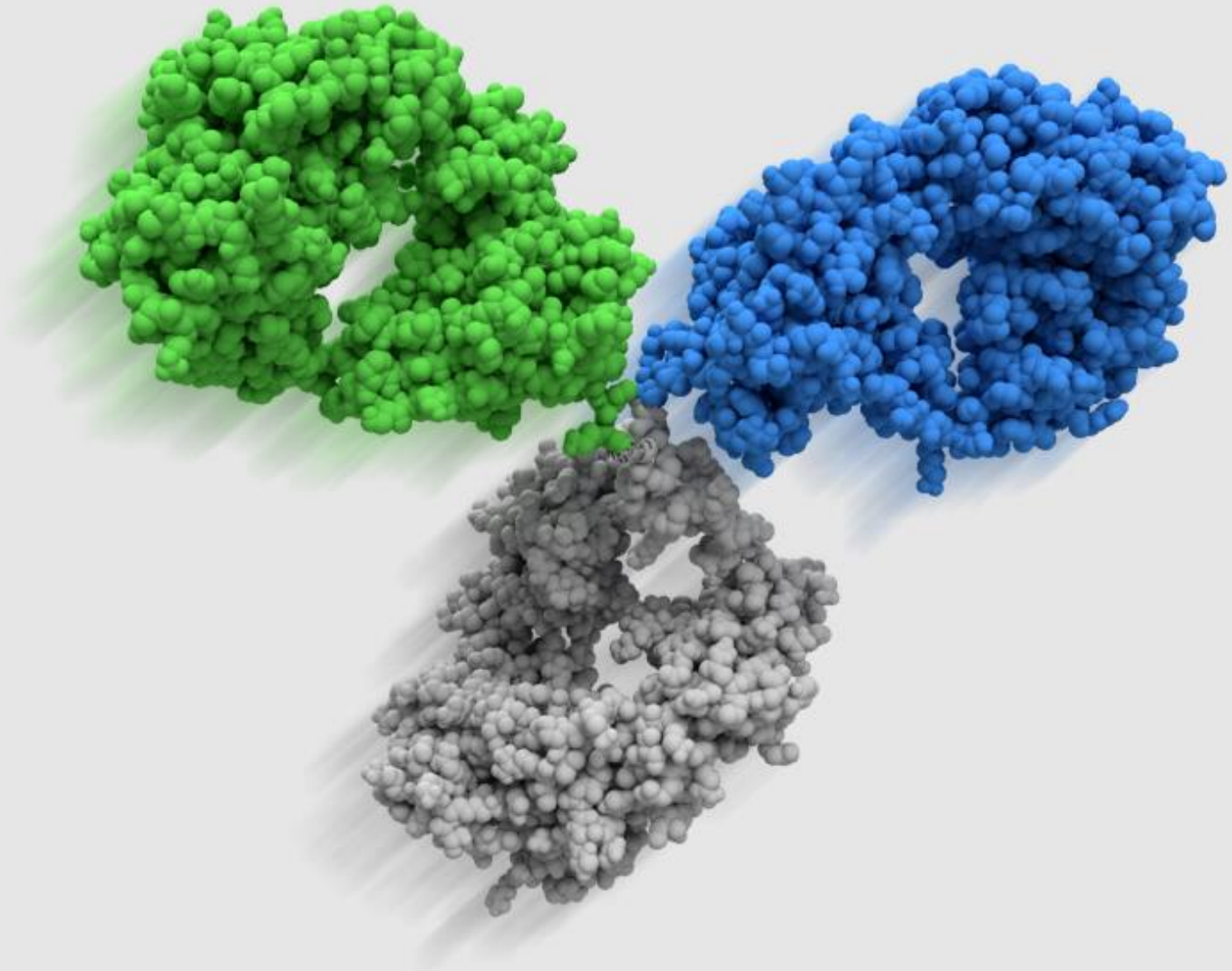
Engineering Trispecific CD3×CD28 T-Cell Engagers: Systematic Optimization of Affinity, Format, and Co-Stimulation



Bryan Glaser, PhD

PEGS Conference

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Nonconfidential

Novel Monoclonal, Bispecific, and Trispecific Antibodies: Discovered, Optimized, and Ready for the Clinic

Why Invenra?

Innovative

Science–first mentality | 10+ years multispecific expertise

Proven

20+ partners | 2 clinical-stage (2025)

Easy

Flexible and transparent business terms from fee-for-service to strategic collaboration

	Accelerated timelines with transparent pricing and terms
1 **Best multispecifics start with quality mAbs**	>30 diverse, optimized human libraries
	Achieve desired affinity, specificity, and functional profile goals
	Transfer easily to our multispecifics platforms
2 **B-Body® bispecific antibodies deliver unrivaled yield**	Accelerate lead identification
	Reduce manufacturing risks
	Make IgG modifications using a fully human IgG-like scaffold
3 **T-Body™ trispecific antibodies yield powerful solutions for complex disease**	Overcome manufacturability challenges
	Optimize performance based on desired target density, therapeutic window, and manufacturing
	Support advanced therapeutics: ADCs, next generation Immuno-Oncology & Cell Engagers

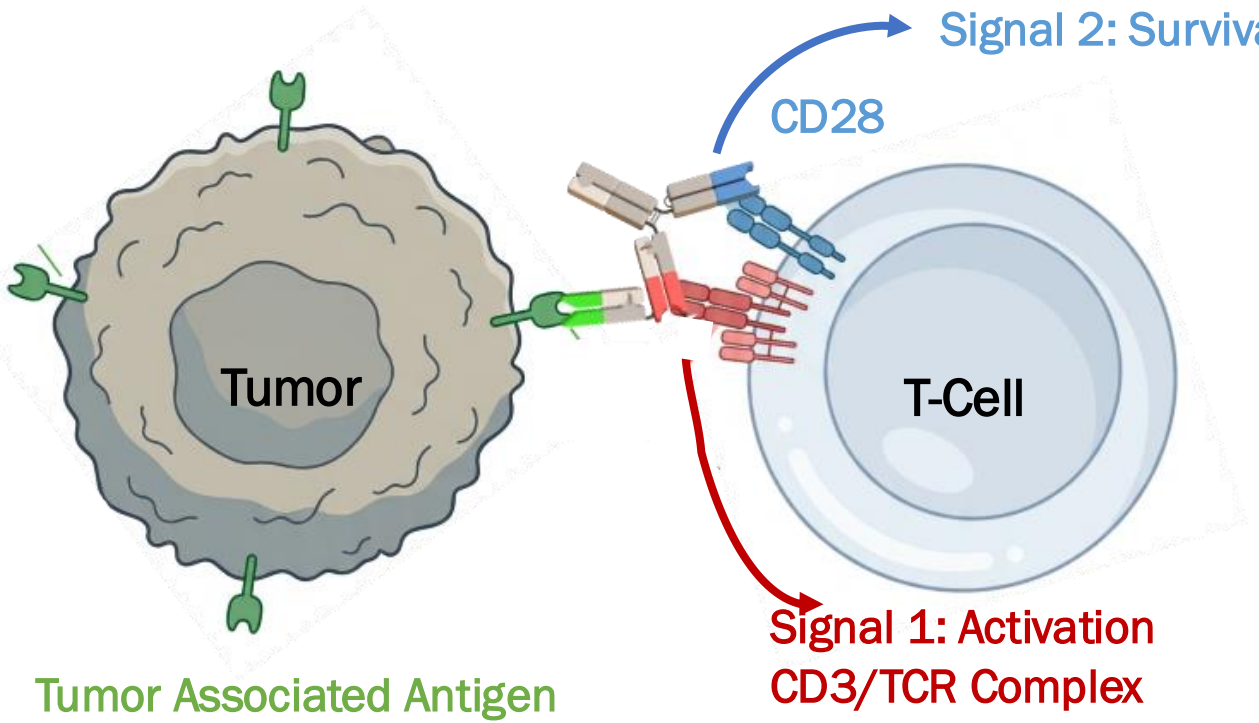
T-Cell Engager Challenges: Optimizing Therapeutic Index for Treating Solid Tumors

Advancing next-generation TCEs requires addressing three interconnected challenges

Narrow Therapeutic Window	T-Cell Exhaustion in Solid Tumors	Development Bottlenecks
CRS remains dose-limiting despite step-up dosing strategies	35-65% of tumor-infiltrating CD8+ T-cells express high PD-1 (exhausted phenotype)	Traditional antibody discovery and optimization: 6-12 months
On-target, off-tumor toxicity limits maximum tolerated doses	PD-1+/TIM-3+ co-expression indicates severe exhaustion	Affinity, epitope, format, and valency require empirical testing
CD3 affinity tuning critical but complex—narrow optimization window	CD3 engagement alone may be insufficient to reactivate exhausted T-cells	Iterative cycles extend timelines by 4+ months per reformatting
Need enhanced tumor selectivity to decouple efficacy from toxicity	Co-stimulatory signals needed for durable anti-tumor responses	Critical barrier to exploring new targets and disease indications

Bispecific TCE programs in solid tumors have achieved low ORR across multiple Phase 1 trials, with many discontinued before reaching therapeutic dose due to toxicity

Two Signals Drive Superior T-Cell Responses



Signal 1 (CD3): T-Cell Activation
- Initiates cytotoxicity
- Limited proliferation alone
- Susceptible to apoptosis

