Engineering Trispecific T Cell Engager Leveraging Conditional CD28 Co-Stimulation to Treat Solid Tumors

Immuno-Oncology Summit 2025
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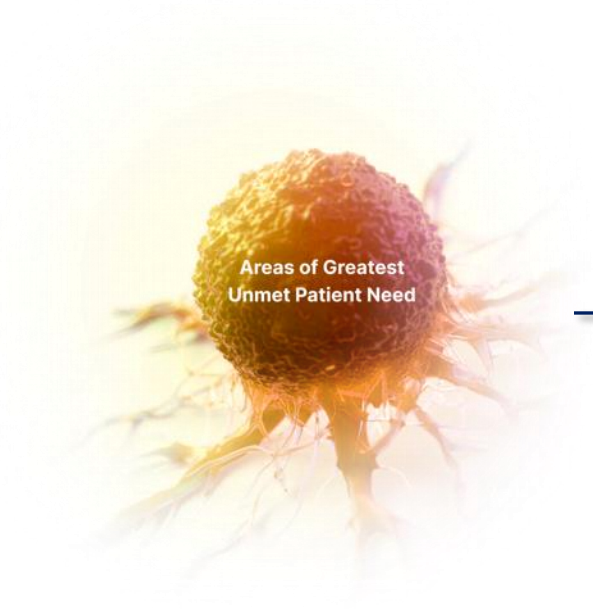
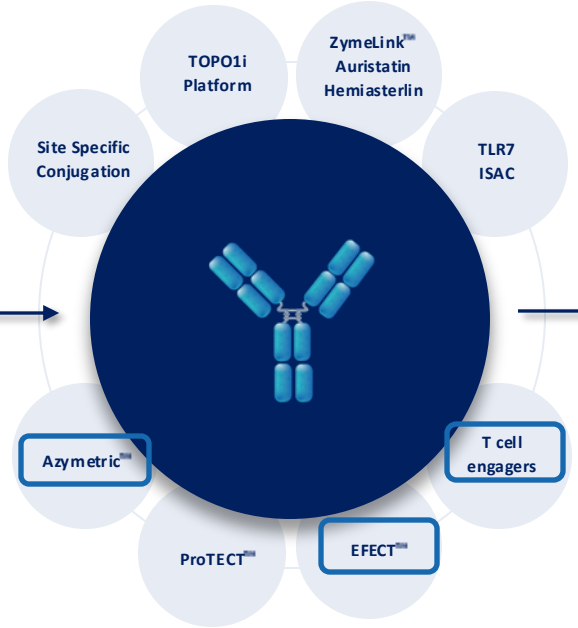
Geneviève Desjardins, PhD
Principal Scientist, Multispecific Antibody Therapeutics

ADC and Multispecific Modalities Driving Zymeworks' Pipeline

Select Difficult-to-Treat Cancers & Target

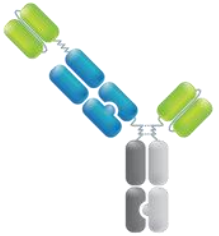


Design with Complementary Technology



Optionality with Two Foundational Fit-for-Purpose Modalities

Antibody Drug Conjugates
Customization:
- Antibody properties
- Antibody format
- Payload
- DAR



Multispecifics
Customization:
- Multiple MOA in single molecule
- Synergistic biology
- Precision targeting through multivalency

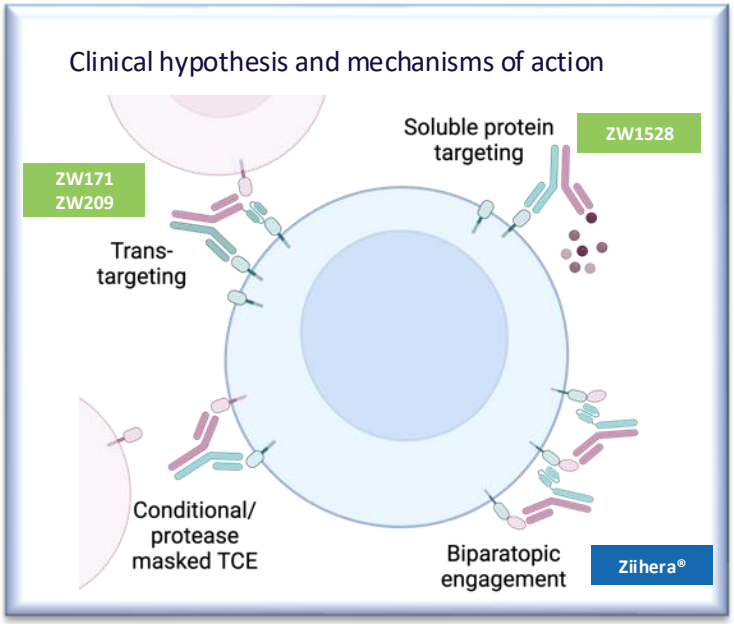
DAR: drug to antibody ratio; ISAC: immune stimulating antibody conjugate; MOA: mechanism of action

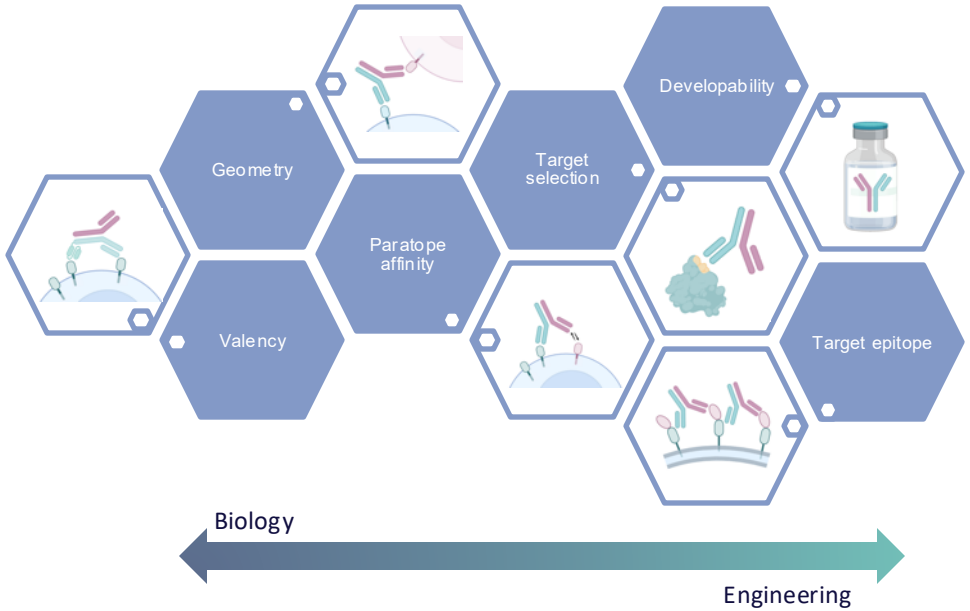
Differentiated Development of Multifunctional Therapeutics

