Leveraging Humanized Mouse Models to Support T-Cell Engager Development

Tumor Models Summit San Francisco

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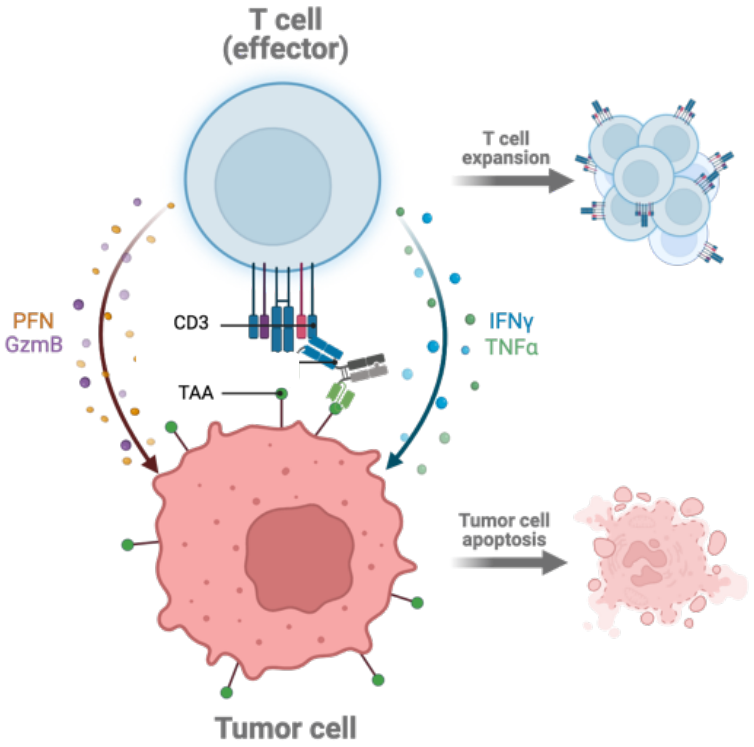
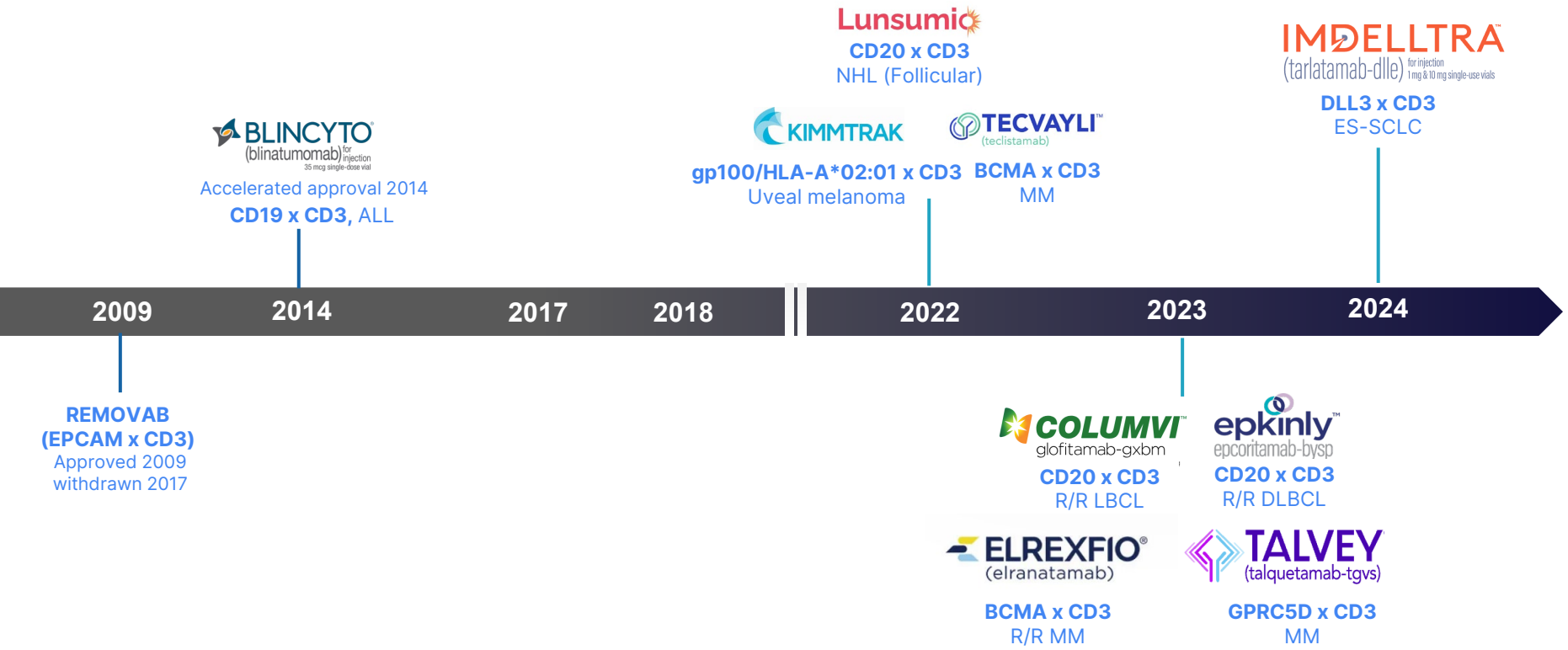
Nichole Escalante, PhD
Senior Scientist and Group Lead
in vivo Pharmacology and Pharmacokinetics