Signal 2 (CD28): Co-Stimulation & Survival
- Prevents apoptosis (Bcl-xL ↑)
- Sustained proliferation
- Memory formation
- **Reactivates exhausted T-cells**

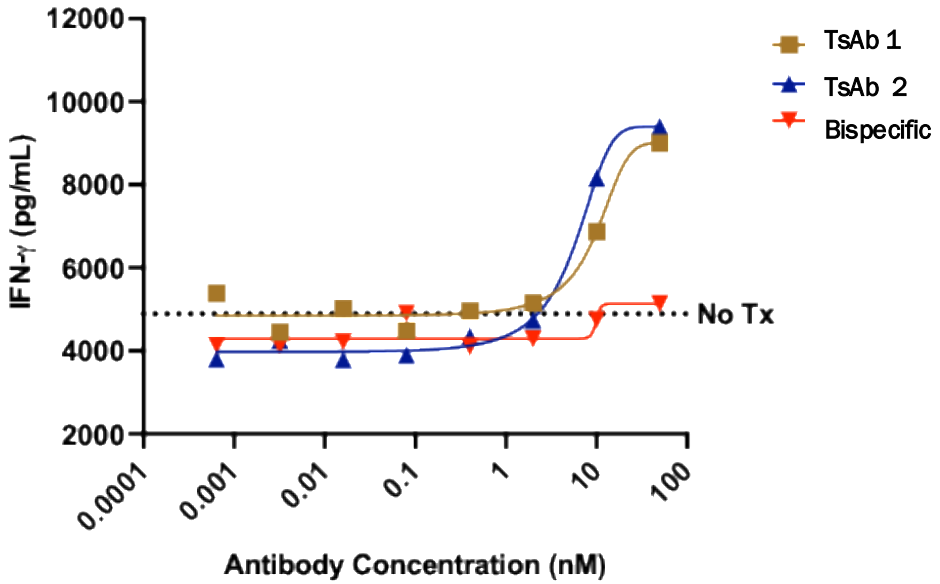
Impact in Trispecific TCEs: Enhanced Activity
- >30-fold increased T-cell activation
- **2-4 fold** improved T-cell survival
- Critical for PD-1+ exhausted T-cells

CD28 co-engagement transforms TCE activity and is particularly critical for reactivating exhausted T-cells in solid tumors

CD3/CD28 Co-Engagement Reactivates Exhausted T-Cells

PD-1+ exhausted T-cells require CD28 co-stimulation for reactivation

T-Body Trispecific TCE vs B-Body Bispecific TCE



Trispecific (CD3×CD28×TAA) Performance:

✓ IFN-γ production from PD-1+ exhausted T-cells
- TsAbs have significant increase vs. bispecific

✓ Enhanced exhaustion marker modulation
- Demonstrates engagement of PD-1+ T-cell population
- Bispecifics (CD3×TAA): minimal activation

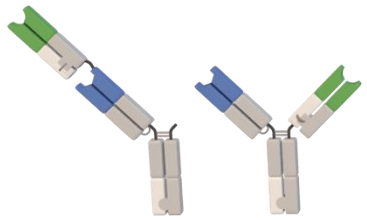
✓ Maintained safety profile
- No cytokines detected in absence of tumor cells
- Tumor-dependent activation preserved

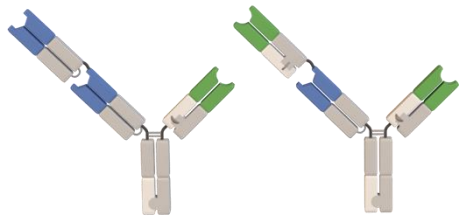
How do we identify optimal CD3/CD28 combinations?

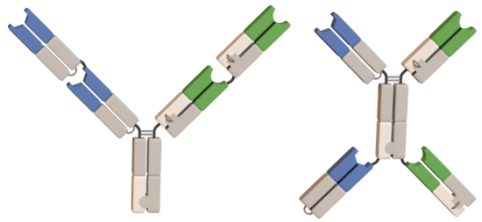
TCE Format Selection: Context-Dependent Optimization

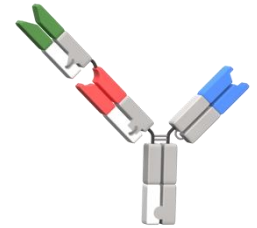
Target density, therapeutic window, and manufacturing drive choice of optimal format

Invenra's B-Body® Bispecific & T-Body™ Trispecific Antibody Family





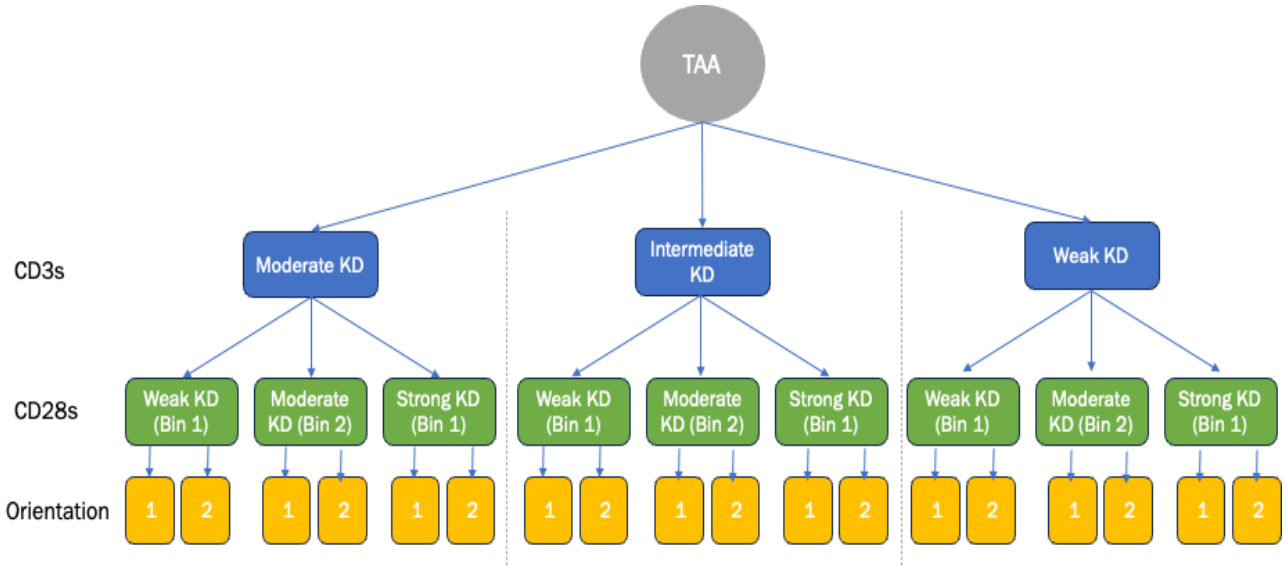




	B-Body® Bispecific 1x1	B-Body® Bispecific 2x1	B-Body® Bispecific 2x2	T-Body™ Trispecific
Key Feature	Monovalent CD3 & TAA	Bivalent TAA	Bivalent CD3 & TAA	CD3 + CD28 + TAA
Advantage	Simple, IgG-Like	Stronger tumor binding	Avidity to both	Co-stimulation enabled
Trade Off	No Avidity	More complex	CD3 avidity toxicity risk	Molecular complexity

Invenra's platforms enable parallel format testing in final configuration & functional data in weeks, not months

T-Body Trispecific Platform Enables Rapid Systematic Optimization of TCEs



Performed in collaboration with Alloy Therapeutics

Experimental Design:

- 18 variants in final T-Body trispecific format
  - 3 CD3 affinities (Low, Moderate, High)
  - 3 CD28 affinities across 2 epitope bins
  - 2 molecular orientations

Platform Advantages:

- Plug-and-play variable domain design
- All variants tested in final trispecific format
- No reformatting required between screening and characterization
- Parallel expression and functional testing

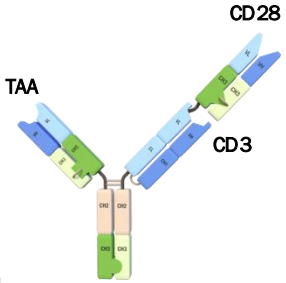
Timeline:

- All 18 variants expressed and characterized in 10 weeks

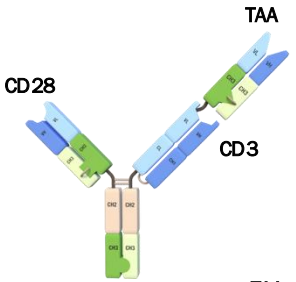
All variants expressed and functionally characterized in 10 weeks