Program	Technology	Target	Indication	Discovery	Preclinical	Phase 1	Phase 2	Phase 3
Solid Tumor Oncology: Antibody-Drug Conjugates (ADC)								
ZW191 Topo1i ADC | DAR 8 | Fc WT	ZD06519 Payload	FRα	Gynecological Thoracic			NCT06555744		
ZW220 Topo1i ADC |DAR 4 | Fc Mut	ZD06519 Payload	NaPi2b	Gynecological Thoracic					
ZW251 Topo1i ADC | DAR 4 | Fc WT	ZD06519 Payload	GPC3	Digestive System (HCC)			FDA IND Clearance Received		
Solid Tumor Oncology: Multipecifics								
Zanidatamab Bispecific	Azymetric™	HER2	Multiple indications			HERIZON-BTC-302, HERIZON-GEA-01, EMPOWHER, +8 ongoing P1 and P2		
ZW171 Trivalent TCE | 2+1 Format	Azymetric™ Novel anti-CD3	MSLN x CD3	Gynecological Thoracic			NCT06523803		
ZW209 Trispecific TCE | Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	DLL3xCD3xCD28	Thoracic			Anticipated IND 1H 2026		
ZW239 Trispecific TCE | Tri-TCE Costim	Azymetric™ Novel anti-CD3 Conditional CD28	CLDN18.2x CD3xCD28	Digestive System					
AIID								
ZW1528 Dual Cytokine Blocker	Azymetric™ Hetero-Fab | YTE	IL4RαxIL33						
ZW1572 Dual Cytokine Blocker	Azymetric™ Hetero-Fab | YTE	IL4RαxIL-31						

Multispecific Antibody Development Requires Optimization of Multiple Parameters to Engineer the Desired Mechanism of Action

- Understanding the interplay of antibody geometry with optimal paratope affinity, valency, and target epitope is critical to identify molecules with novel or improved biology.

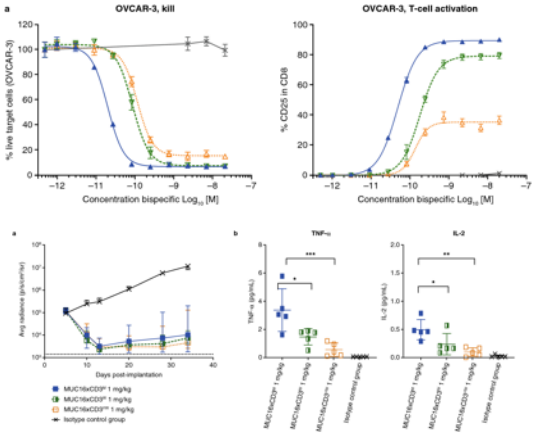




Optimization of T Cell Engager Properties and Geometry Is Critical to Driving Intended Biological Effect – No "One-Size Fits All"

Affinity

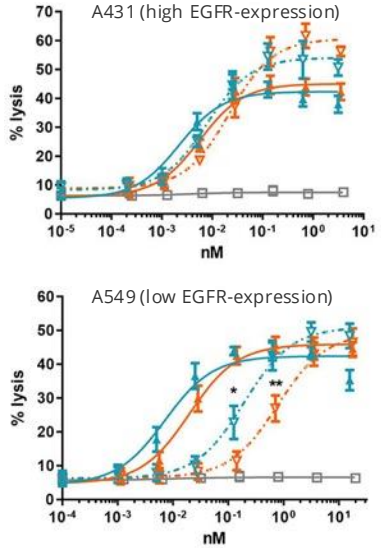
αMUC16 x **αCD3 (medium affinity)** · αMUC16 x **αCD3 (weak affinity)** · αMUC16 x **αCD3 (very weak affinity)** · Isotype control



Haber et al. Sci Rep. 2021

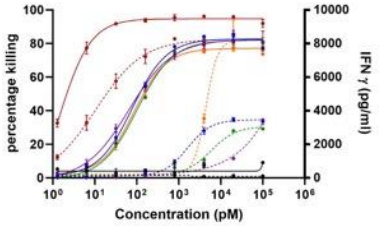
Valency

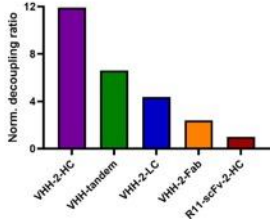
Bivalent αEGFR x αCD3 (2+1) · Monovalent αEGFR x αCD3 (1+1) · Negative control