T Cell Engagers for Cancer Immunotherapy

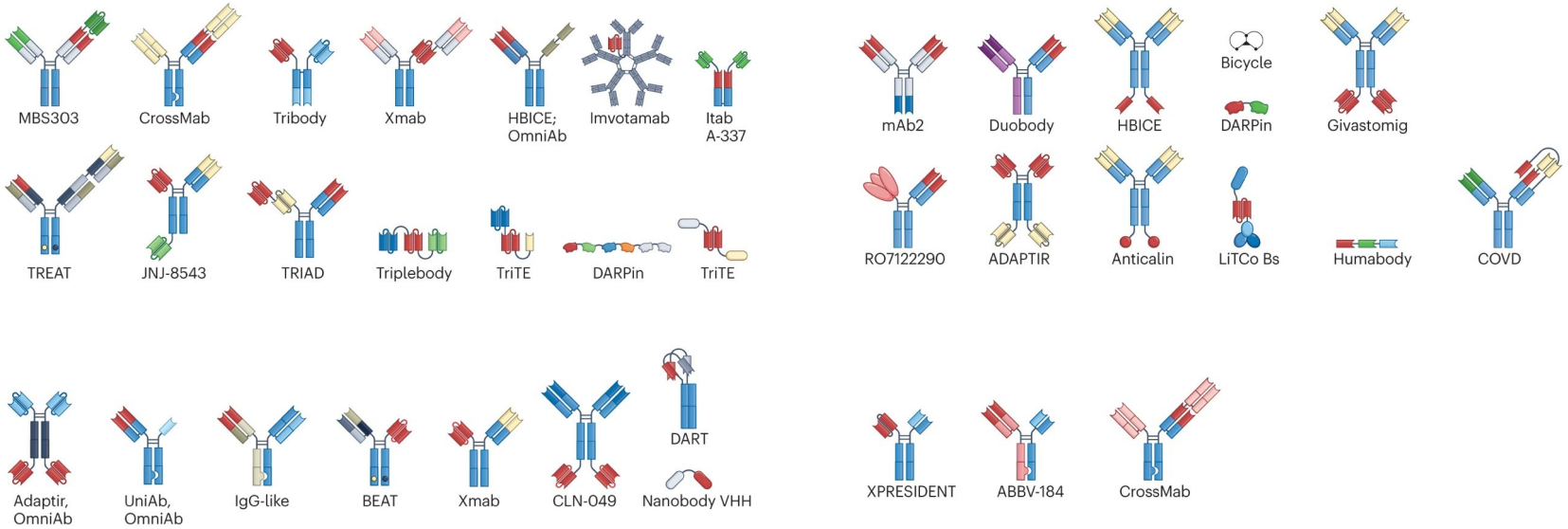


- T cell Engagers (TCEs) are classically designed as bi-specific antibody-like proteins that simultaneously engage T cells and tumor cells leading to targeted tumor cell death
- Targeting of T cells through anti-CD3 arm leads to T cell recruitment and activation
- Targeting of tumor cells through anti - Tumor Associated Antigen (TAA) arm increases specificity of killing

Accelerated Pace of T Cell Engager Approvals in Oncology



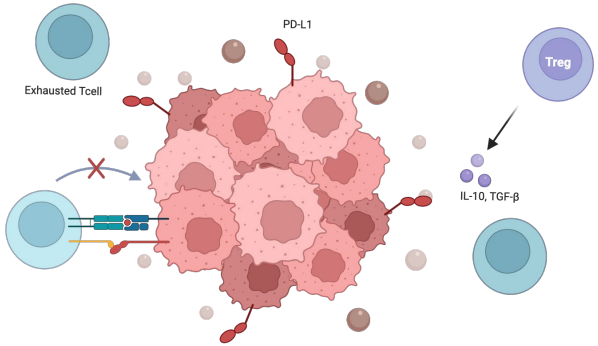
TCEs are Rapidly Evolving in Format and Functionality

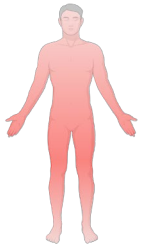


Fenis, A., Demaria, O., Gauthier, L. *et al.* New immune cell engagers for cancer immunotherapy. *Nat Rev Immunol* **24**, 471–486 (2024).

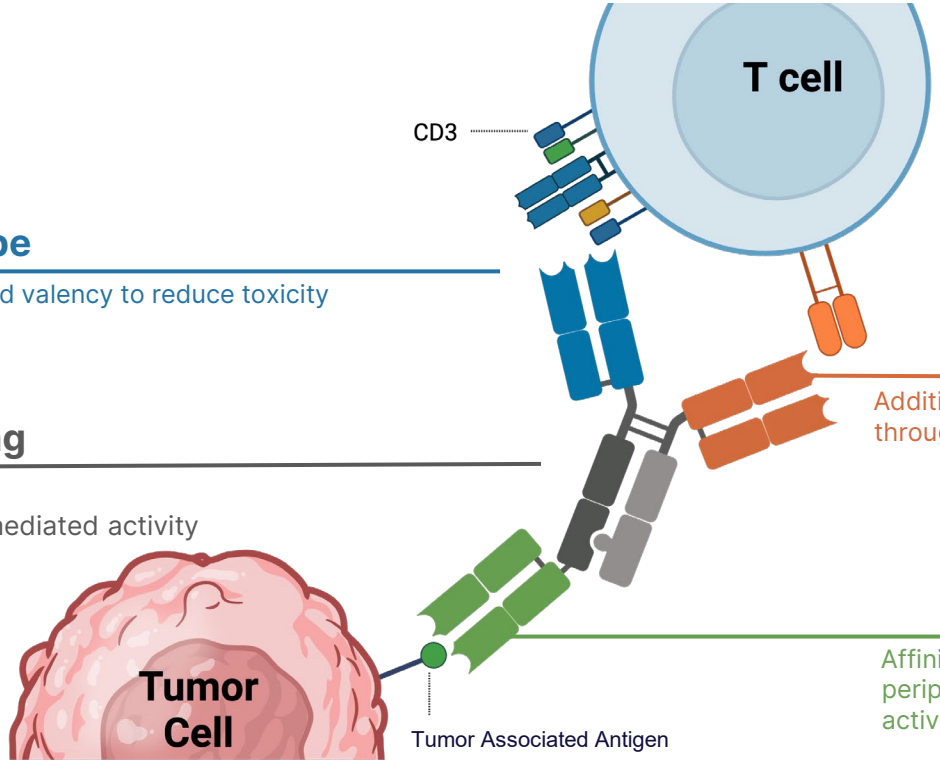
Challenges Exist for TCEs in the Clinic

- Dose limiting toxicities (CRS, neurotoxicity, on-target off tumor)
- Post-treatment relapse (PD-L1 upregulation, Treg increases, T cell exhaustion, Loss of TAA)
- Short serum half life of first generation of T cell engagers
- Poor efficacy in solid tumors (less penetration and T cell presence)