B-Body Bispecific and T-Body Trispecific Expression and Developability

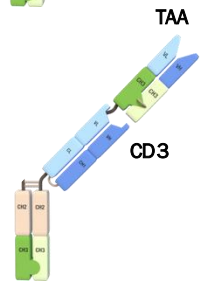
Identity	Orientation	Expression (ug/mL)	PDI	Z-Ave D (nm)	Tm (DegC)	Tagg 266 (degC)	Tagg473 (DegC)	SEC	SCX	HIC
CD3 (Mod) + CD28 (Weak, Bin 1)	TAA x CD28 + CD3	28.85	0.045	13.34	53.73	64.54	66.01	Pass	Pass	Pass
CD3 (Mod) + CD28 (Strong, Bin 1)	TAA x CD28 + CD3	72.1	0.01	13.17	65.56	66.79	65.67	Pass	Pass	Pass
CD3 (Mod) + CD28 (Mod, Bin 2)	TAA x CD28 + CD3	52.15	0.035	13.11	64.76	65.56	65.21	Pass	Pass	Pass
CD3 (Low) + CD28 (Weak, Bin 1)	TAA x CD28 + CD3	32.3	0.021	13.09	53.33	66	65.38	Pass	Pass	Pass
CD3 (Low) + CD28 (Strong, Bin 1)	TAA x CD28 + CD3	69.9	0.018	13.38	65.88	67.18	66.82	Pass	Pass	Pass
CD3 (Low) + CD28 (Mod, Bin 2)	TAA x CD28 + CD3	125.25	0.093	13.03	65.2	65.84	65.8	Pass	Pass	Pass
CD3 (Int) + CD28 (Weak, Bin 1)	TAA x CD28 + CD3	54.3	0.01	13.52	53.23	65.43	64.81	Pass	Pass	Pass
CD3 (Int) + CD28 (Strong, Bin 1)	TAA x CD28 + CD3	116	0.025	13.23	65.39	66.52	65.51	Pass	Pass	Pass
CD3 (Int) + CD28 (Mod, Bin 2)	TAA x CD28 + CD3	57.15	0.07	13.54	65.8	66.2	65.48	Pass	Pass	Pass



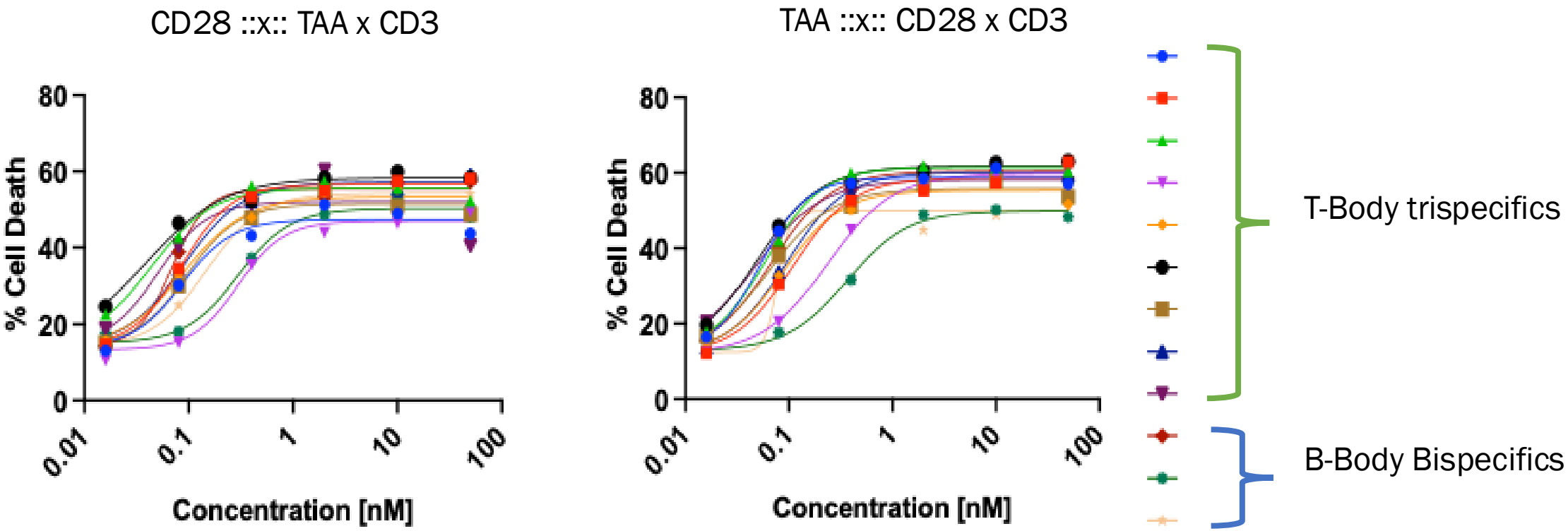
Identity	Orientation	Expression (ug/mL)	PDI	Z-Ave D (nm)	Tm (DegC)	Tagg 266 (degC)	Tagg473 (DegC)	SEC	SCX	HIC
CD3 (Mod) + CD28 (Weak, Bin 1)	CD28 x TAA + CD3	112.8	0.023	13.63	54.18	65.9	65.95	Pass	Pass	Pass
CD3 (Mod) + CD28 (Strong, Bin 1)	CD28 x TAA + CD3	75.1	0.06	13.69	62.24	65.91	65.11	Pass	Pass	Pass
CD3 (Mod) + CD28 (Mod, Bin 2)	CD28 x TAA + CD3	91.75	0.006	14.23	63.29	64.81	64.94	Pass	Pass	Pass
CD3 (Low) + CD28 (Weak, Bin 1)	CD28 x TAA + CD3	65.7	0.041	13.76	54.07	66.3	66.39	Watch	Pass	Pass
CD3 (Low) + CD28 (Strong, Bin 1)	CD28 x TAA + CD3	48.7	0.01	13.89	62.45	65.7	65.78	Pass	Pass	Pass
CD3 (Low) + CD28 (Mod, Bin 2)	CD28 x TAA + CD3	66.9	0.041	14.19	63.66	65.47	65.83	Pass	Pass	Pass
CD3 (Int) + CD28 (Weak, Bin 1)	CD28 x TAA + CD3	46.95	0.031	13.77	54.27	65.5	66.13	Watch	Pass	Pass
CD3 (Int) + CD28 (Strong, Bin 1)	CD28 x TAA + CD3	65.3	0.038	13.94	62.15	65.5	65.84	Pass	Pass	Pass
CD3 (Int) + CD28 (Mod, Bin 2)	CD28 x TAA + CD3	65.2	0.019	14.5	63.46	64.46	65.07	Pass	Pass	Pass



Identity	Orientation	Expression (ug/mL)	PDI	Z-Ave D (nm)	Tm (DegC)	Tagg 266 (degC)	Tagg473 (DegC)	SEC	SCX	HIC
TAA x CD3 (Mod)	Null x TAA X CD3	116.5	0.03	12.37	66.97	68.07	68.54	Pass	Pass	Pass
TAA x CD3 (Low)	Null x TAA x CD3	70.95	0.039	12.6	67.97	68.78	69.47	Pass	Pass	Pass
TAA x CD3 (Int)	Null x TAA x CD3	74.3	0.009	12.57	68.16	68.79	69.25	Pass	Pass	Pass



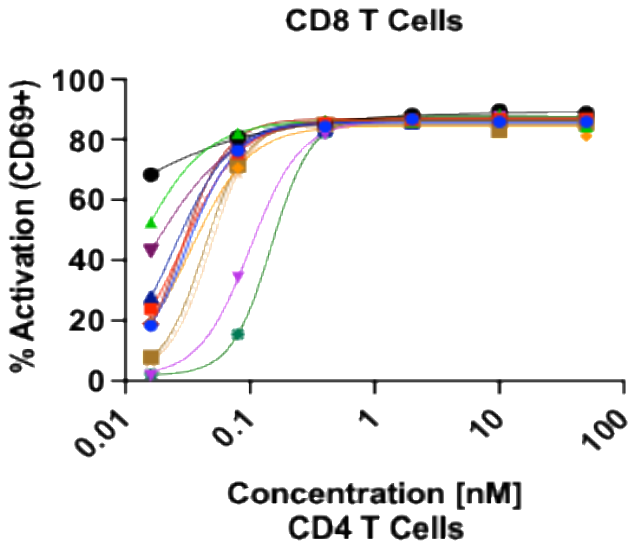
Comparison of T-Body Trispecific vs B-Body Bispecific TCEs Killing of Target Cells

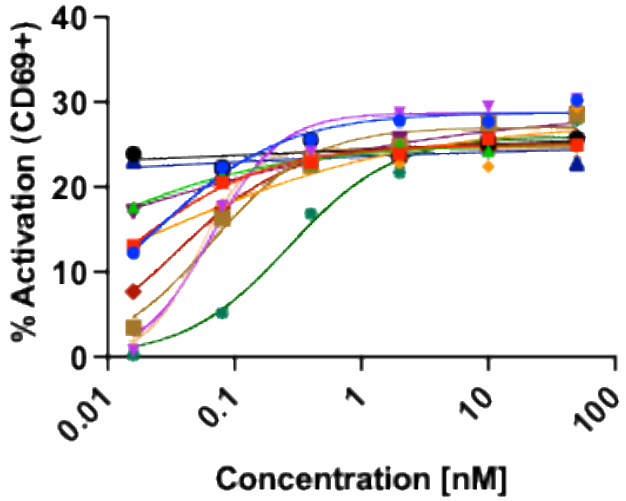


- Trispecific TCEs outperformed bispecific TCEs
- Optimization of CD3 and CD28 KDs and epitope was Important for enhancing efficacy