Boje et al. mAbs. 2024

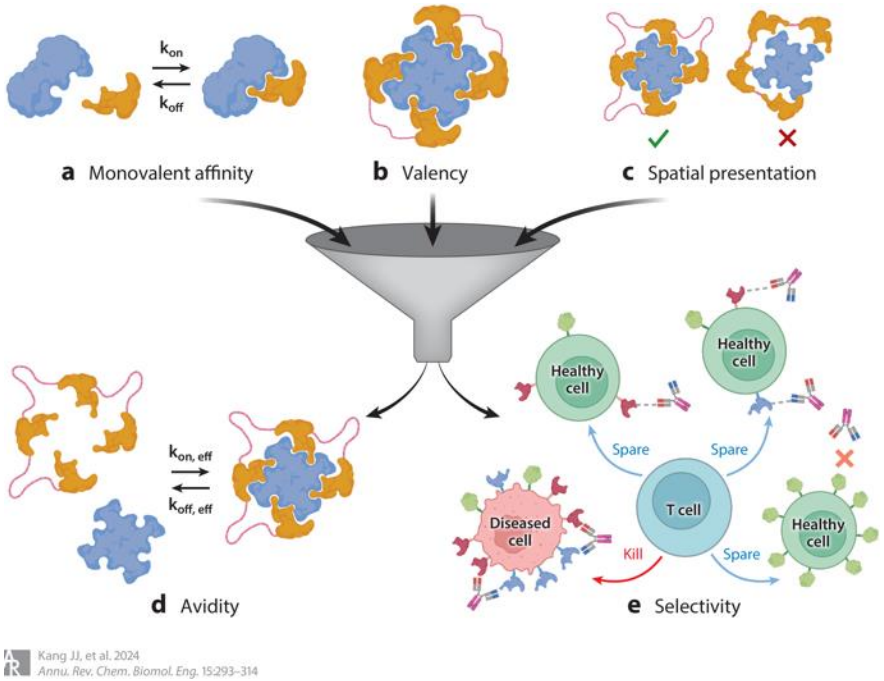
Geometry





Zhou et al. Front Immunol. 2024

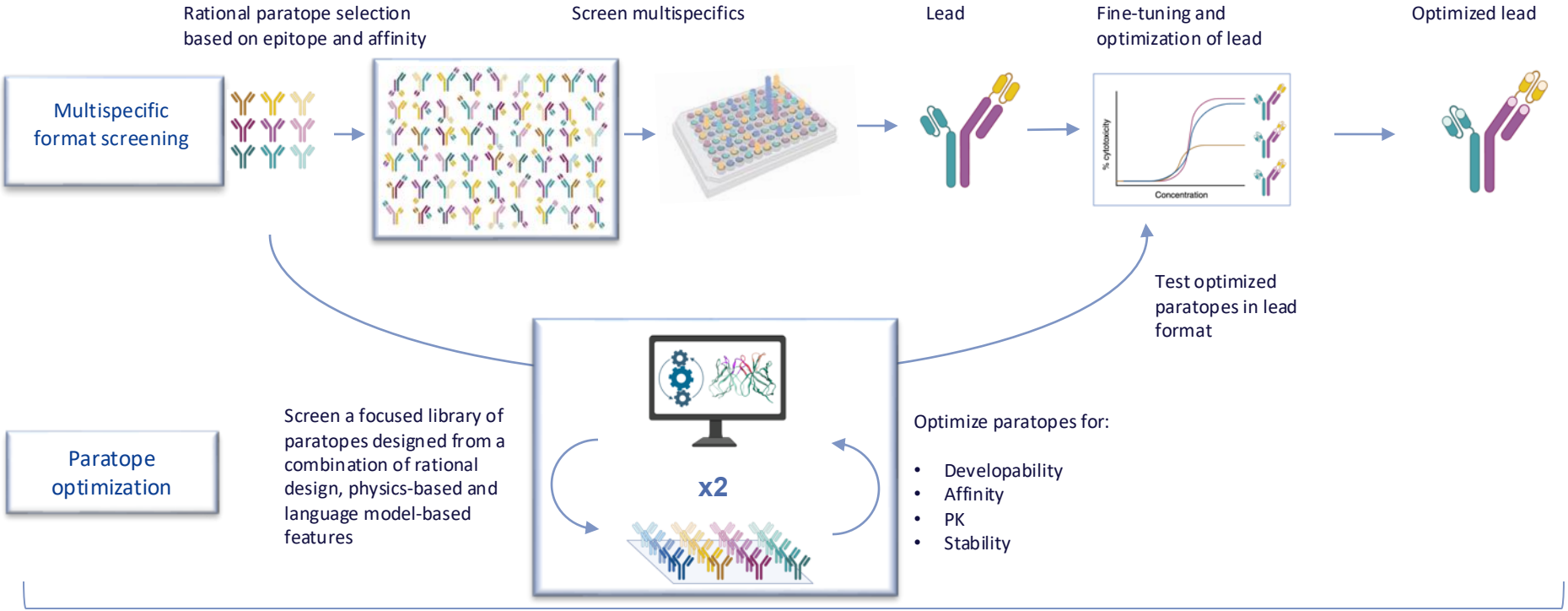
Understanding How These Tunable Parameters Come Together to Design Mechanistic-driven Multispecific Antibody is Challenging



Kang JJ, et al. 2024
Annu. Rev. Chem. Biomol. Eng. 15:293–314

- Creating a molecule with optimal activity and developability requires:
  - robust protein engineering strategies
  - empirical format screening
  - biophysical characterization
  - integration of AI tools
- Testing multiple parameters quickly in a comprehensive manner is challenging

Parallelization of Format Selection and Paratope Optimization using HTP Screening Together with AI/ML Methods

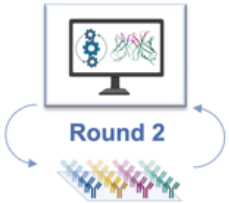


Hit-to-lead + Candidate selection

Paratope Optimization Using Two Rounds of *In Silico* Engineering Yielded a Focused Library with Improved Properties

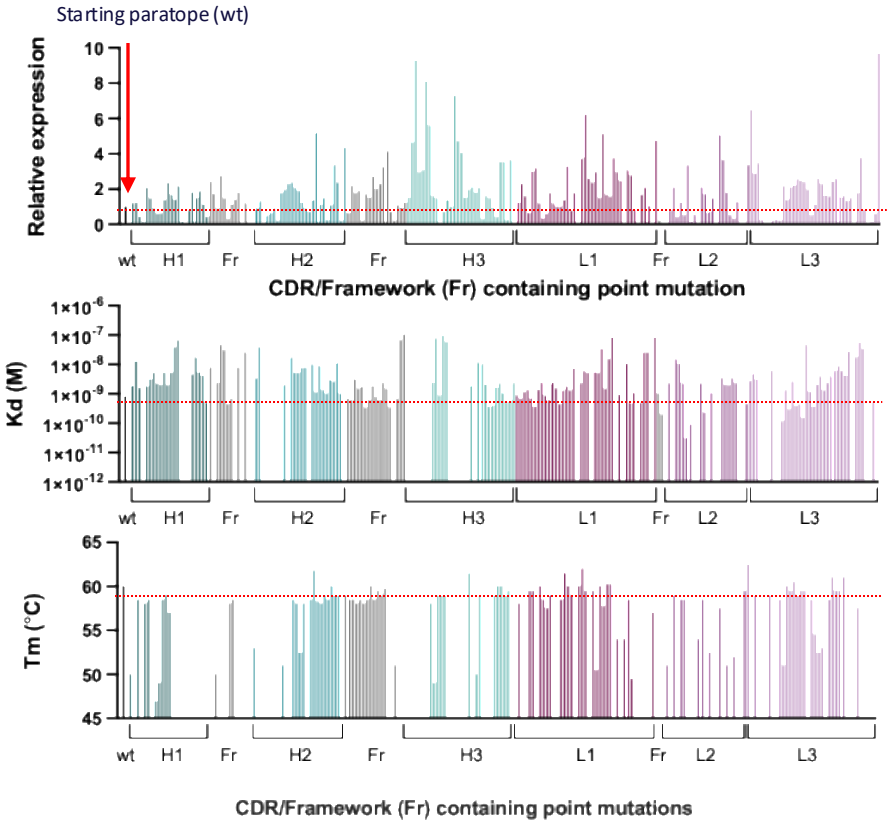
- **Focused 1x screen** to gain information on CDR mutational tolerance, affinity, thermal stability, expression characteristics, etc
- 284 variants tested from ~10^4 computationally evaluated mutations

- 80% of constructs expressed
- 81% bound human and cynomolgus monkey antigen



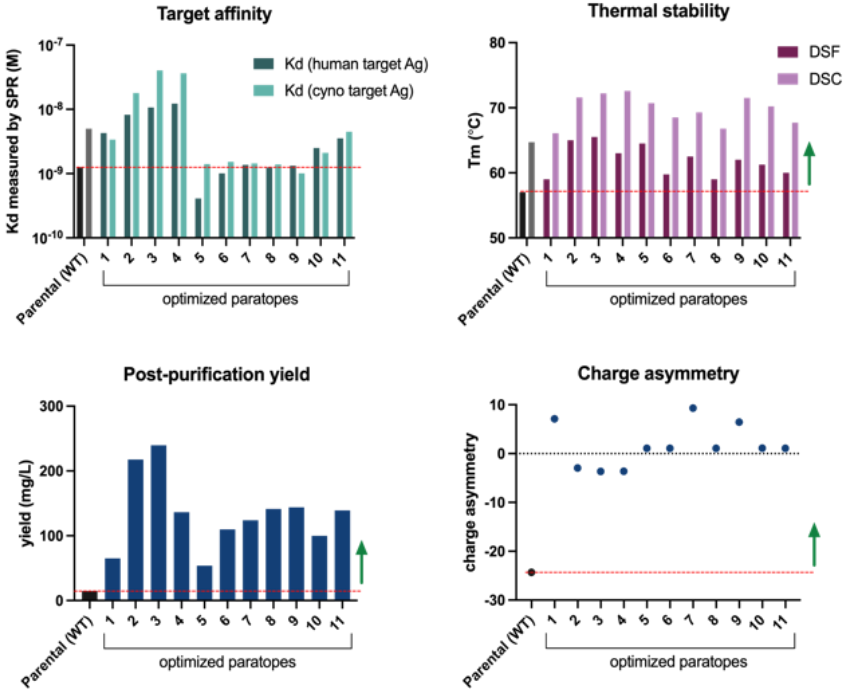
- Identify and **combine best point mutations** from 1x results into optimized paratopes
- 285 combinations selected for testing out of ~10^7 computationally evaluated designs