Innovative Engineering of TCEs to Overcome Clinical Challenges



αCD3 Paratope

Fine tuned affinity and valency to reduce toxicity from CRS.

Fc engineering

Half-life extension
Modulation of Fc mediated activity

Checkpoint blockade or Co-stimulation Paratope

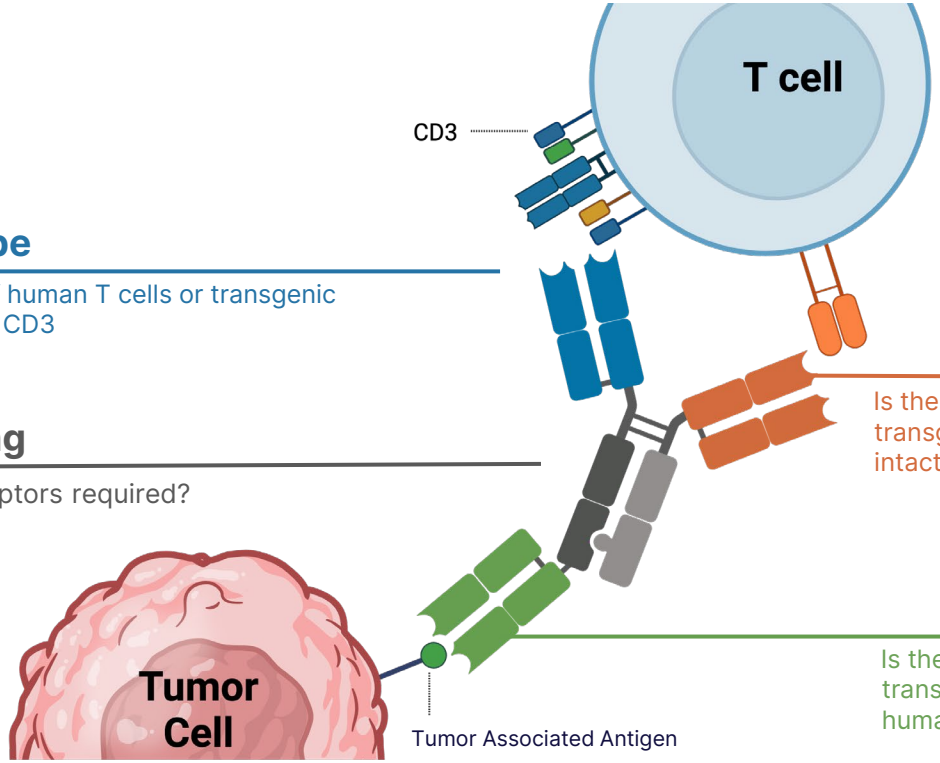
Additional functionality that enhances anti-tumor activity through a complimentary pathway

αTAA Paratope

Affinity and valency adjusted to mitigate the risk of peripheral T cell activation and on-target off tumor activity

Zymeworks Data References:
L Newhook, P Bhojane et al. SITC 2023. Poster#1372
L. Newhook, P. Bhojane et al. AACR 2023. Poster#5121.

Engineering of TCEs Increases Complexity of *in vivo* Modeling



αCD3 Paratope

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

Fc engineering

Binding to Fc Receptors required?

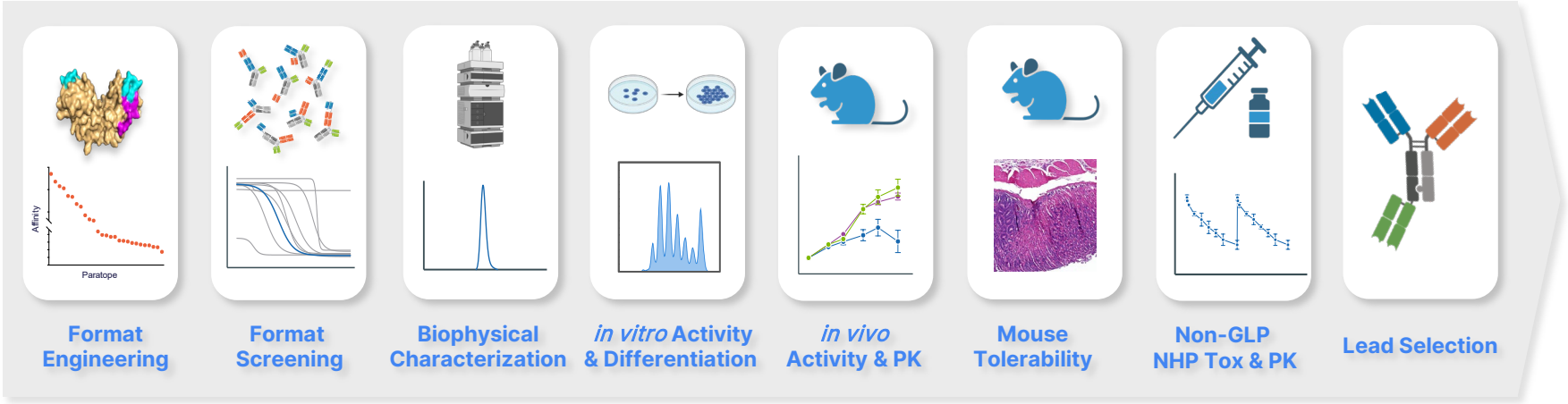
Checkpoint blockade or Co-stimulation Paratope

Is there cross-reactivity to mouse proteins or is a transgenic mouse required? Is your signaling pathway intact?