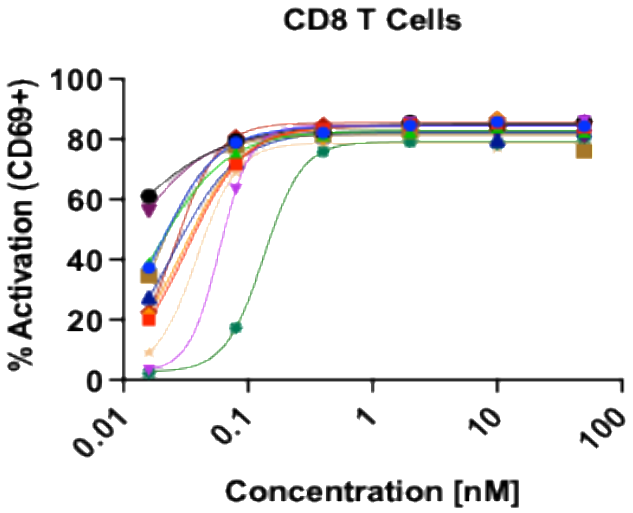
Comparison of T-Body Trispecific vs B-Body Bispecific Activation of CD8 and CD4 T Cells in Presence of Tumor Cells

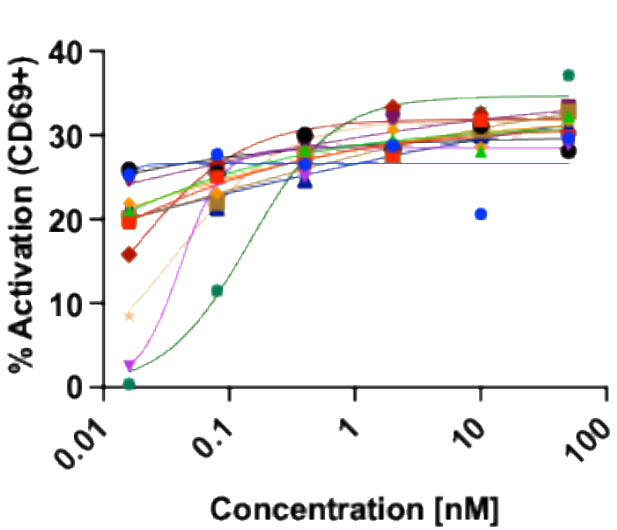
CD28 ::x:: TAA x CD3

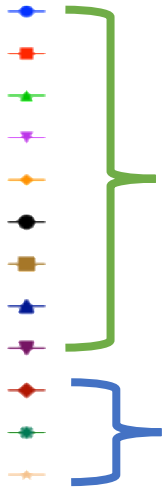




TAA ::x:: CD28 x CD3







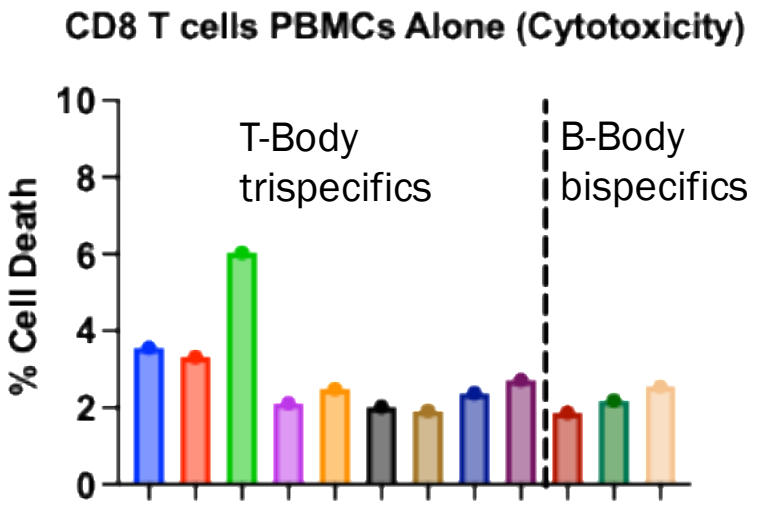
T-Body trispecifics

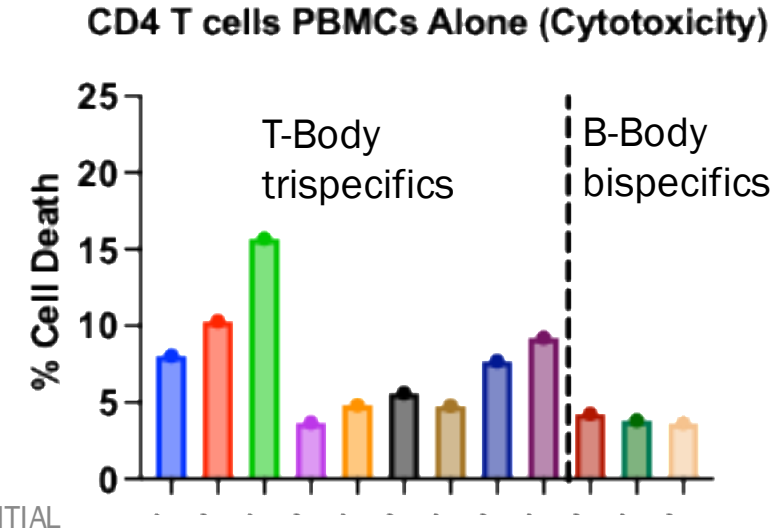
B-Body Bispecifics

- Combinations of CD3 and CD28 KDs and epitope generated a range of T-cell activation

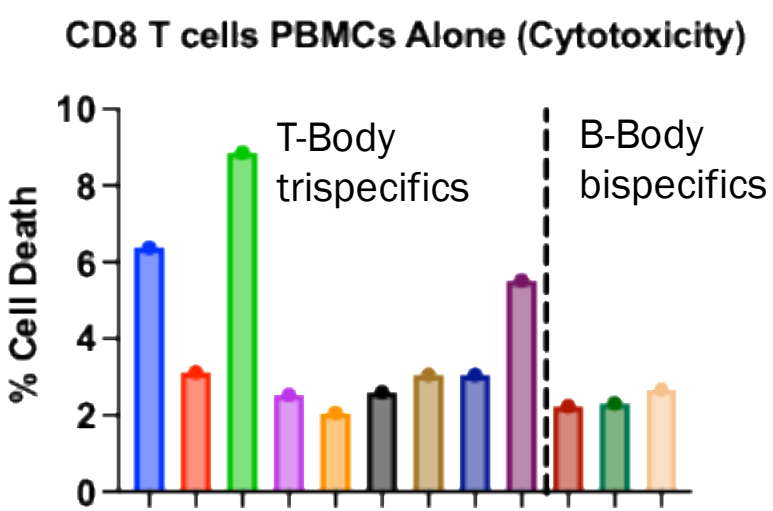
Comparison of T-Body Trispecific vs B-Body Bispecific TCE Induced Fratricide of CD8 + CD4 T-Cells (No Tumor)