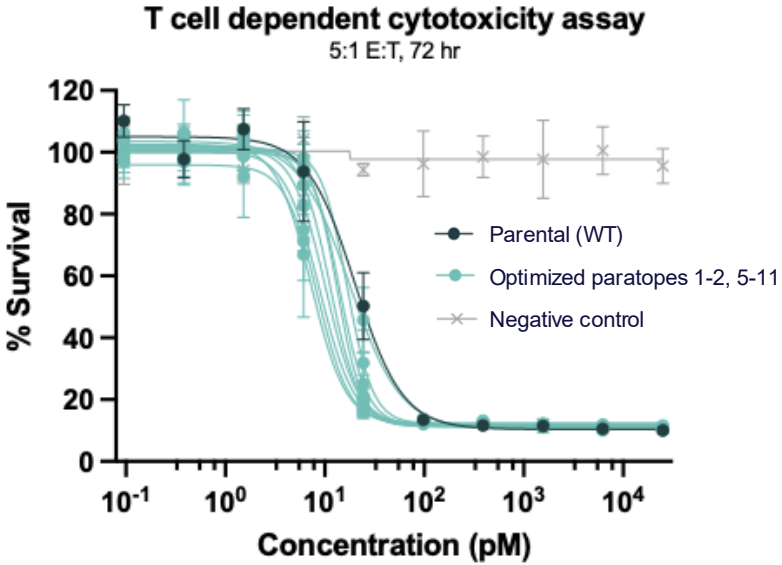
- 61% expressed better than parental
- 43% bound antigen within 10-fold (above or below) parental



Optimized Paratopes Yield Improved Biophysical Properties and Functional Activity in Lead Multispecific Format

- Combination of language models and rational design provides an opportunity to optimize multiple parameters in parallel and yields paratopes with improved expression, thermal stability, developability and diverse affinities





Next Generation CD28 Co-stimulatory Trispecific T cell Engager Platform

Designed to provide more durable responses in solid tumors and superior activity in ‘cold’ tumors

Therapeutic Rationale

- Next Gen TriTCE Co-stim can provide increased T cell fitness, activation, and proliferation via tumor-dependent T cell co-stimulation

Product Differentiation

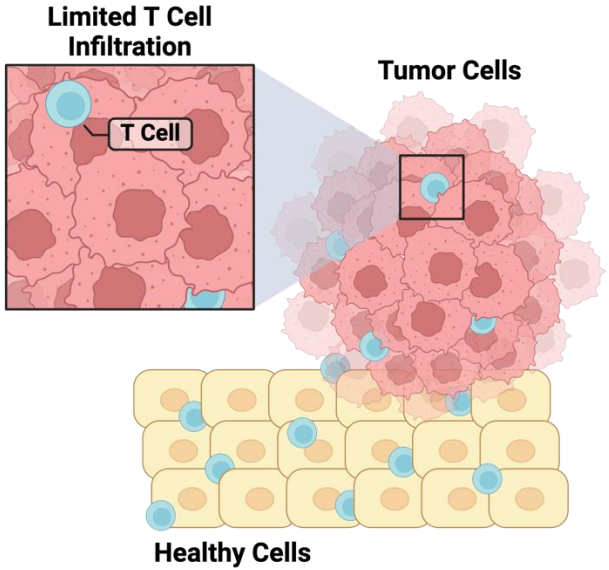
- Novel approach of modular geometry and avidity screening of trispecifics to optimize T cell activation by Signal 1 and Signal 2
- TriTCE Co-stim show superior anti-tumor activity to bispecific benchmarks and exhibit no activation of T cells in absence of tumor cells

Next Milestones

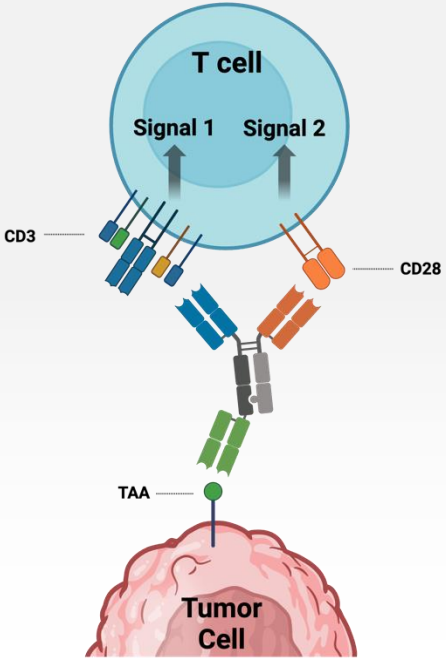
- Continue to expand utility to additional tumor targets

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 T cell anergy remain challenges in the treatment of solid tumors



Zymeworks Trispecific Co-stimulatory Program



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

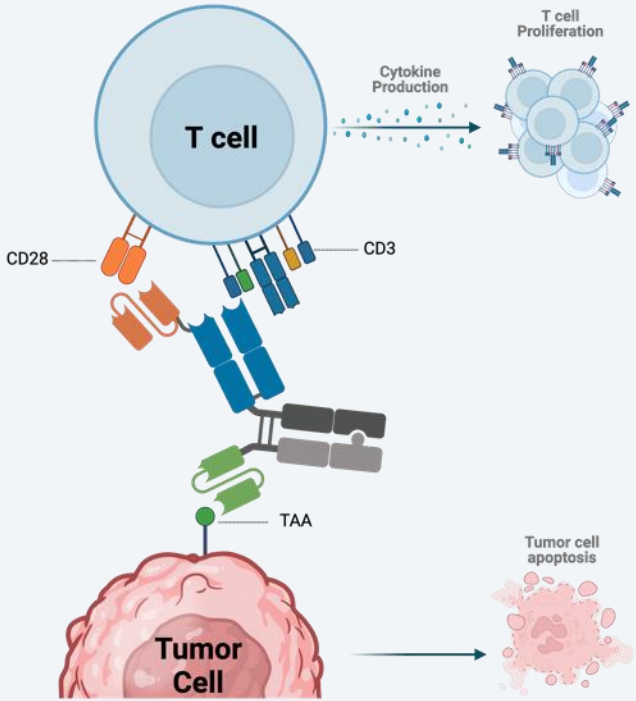
Engineered to balance signal 1 and 2 for optimized **TAA-dependent T cell activation** and expansion