αTAA Paratope

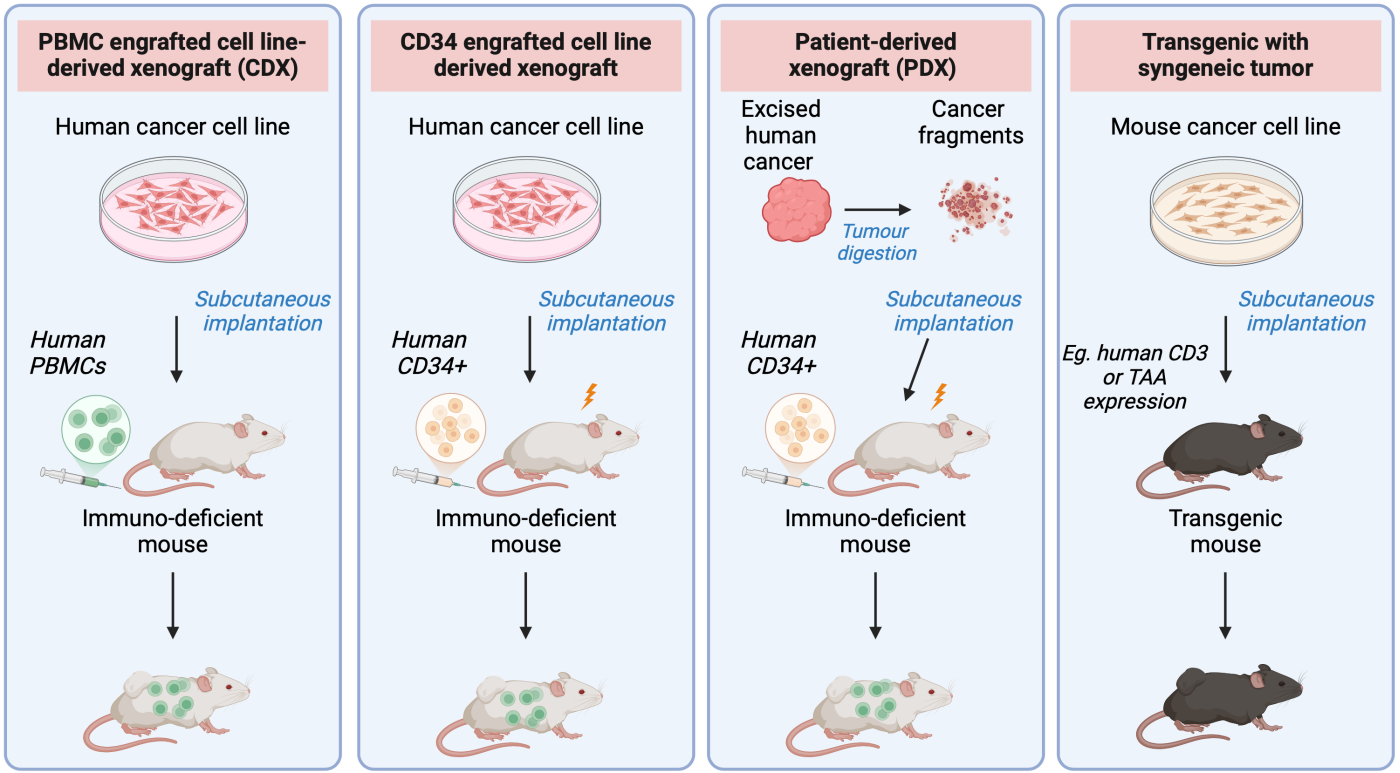
Is there cross-reactivity to mouse protein or is a transgenic mouse required? Cell line expression of human protein? Expression patterns.

Workflow For Preclinical Development of Multispecific TCEs



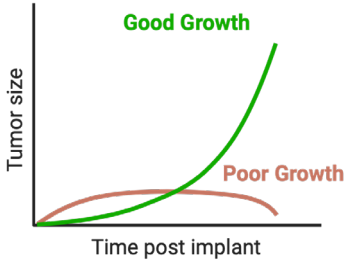
Why Humanized Mouse Models?

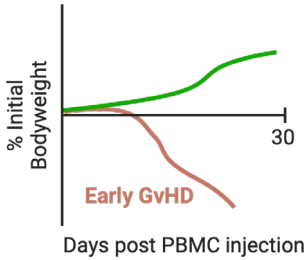
Humanized *in vivo* Models for Evaluating TCE Anti-tumor Activity

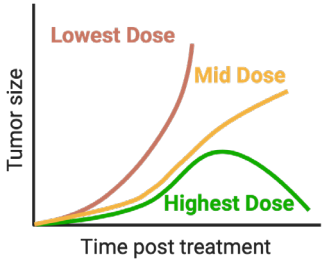


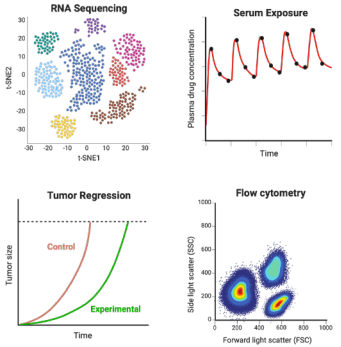
PBMCs = Peripheral Blood Mononuclear Cells