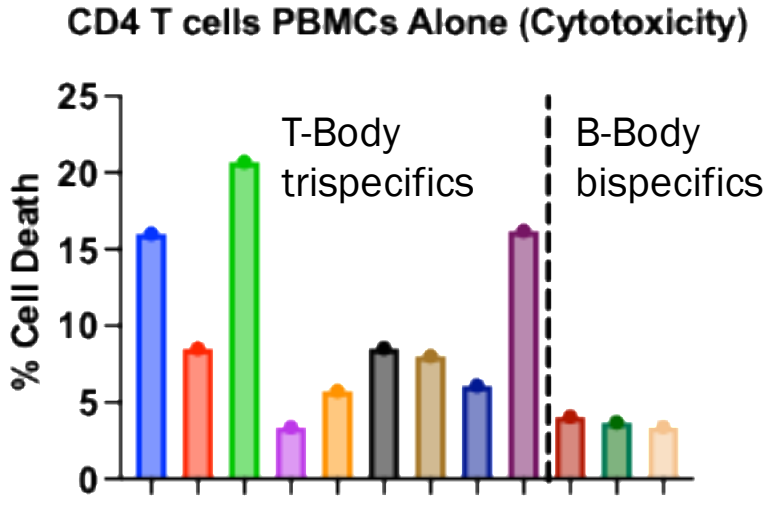
CD28 ::x:: TAA x CD3





TAA ::x:: CD28 x CD3

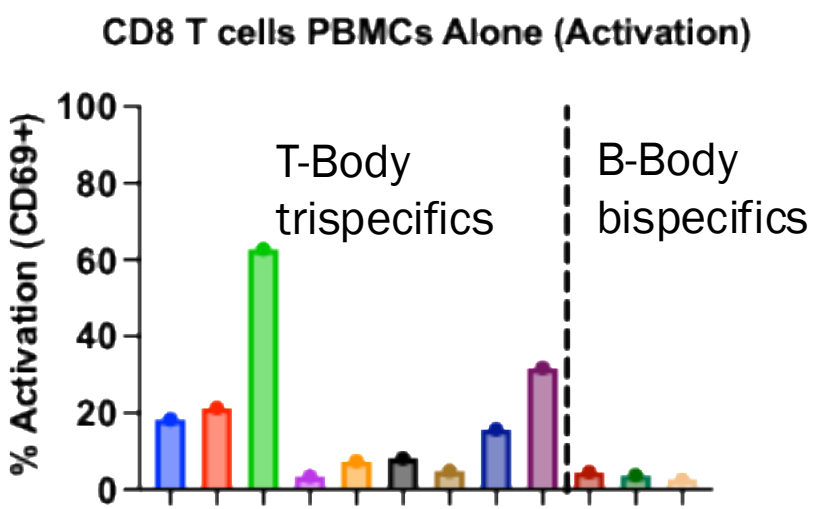


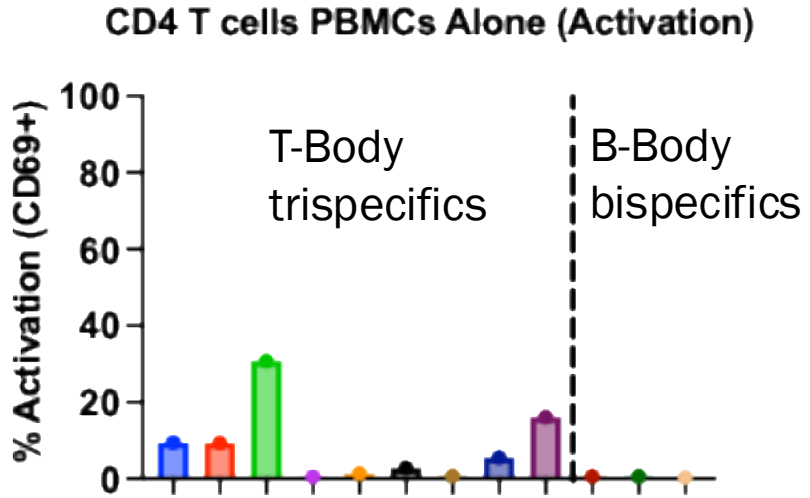


- Testing of CD3 and CD28 combinations was important to identify low T-cell Fratricide

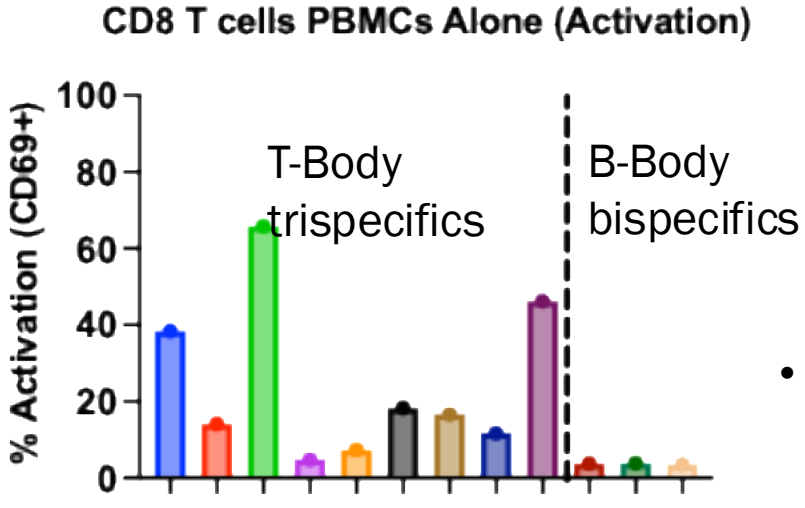
Comparison of T-Body Trispecific vs B-Body Bispecific TCE Auto-Activation of CD8 and CD4 T Cells (No Tumor)

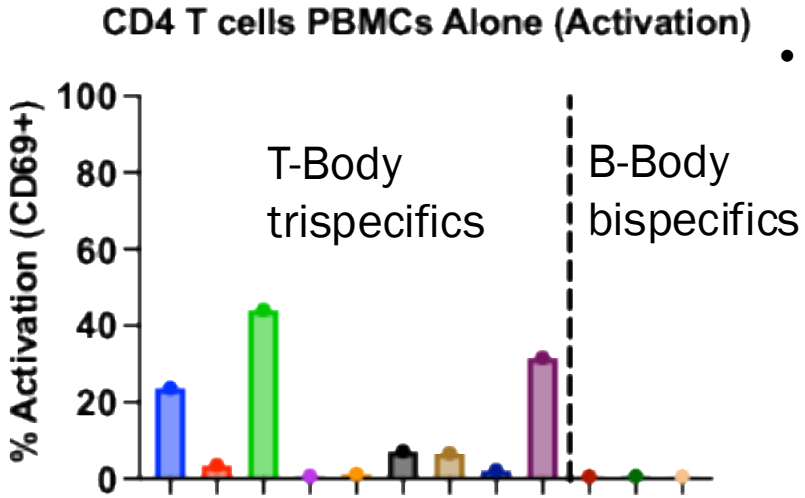
CD28 ::x:: TAA x CD3





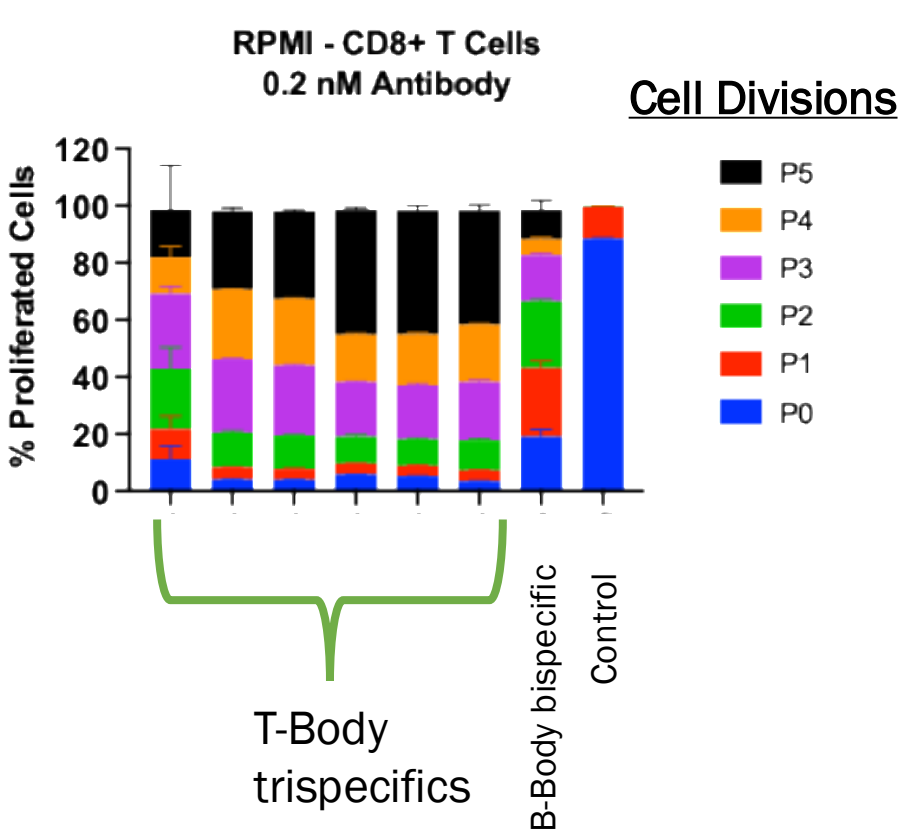
TAA ::x:: CD28 x CD3

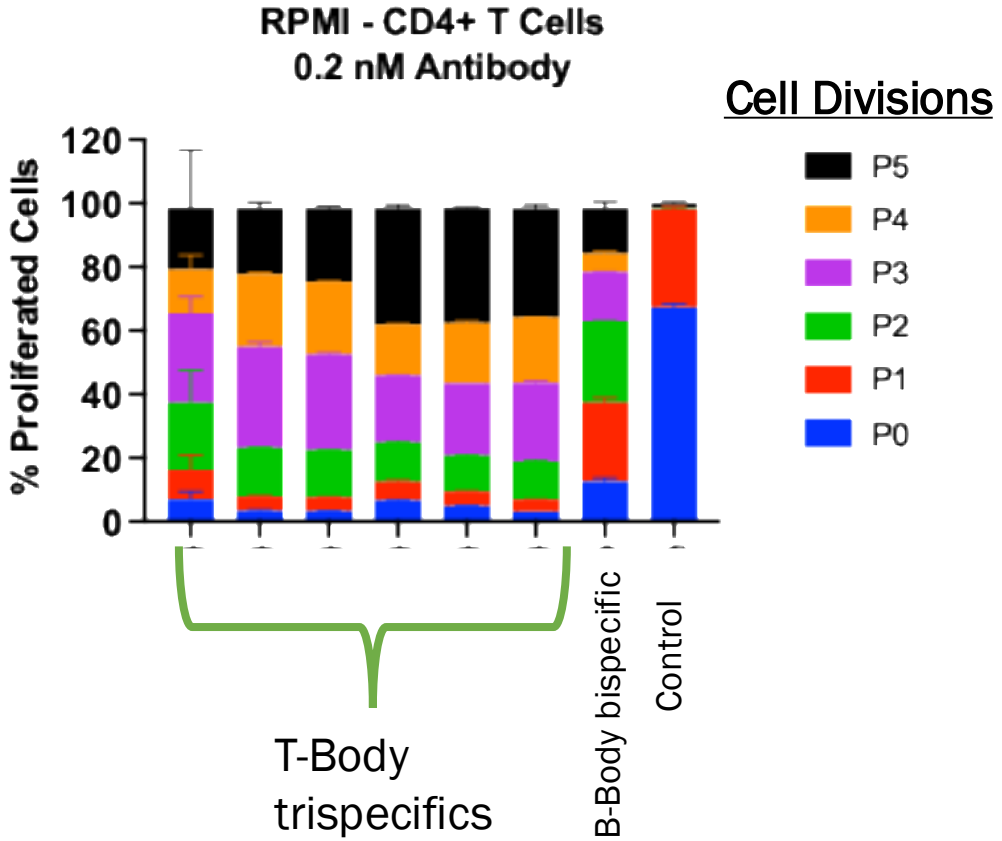




- Most CD3/CD28 combinations activated T-cells more than bispecifics when no tumor was present
- Optimized combination were rare in this assay