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

Arvedson T et al Ann Rev Cancer Biol 2022

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

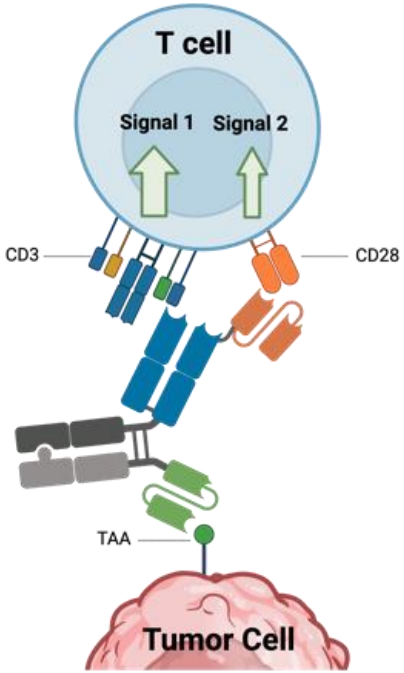
Conditional CD28 Co-stimulation and Obligate *cis* T cell Binding



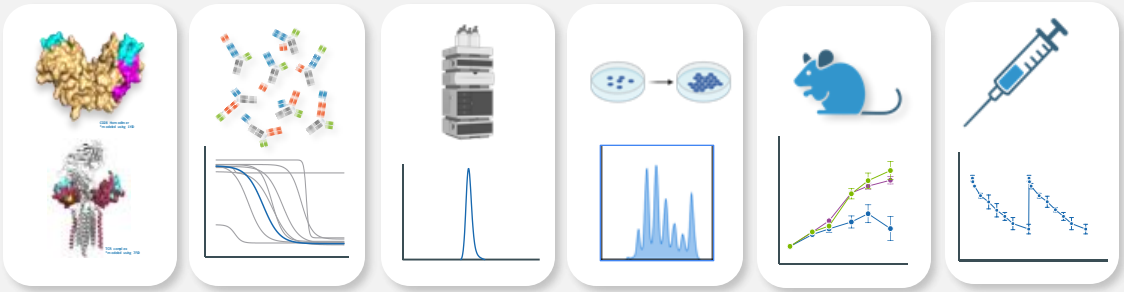
	Design Feature	Expected Benefit
1	**Balanced activation of CD3 and CD28**	Potential to provide more durable responses and activate T cell responses in 'cold' tumors with lower T cell infiltration
2	**Low affinity CD3 and CD28 binding**	Prevents overactivation of T cells and reduces risk of CRS and irAEs
3	**Obligate *cis* T cell binding**	No T cell-to-T cell bridging or T cell fratricide
4	**Conditional CD28 engagement**	Requires co-engagement of CD3
5	**Enhanced target-dependent activity**	Low T cell binding and no T cell activation in periphery or absence of tumor target

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

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



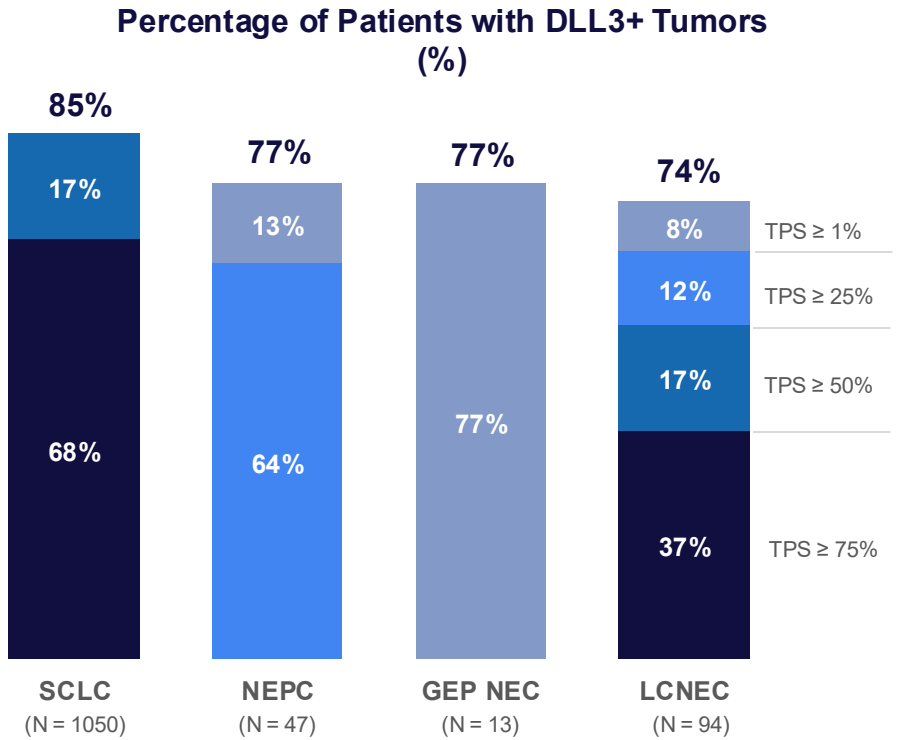
TriTCE Co-Stim Platform Workflow



TriTCE Co-Stim Lead Format Selection

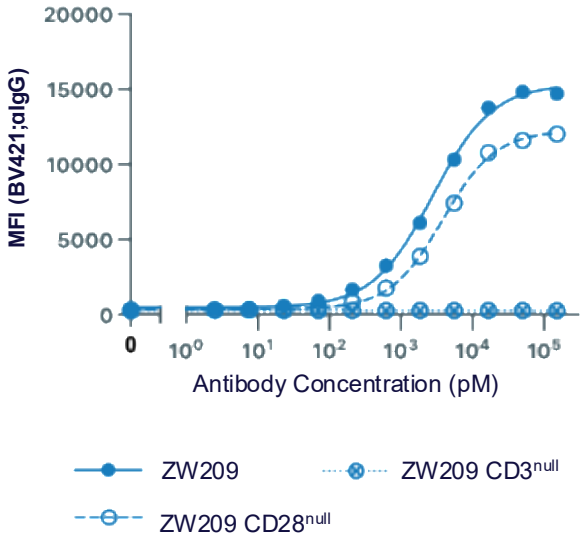
DLL3 is an Ideal Target to Evaluate TriTCE Co-stimulation Platform, with Opportunities in Multiple Cancers

- 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

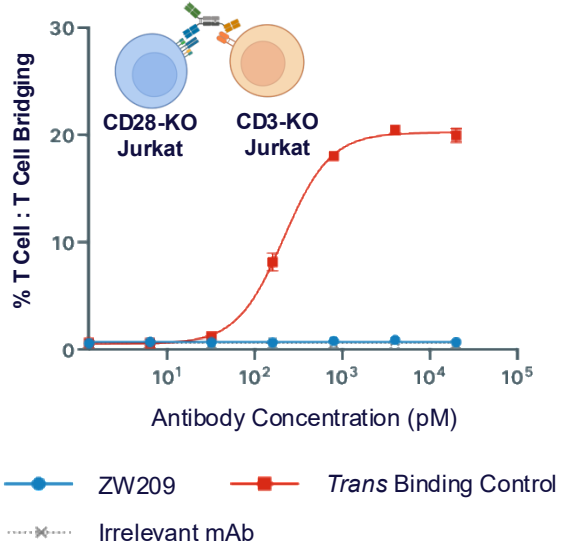


ZW209 Design Facilitates Desirable T Cell Engagement

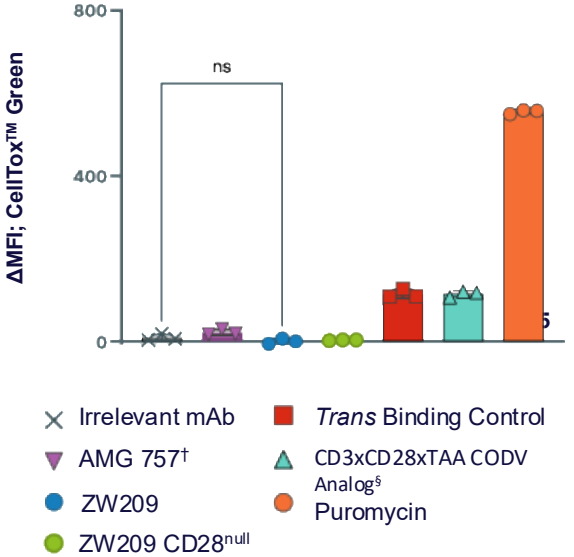
Conditional binding of CD28, requiring co-engagement of CD3