Focus in: PBMC Humanized Models









Model Selection / Growth Kinetics

- Expression of TAA
- Indication of interest
- Previous use with humanized mice
- Evidence of activity with similar molecules

PBMC Donor Screen

- Timeline for graft versus host disease
- Non-treatment PBMC vs Tumor activity
- Responsiveness to positive control

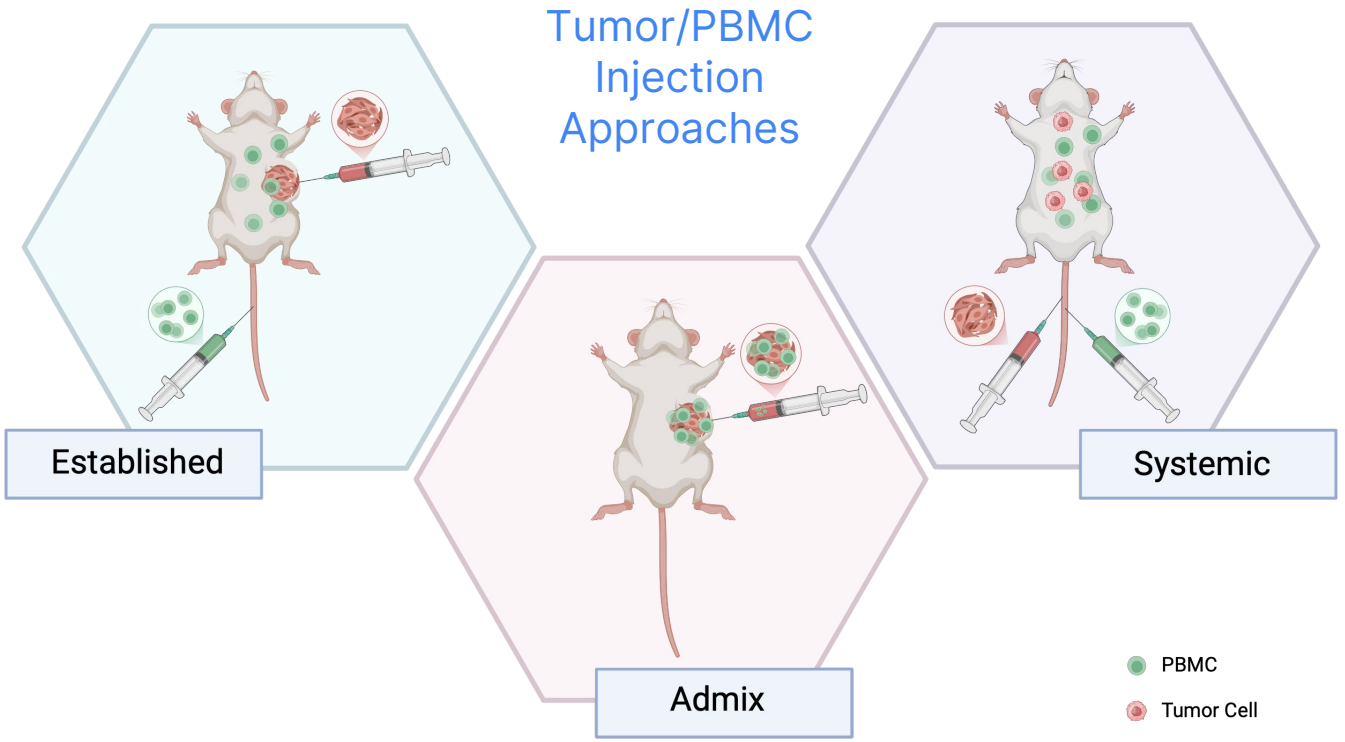
Dose Range Finding Study

- Active dose ranges for our positive control or lead molecule

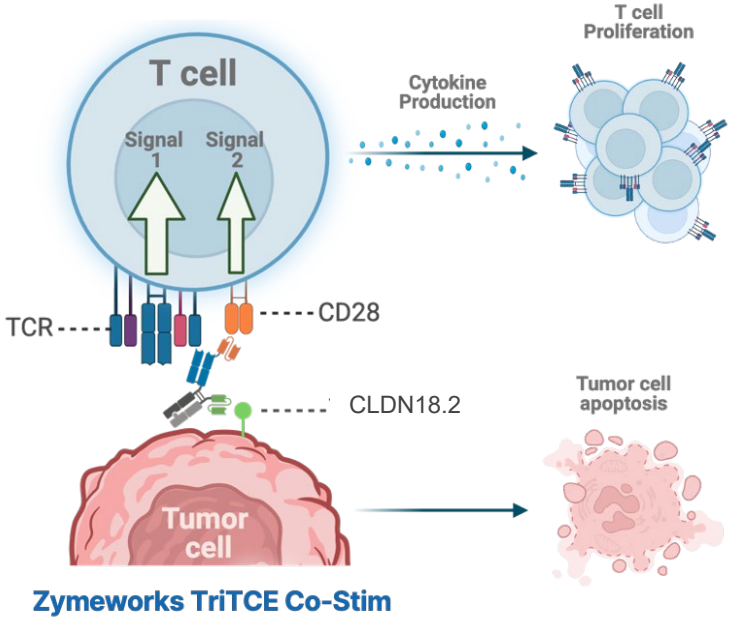
In vivo Anti-tumor Study

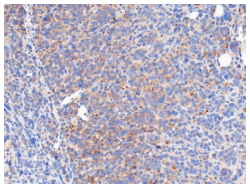
- Activity compared to controls and benchmarks
- Blood Pharmacokinetics
- Bodyweight loss
- Clinical health
- Pharmacodynamic analysis