Comparison of T-Body Trispecific vs. B-Body Bispecific Impact on T cell Proliferation (5 Days)

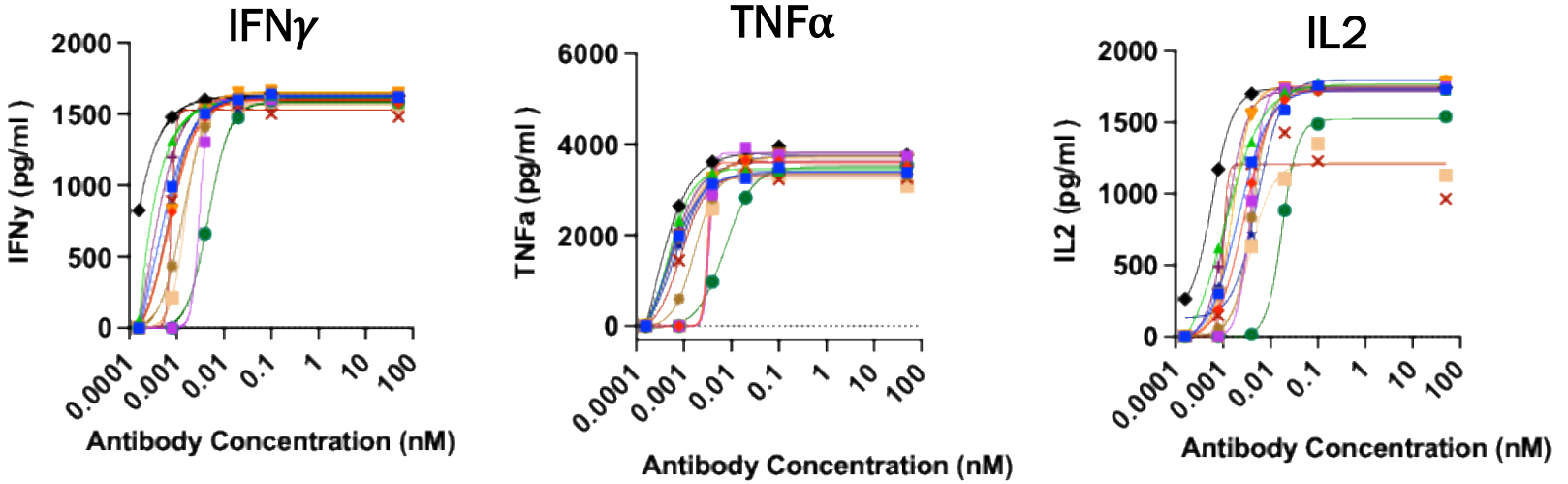




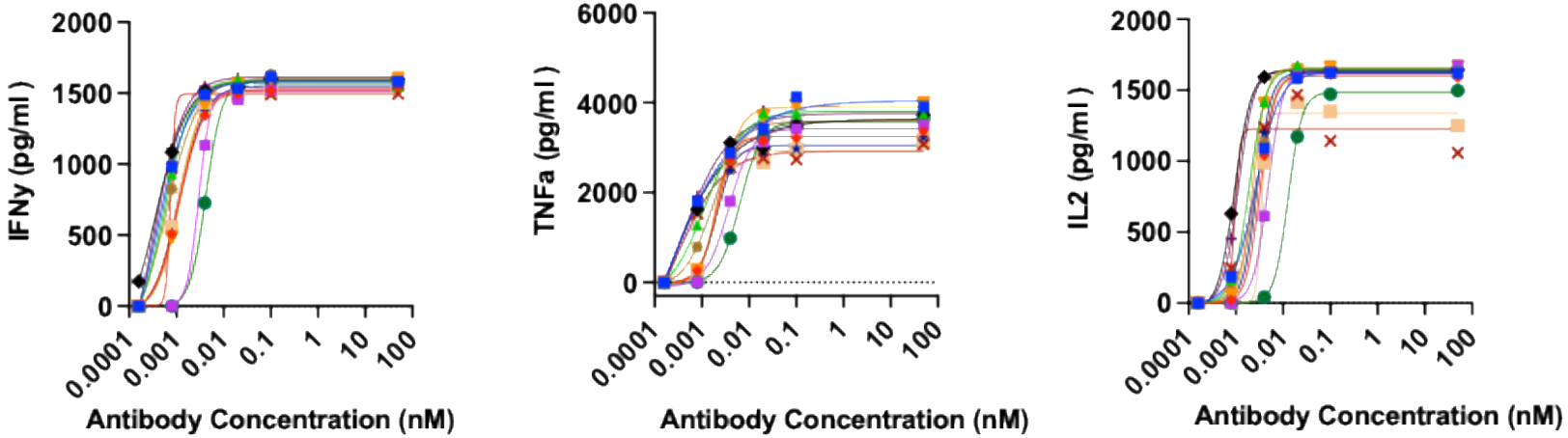
- Trispecific TCEs outperformed bispecific TCEs
- Optimization of CD3 and CD28 KDs and epitope was Important for enhancing proliferation

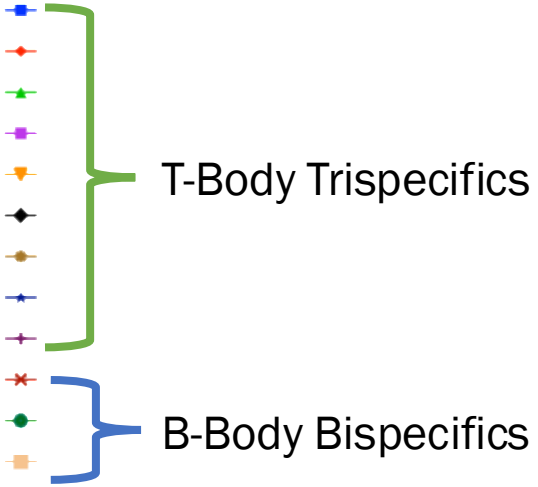
Comparison of T-Body Trispecific vs B-Body Bispecific TCE Induction of Cytokines

CD28 ::x:: TAA x CD3



TAA ::x:: CD28 x CD3

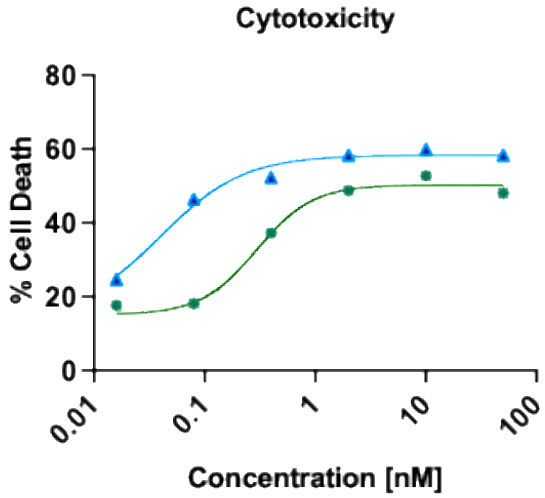


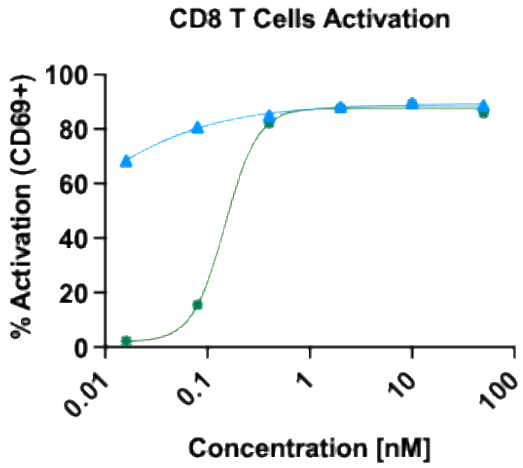


Note: No Cytokines were detected in the absence of tumor cells

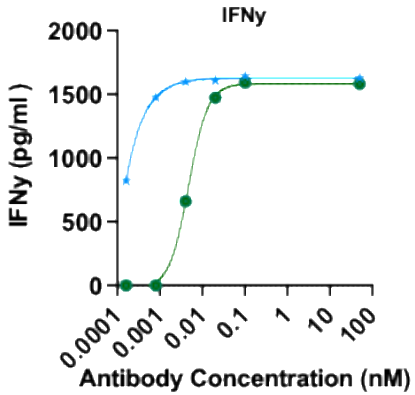
Comparing the Optimized T-Body Trispecific TCE to the Top B-Body Bispecific TCE

