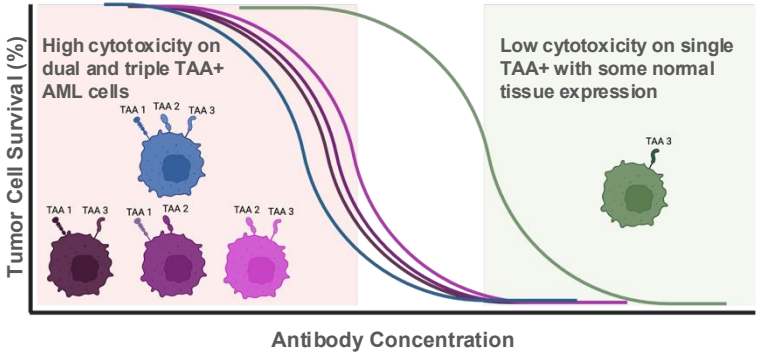
Biological Challenge	Limitation of Mono-antigen Targeted Therapies
Heterogeneous intertumoral antigen expression	Antigen escape
Lack of a clean single target between AML blast, LSCs and healthy cells	Narrow therapeutic window



*representative format

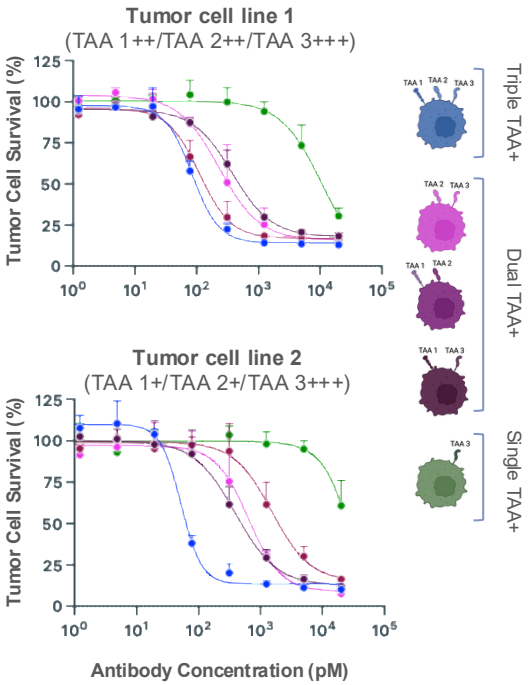
Screened logic-gated TriTCE antibody formats for selective tumor cytotoxicity in the presence of two or three TAAs

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	❌



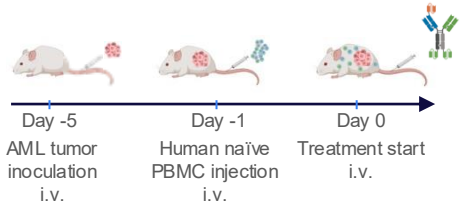
Logic-gated TriTCE Exhibits TAA-selective *In-Vitro* Cytotoxicity and *In-Vivo* Activity in AML Xenograft Model

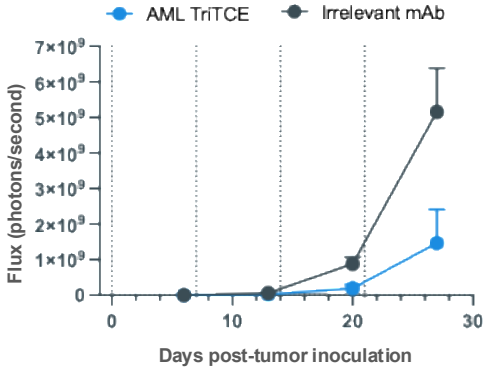
Lead logic-gated TriTCE demonstrates desired TAA-selective cytotoxicity



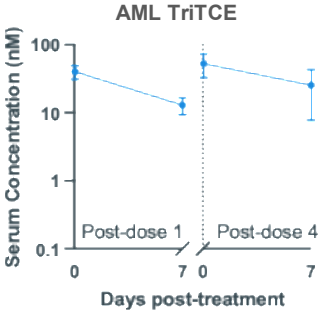
In vivo anti-tumor activity of AML TriTCE in disseminated AML xenograft model

(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

Multispecific Antibody Therapeutic Department

Patricia Zwierzchowski
Wingkie Wong
Janessa Li
Lisa Newhook
Purva Bhojane
Peter Repenning
Diego Perez
Polly Shao
Alec Robinson
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
Richard Kunze
Gavin Storoschuk
Hamed Shirvani
Aditi Deshmukh
Diana Canals Hernaez
John Zhang
Mariana de Souza Rocha
Jan-Philip Meyer
Kurt Stahl
Michelle Chakraborti
Diego Alonzo
Begonia Silva Moreno
Ritesh Korat
Sahar Arbabimoghadam
Karlton Scheu
Siran Cao
Shawna Zhu
Jaqueline Russi
Harpreet Bamra
Annice Li

Nicole Afacan
Genevieve Desjardins
Nina Weisser
Thomas Spreter von Kreudenstein
Charles Chen
Paul Moore

National Research Council (NRC) Canada

Health and Human Therapeutics department