Focus in: PBMC Humanized Models

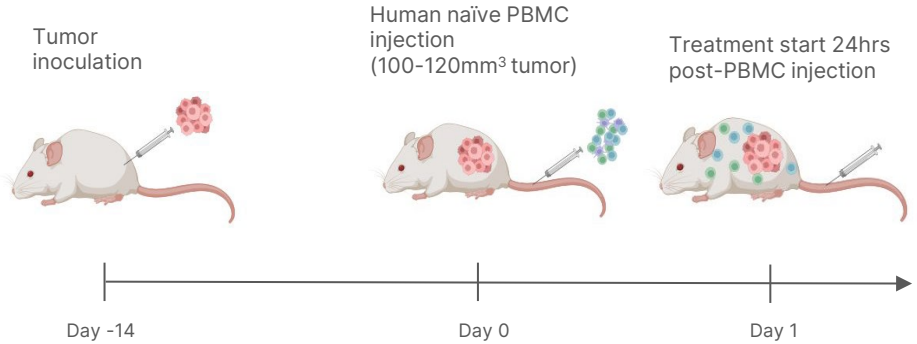


Case Study 1: Established PBMC Humanized Models can be used to Evaluate Activity of Trispecific TCEs with Co-stimulatory Function



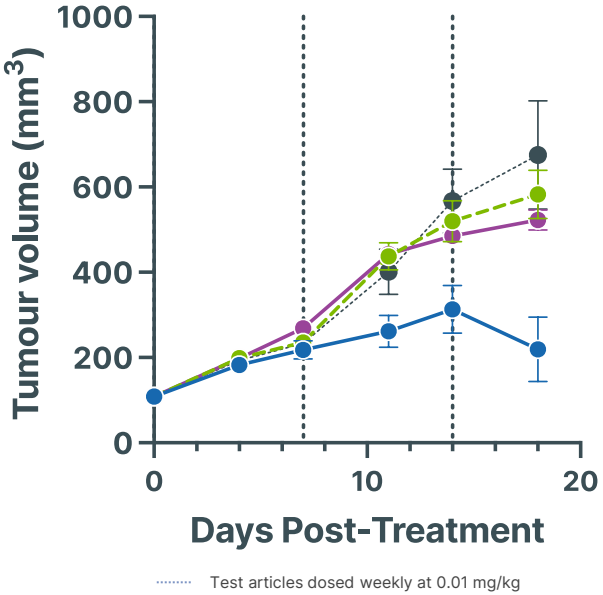


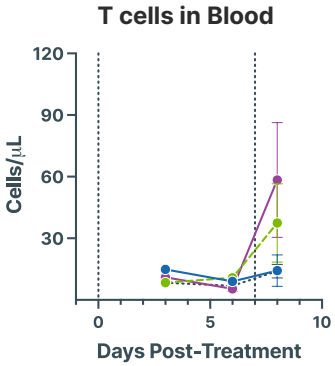
CLDN18.2 IHC of Gastric Cancer Cell line (SNU620)

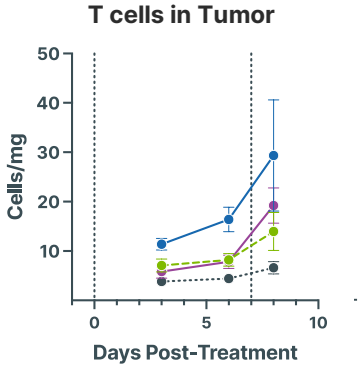


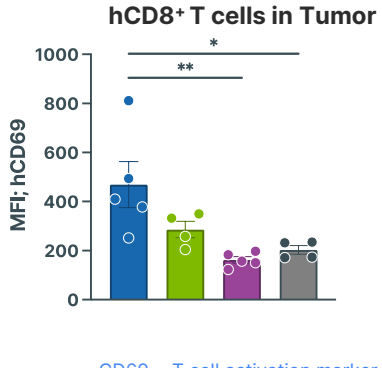
Goal: Evaluate activity of TriTCE molecule compared to Bispecific or Clinical Benchmark

CLDN18.2 Trispecific TCE (ZW239) has Enhanced Activity in an Established PBMC Model of Gastric Cancer









CD69 = T cell activation marker

- CLDN18.2 TriTCE Co-Stim (CD3 x CLDN18.2 x CD28)
- CLDN18.2 Bispecific TCE (CD3 x CLDN18.2)
- Benchmark (CD3 x CLDN18.2)
- Negative Control

Zymeworks Data References:
L Newhook, P Bhojane et al. SITC 2023. Poster#1372
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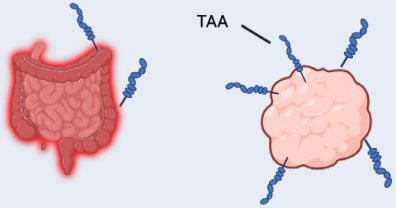
Tolerability Concerns for TCEs

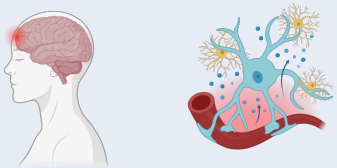
On Target Off Tumor Activity



Low TAA on Organ

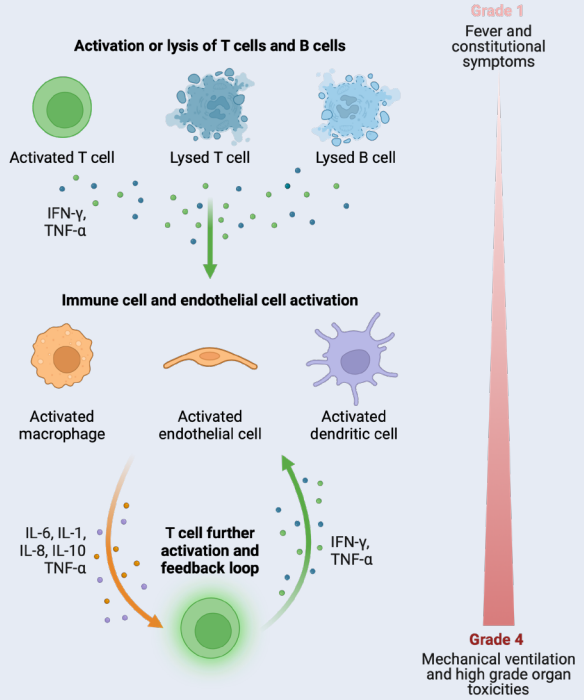
High TAA on tumor

Neurotoxicity



Immune cell-associated Neurotoxicity Syndrome (ICANS)