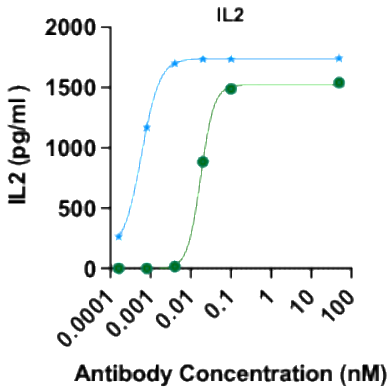
PBMCs + Tumor Cells + Trispecific Abs or Bispecific Abs

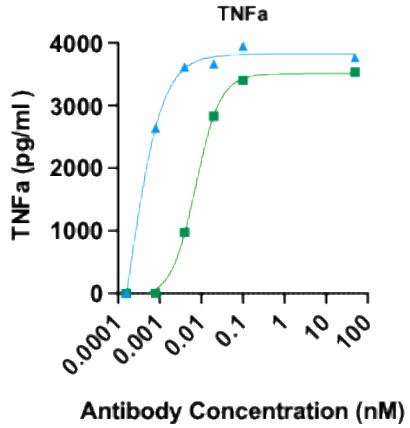




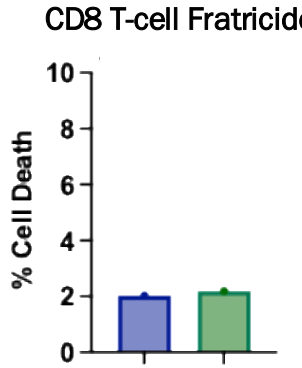
Cytokines

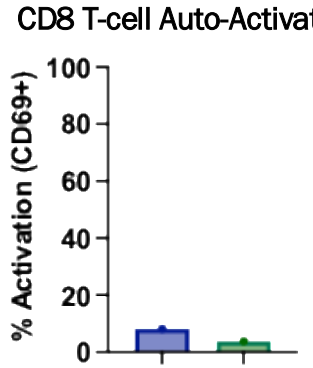






PBMCs Alone + Trispecific Abs or Bispecific Abs





Tested at 50 nM

No cytokines detected in absence of tumor cells

Platform Power: From Concept to Candidate in Weeks

What you just saw:

- Rapid optimization of 18 T-Body trispecific TCE variants
- Systematic CD3×CD28 optimization
- Complete developability profiling
- Timeline: 10 weeks

This same platform approach applies to:

- Any bispecific or trispecific combination
- Any therapeutic modality (TCEs, ADCs, IO, checkpoints)
- Any new target or indication

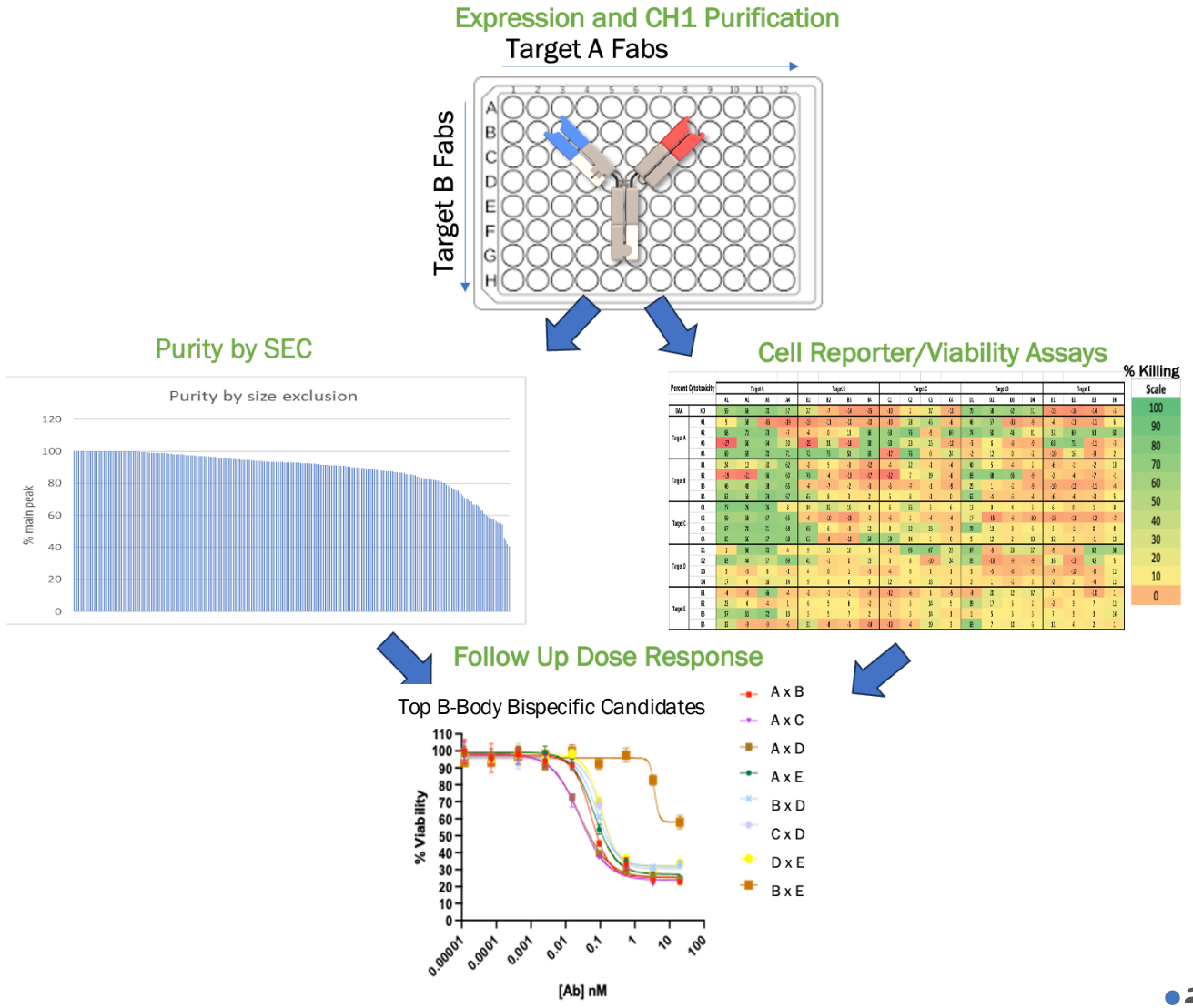
Next: How we enable rapid prototyping across our portfolio

High-Throughput Discovery: Test Hundreds of Combinations in Parallel

Traditional approach: Sequential testing, 1-2 combinations at a time

Invenra's platform: Matrix screening of Target A Fabs × Target B Fabs

- Express and purify in 96-well or 50 mL format
- Parallel SEC, binding, functional assays
- Identify optimal combinations in weeks, not months



Invenra's Three Core Focus Areas

ADCs

Antibody-Drug Conjugates

- **Three-TAA:** Triple tumor antigen targeting to overcome resistance
- **Stromal Targeting:** Penetrate tumor microenvironment barriers
- **BP+ (Biparatopic-Plus):** Enhanced internalization + resistance blockade
- **Novel MOAs:** Novel ADC mechanisms available under CDA

52 View of Cancer

TCEs

T-Cell Engagers

- **Dual Tumor Targeting:** Engage two tumor antigens simultaneously for specificity
- **CD3/CD28 Engagement:** Redirect T-cells to tumor sites with precision
- **Reduced On-Target/Off-Tumor:** Added specificity minimizes healthy tissue engagement
- **IgG-like Half-Life:** T-Body architecture enables optimized PK profiles

Discovery Collaborations or Assets for Partnering

Immuno-Oncology

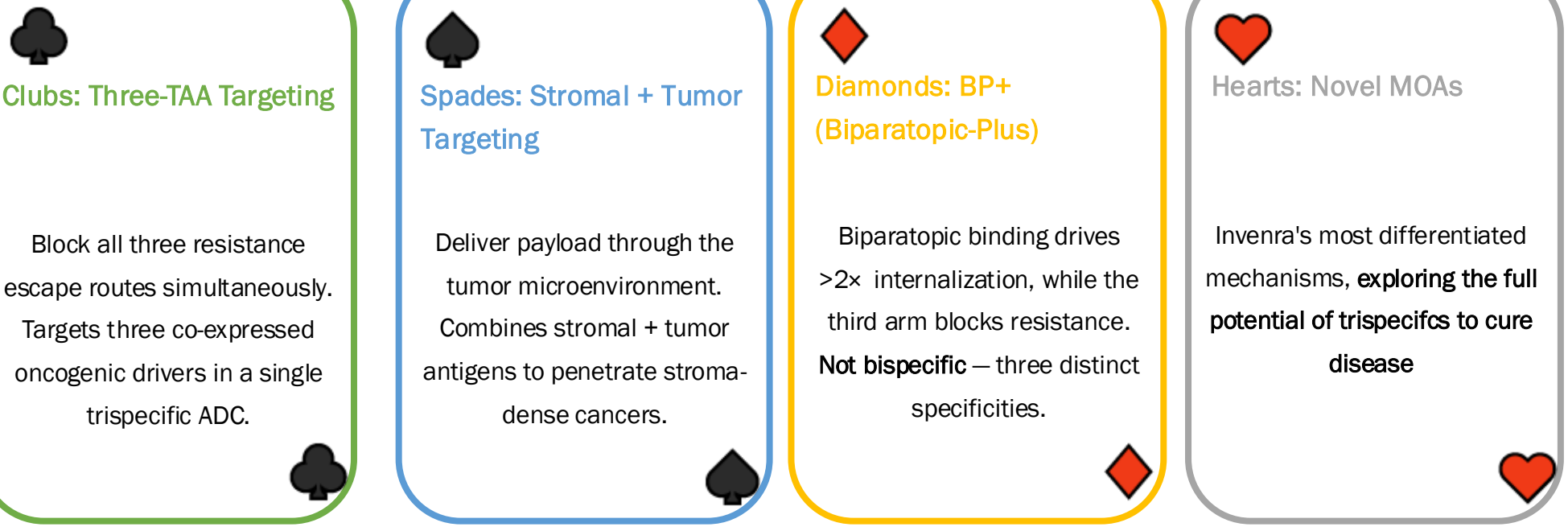
Checkpoint & Co-Stimulation

- **Checkpoint + Tumor Antigen:** Localize immune activation to tumor microenvironment
- **Co-Stimulation + Checkpoint:** Activate T-cells while blocking inhibitory signals
- **Tri-Functional IO:** Novel three-mechanism combinations for cold tumors