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

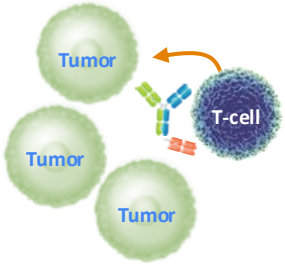


ZW209 does not mediate T cell lysis



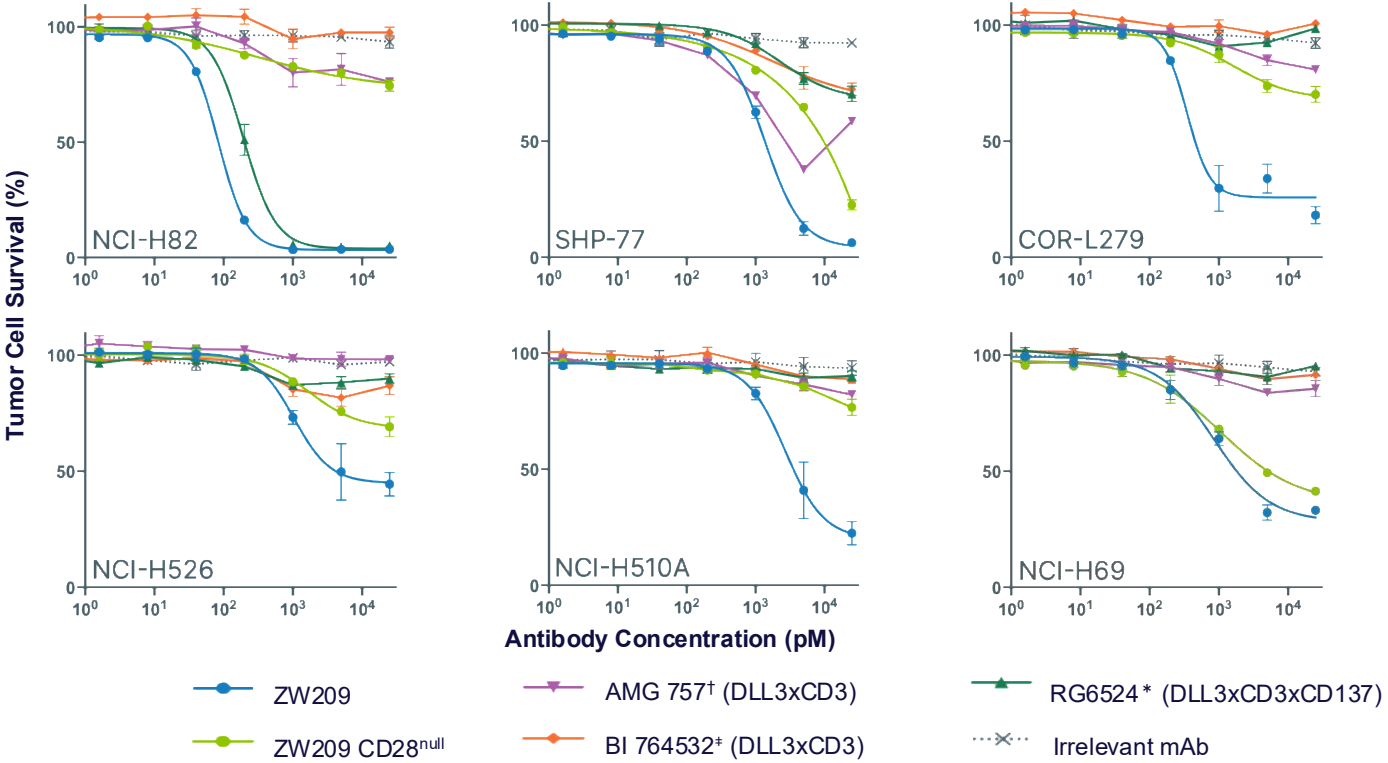
† AMG 757 (DLL3/CD3 BiTE) produced in-house
§ CD3xCD28xTAA CODV Analog is a CD3xCD28xMSLN trispecific with the same format as the Sanofi Trispecific containing a CD3xCD28 CODV-Fab; produced in-house.

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



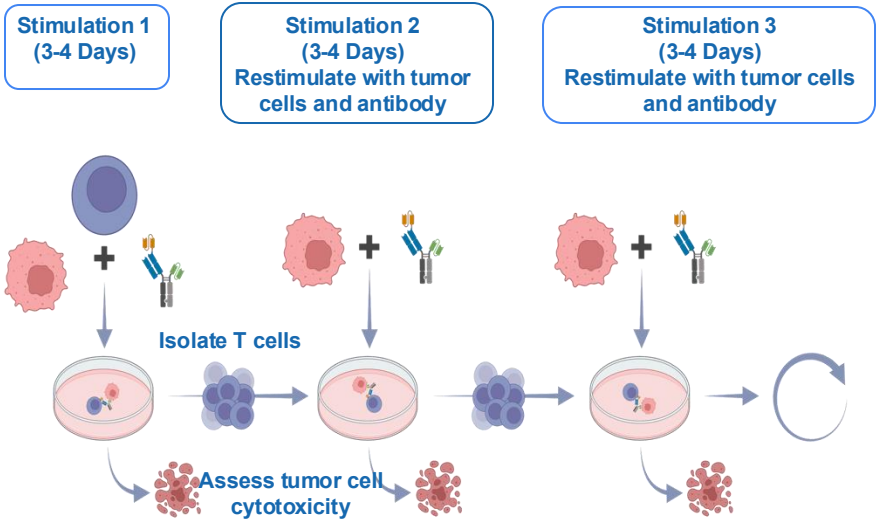
Low E:T Assay System

Cytotoxicity and T cell proliferation

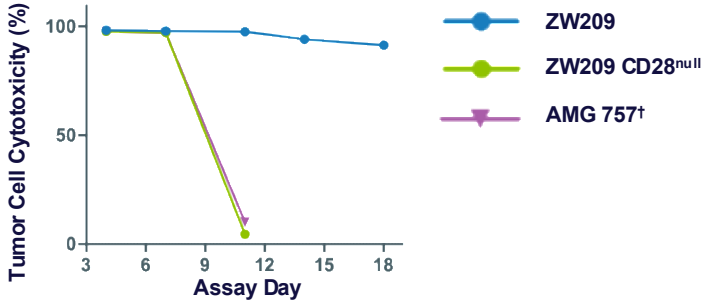


† AMG 757 (DLL3/CD3 BiTE) produced in-house, ‡ BI 764532 (DLL3/CD3 bispecific TCE) produced in-house, * RG6524 (DLL3/CD3/CD137 trispecific TCE), § CD3xCD28xTAA CODV Analog is a CD3xCD28xMSLN trispecific with the same format as the Sanofi Trispecific containing a CD3xCD28 CODV-Fab; produced in-house.