Cytokine Release Syndrome



In vivo Modeling of Cytokine Release Syndrome Risk

In vivo Model	Features of CRS	
PBMC Cytokine Release High cytokine producing PBMCs in immunocompromised mouse	✓	T cell cytokine response (human)
	X	Myeloid cell activation and signal amplification from cytokines
	X	Non-immune cell response and activation by cytokines (e.g. Endothelial cells)
CD34⁺ Human w/ Cytokine Human stem cells engrafted in immunocompromised mouse with human cytokines (supports non-T cell immune engraftment)	✓	T cell cytokine response (human)
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Transgenic Human protein expression in immunocompetent mouse (eg. Human CD3 & Human TAA)	✓	T cell cytokine response (mouse)
	✓	Myeloid cell activation and signal amplification from cytokines
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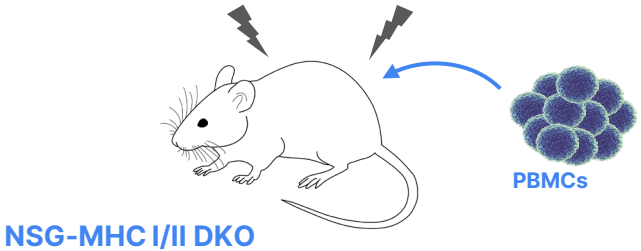
Case Study 2: PBMC Humanized Cytokine Release Model can be used to Assess Risks of Human T Cell Activation

- Utilizes PBMCs pre-screened and identified as high cytokine producers



Day 0:
- Irradiate Mice
- Engraft PBMCs

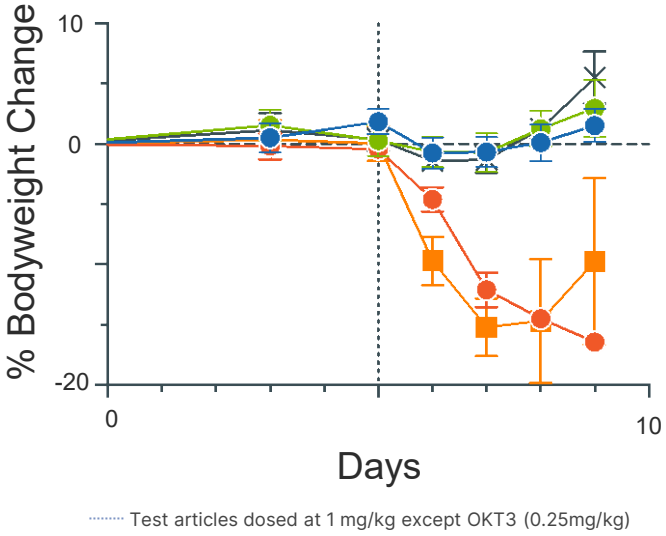
Day 6:
- Single dose Test Article Treatment

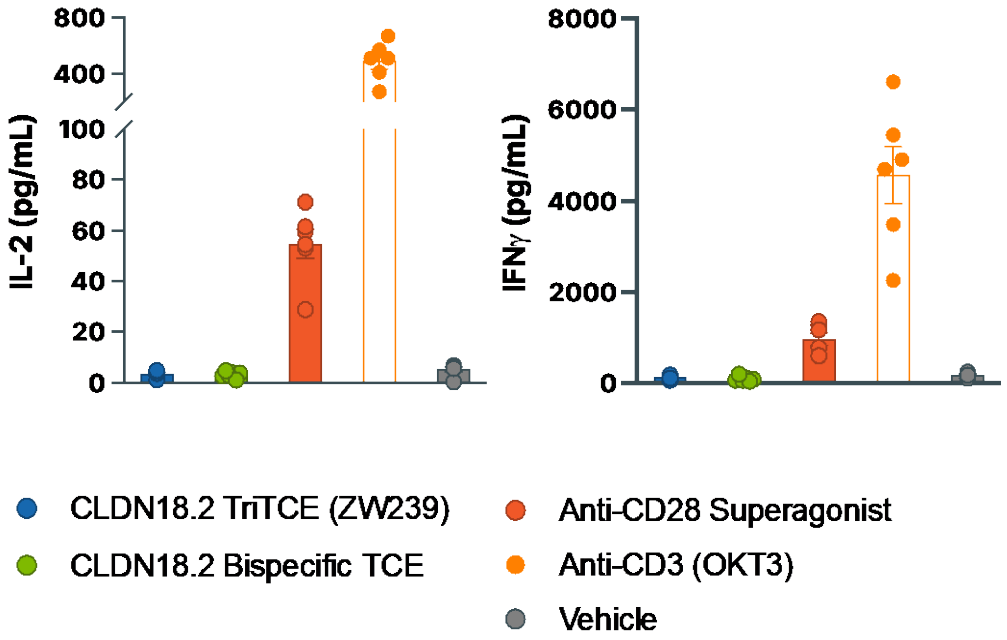
Assess Risk by:
- Systemic Cytokine Production (2&6 h post-treatment)
- Body weight loss

Goal: Evaluate T cell mediated tolerability risk of CD3 and CD28 engagement

Model References: A rapid, sensitive, and reproducible in vivo PBMC humanized murine model for determining therapeutic-related cytokine release syndrome. C. Ye et al. FASEB J. 2020 Aug 9;34(9):12963–12975.