Discovery Collaborations or Assets for Partnering

All Areas Leverage B-Body Bispecific and T-Body Trispecific Platforms for Rapid Discovery/Optimization

The T-Body Trispecific ADC Portfolio: 52 Programs, Four Mechanisms



Invenra is developing 52 programs across four strategic approaches to overcome resistance and improve tumor selectivity – Attend Bonnie Hammer's talk on Thursday for more information

A Unique Partnership

Twist Bioscience Expands Antibody Discovery Offering with Bispecific Licensing Agreement

February 17, 2026

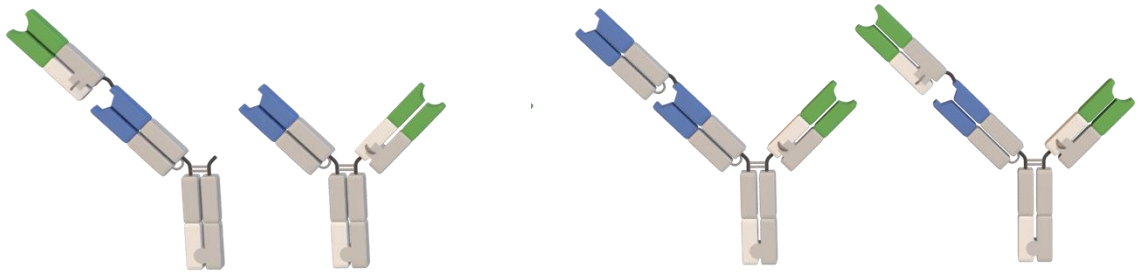
B-Body® platform complements Twist's antibody discovery offering enabling rapid, efficient and precise expression and discovery of complex bispecific antibody therapeutics

- Marries Twist Bioscience scale and throughput with Invenra's antibody engineering expertise
  - Enables facile and democratizing access to a robust and enabling bispecific platform
    - Flexible terms without clinical milestones or product royalties

A Unique Partnership

Plug ‘n’ Play

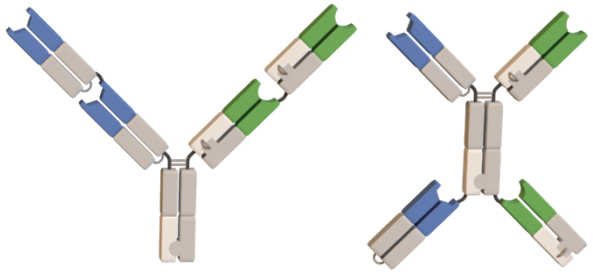
- Access protein production infrastructure for B-Body bispecific prototyping
- Your sequences from gene synthesis through B-Body bispecific formatting and characterization



B-Body Bispecific Antibody Formats

From Discovery

- Access *in vitro* and *in vivo* discovery platforms for binder discovery
- Reformat to B-Body bispecific and screen in matrixed orientations in high throughput formats to identify lead candidates



Industry leading timelines and quality

Three Antibody Platforms x Three Access Points

Invenra Business Model

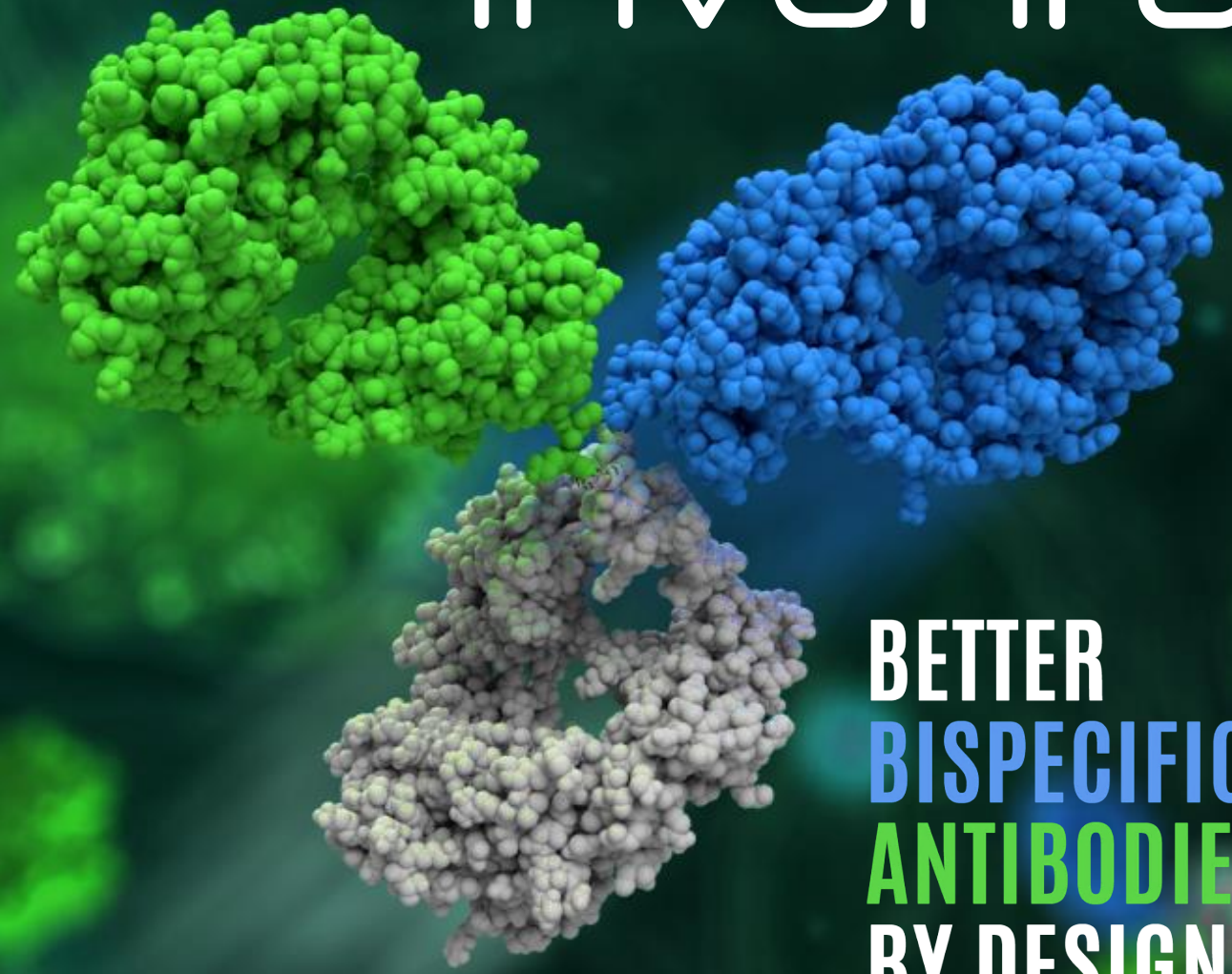
	mAbs	Bispecifics	Trispecifics
Discovery	**Custom Discovery** Target-to-lead generation using Invenra's proprietary display platforms Fee-for-Service	**Custom B-Body Discovery** Tailored dual-targeting antibodies for specific tumor biology Fee-for-Service Twist Partnership	**Custom T-Body™ Programs** Bespoke trispecifics from target-to-lead generation Strategic Collaboration
Express	**Rapid mAb Expression** Production of mAb controls to support multispecific programs Fee-for-Service	**Rapid Bispecific Assembly** Fast-track bispecific development from validated antibodies Fee-for-Service Twist Partnership	**T-Body™ Prototpying** Rapid trispecific testing of partner mAbs, leveraging platform expertise Strategic Collaboration
Assets	**Off-the-Shelf mAbs** Pre-developed antibodies ready for immediate licensing (Coming soon) Fee-for-Service	**Bispecific Catalog** New bispecific candidates generated using Invenra's catalog of mAbs (Coming soon) Fee-for-Service	**52 Views Portfolio** Pre-developed trispecific ADC programs available for partnering Strategic Collaboration

Visit us at Booth #41 and Posters #61 & #64 to discuss your antibody programs

Twist Bioscience is next door at Booth #404

invenra

Thank you!



BETTER
BISPECIFIC
ANTIBODIES
BY DESIGN