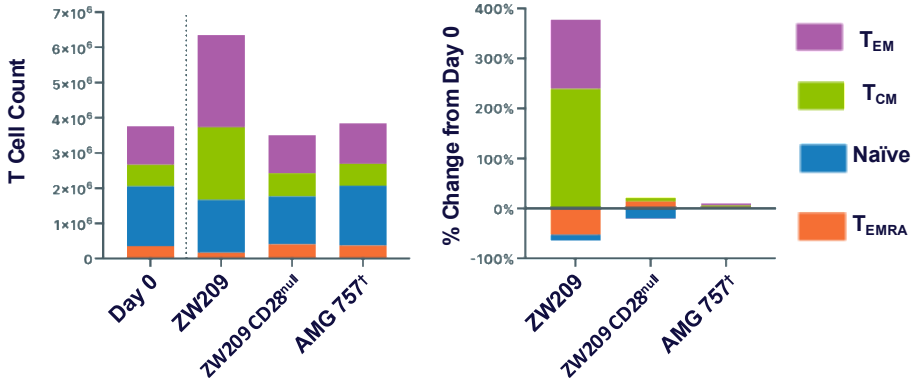
ZW209 Mediates Sustained T Cell-Mediated Cytotoxicity Over Repeated Stimulations



Sustained cytotoxicity relative to bispecific TCEs



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

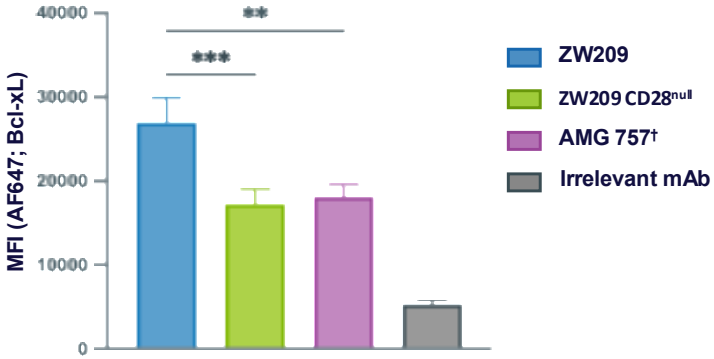


† AMG 757 (DLL3/CD3 BiTE) produced in-house

ZW209 Mediates Enhanced DLL3-dependent T Cell Proliferation and Survival

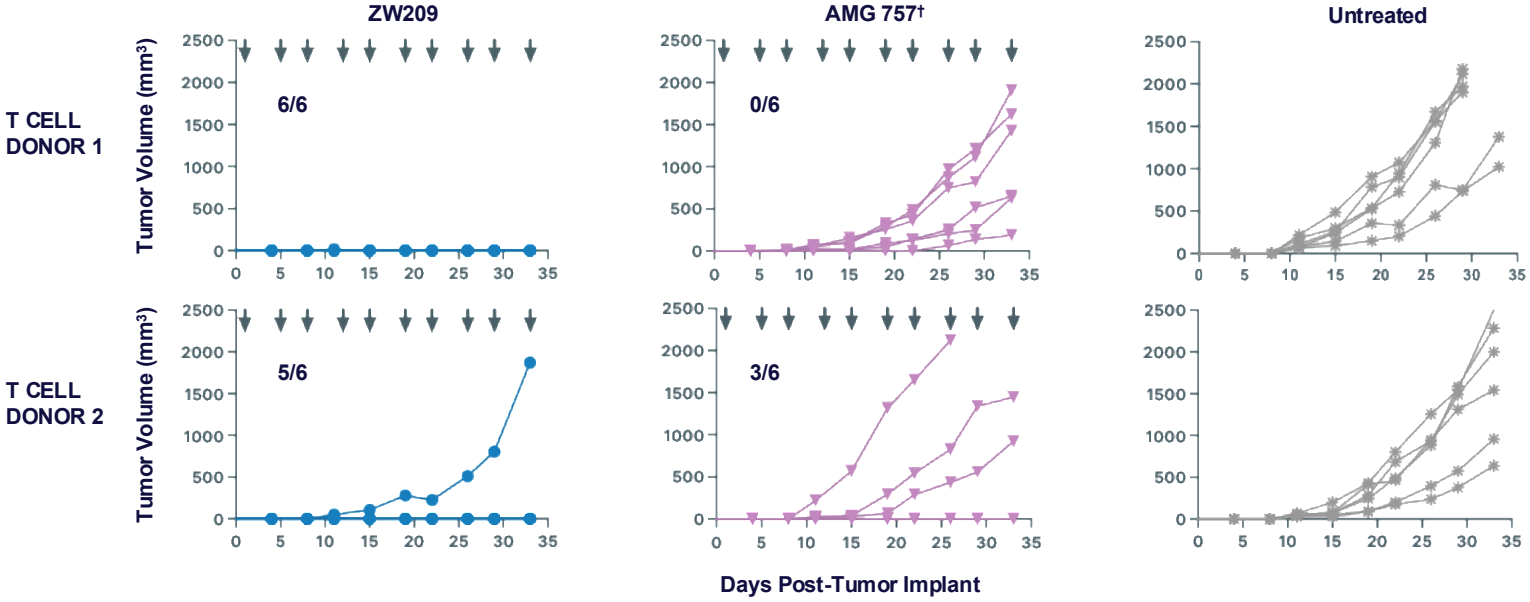
Target-Dependent T Cell Proliferation

T Cell Survival



† AMG 757 (DLL3/CD3 BiTE) produced in-house

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



† AMG 757 (DLL3/CD3 BiTE) produced in-house

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

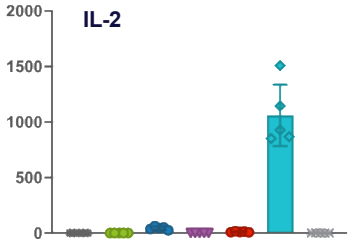
- Antibody Treatment
- All dosed 2 mg/kg except OKT3 (0.25mg/kg)

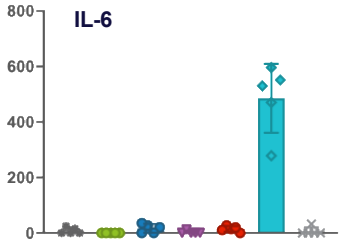
Day 0: Day 6:

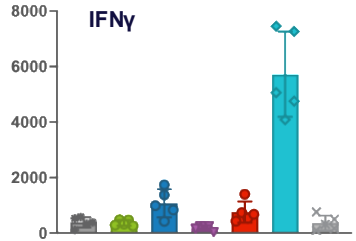
Assess Cytokine Production:

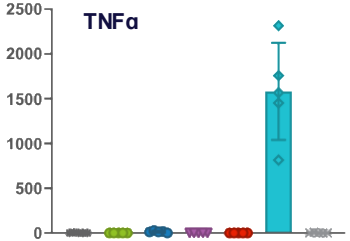
Timepoints:
- **4-hour** – Optimum timepoint for kinetics of CD3 mediated cytokine induction
- **72-hour** – Optimum timepoint for kinetics of CD28 mediated cytokine induction