OKT3 and an Anti-CD28 Superagonist cause Bodyweight Loss and Peripheral Cytokine Production but CLDN18.2 TriTCE Molecule Does Not





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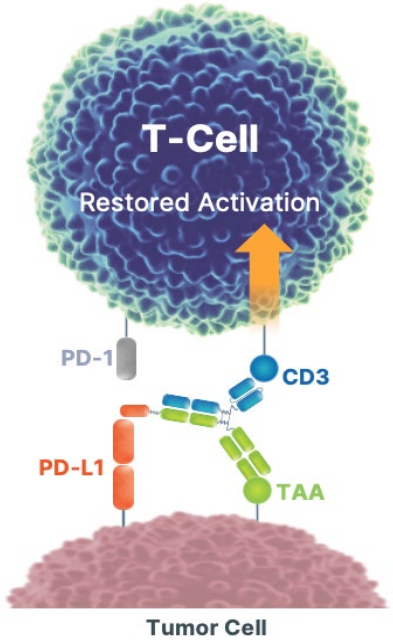
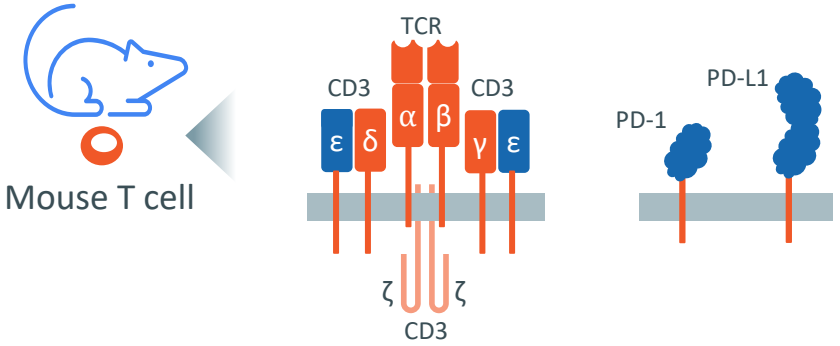
Case Study 3: Transgenic Models can be used to Assess CRS & On-target Off-tumor Activity Risk



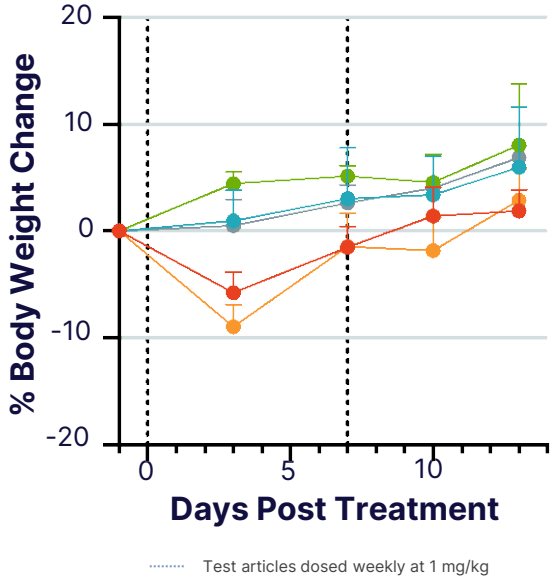
In vivo toxicity model

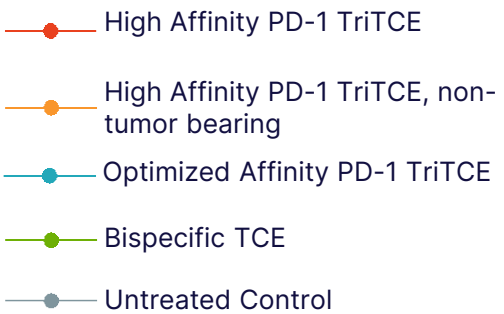
Transgenic mouse with human PD-1, PD-L1, and CD3ε



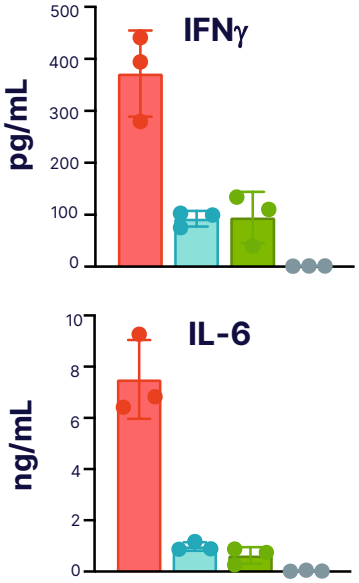
Goal: Assess PD-L1 & CD3 mediated tolerability risk of TriTCE Checkpoint Inhibitor molecules

Tuning PD-1 Domain Affinity Improves Tolerability and Peripheral Cytokine Release





Serum cytokines 24hrs post dosing



Zymeworks Data References: M. Verstraete, M. Poffenberger et al. SITC 2023. Poster#1395

Conclusions and Outstanding Challenges For TCE Screening

Conclusions:

- Innovative engineering of TriTCEs increases the complexity of *in vivo* modelling
- Humanized models can be used to evaluate efficacy and tolerability
- Each model has its limitations, and often multiple preclinical models are required

How to improve translatability?

- Testing primary patient derived tumor tissues (*ex-vivo*, *in vivo*)
- Use of more physiologically relevant models (e.g. Orthotopic, patient derived PBMCs)
- Comparing TCEs in combination with approved standard care

Thanks to the Zymeworks Multispecific Antibody Therapeutics Team

Check Out Our Poster!

Title: TriTCE Co-Stim: A next generation trispecific T cell engager platform with integrated CD28 co-stimulation to improve T cell function and antitumor responses in hard-to-treat cancers