4 hour

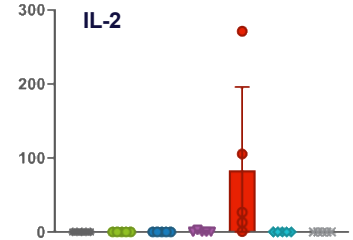


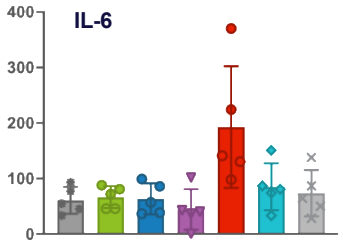


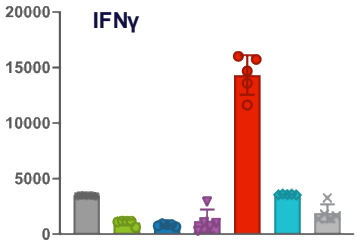


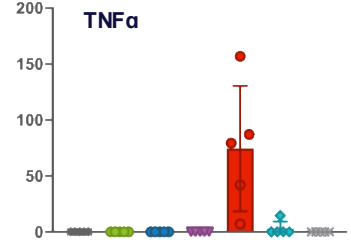


72 hour





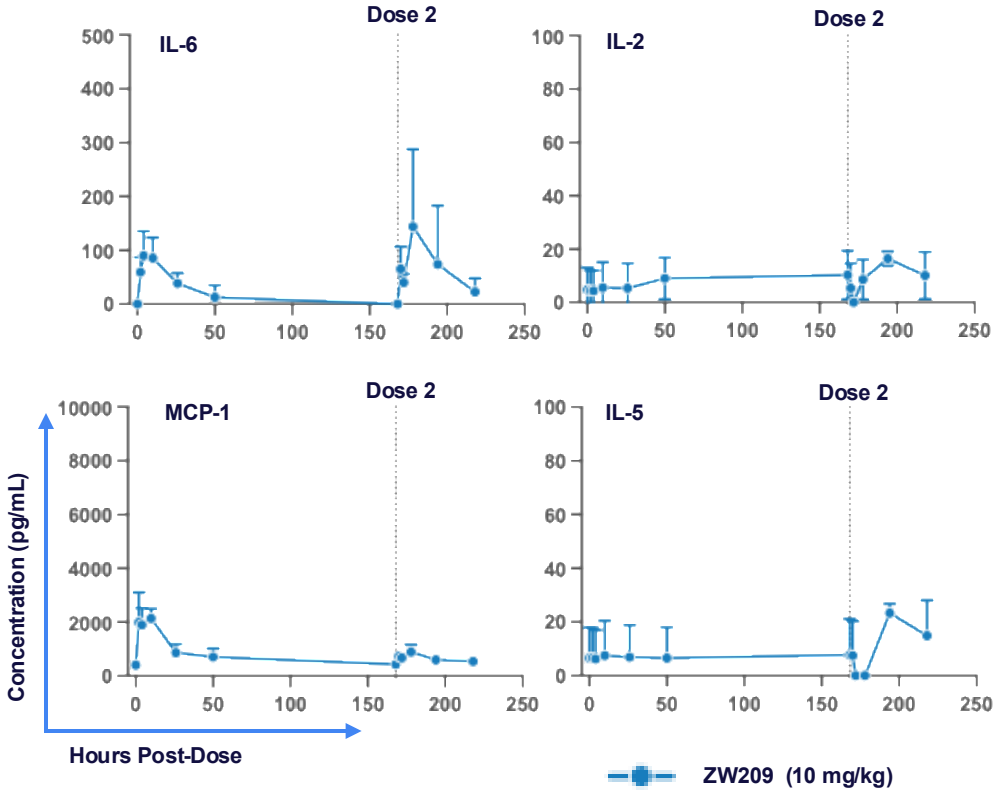


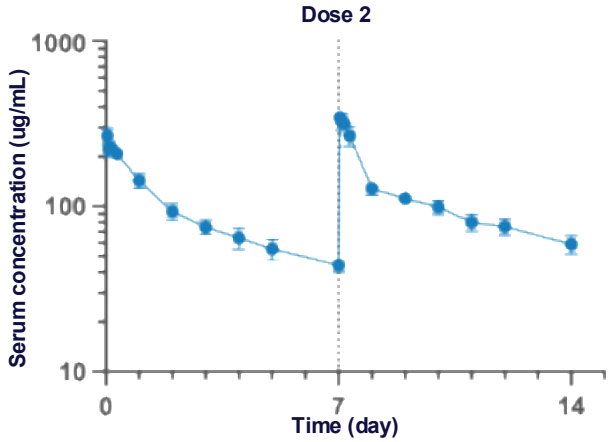


Irrelevant mAb
ZW209
TGN1412♀
PBS
ZW209 CD28^{null}
AMG 757†
OKT3

† AMG 757 (DLL3/CD3 BiTE) produced in-house;
♀ TGN1412 (hIgG4; biosimilar produced in-house)

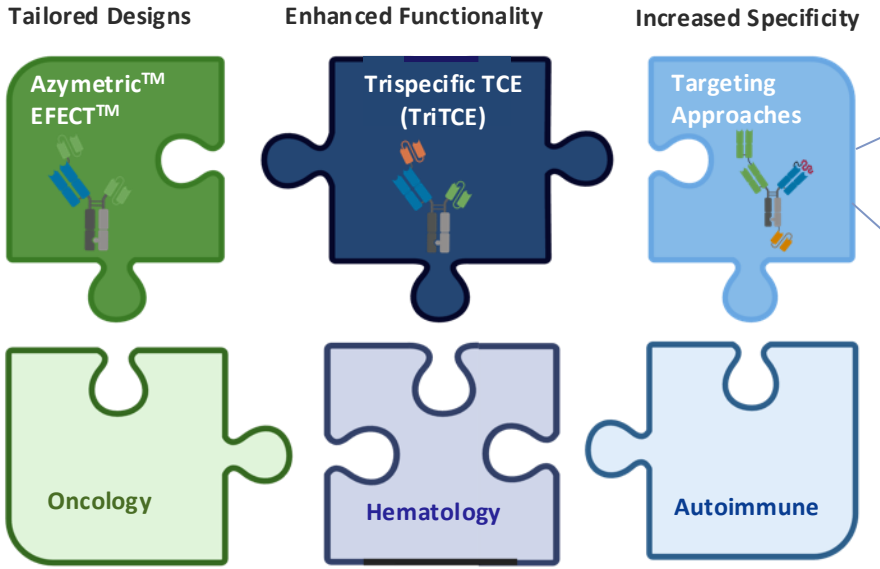
ZW209 is Well-tolerated in Cynomolgus Monkeys





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

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



Targeting Selectivity
- 2+1 formats
- Novel targets
- Multi-antigen targeting

Conditional Activation
- Conditional masking with proprietary cleavage sequence

Focus on indications with high unmet need → Identify biological challenge limiting responses → Tailored engineering solutions

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