Utilizing T-cell Activation Reporter Gene Assays for Potency Monitoring from the Pre-Clinical to the Commercial Stages

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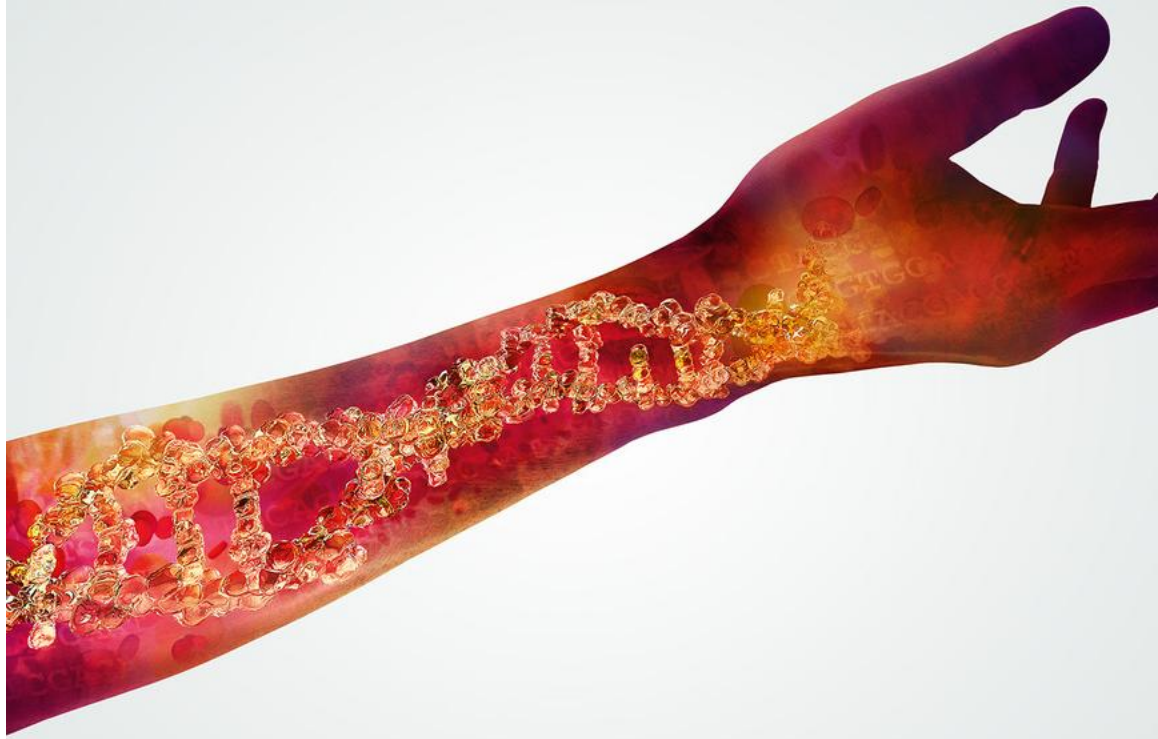


Agenda

1. T-Cell Engager Overview
2. Reporter Gene Assay Overview
3. RGA Development Strategy
4. Methods Comparison
5. Q&A

CMC Potency Assays: What Do Regulatory Bodies Want?

ICH Q6B: Specifications for Biotechnology Products

Potency: The measure of the biological activity using a suitably **quantitative biological assay** (also called potency assay or bioassay), based on the attribute of the product which is **linked to the relevant biological properties**.

21 CFR 600.3(s)

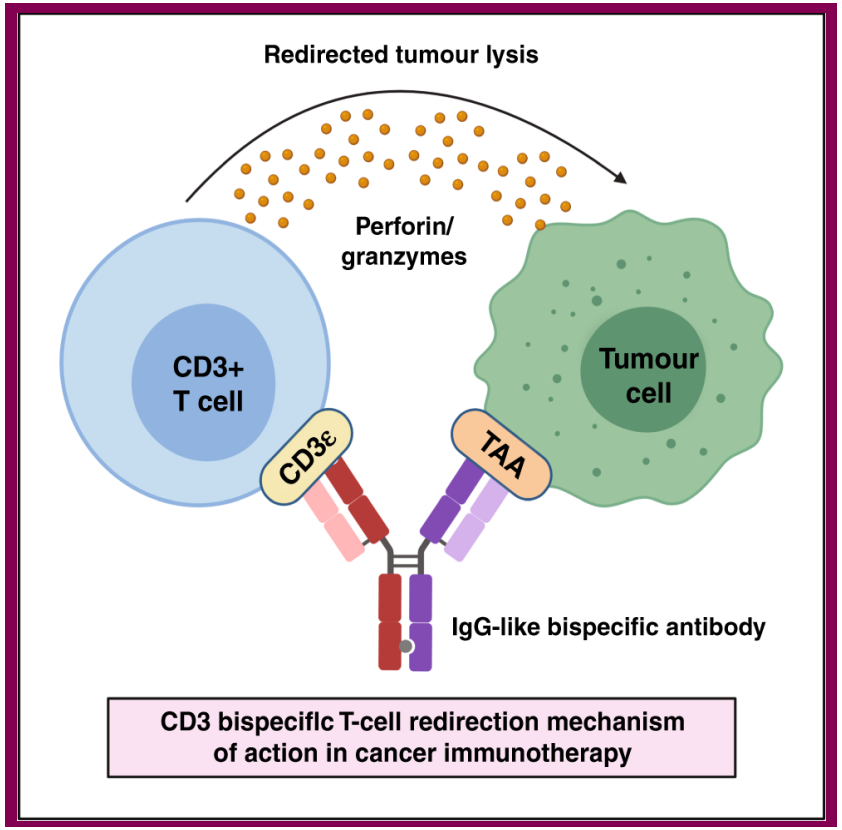
"The word potency is interpreted to mean the **specific ability or capacity of the product**, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, **to effect a given result**."

21 CFR 610.10

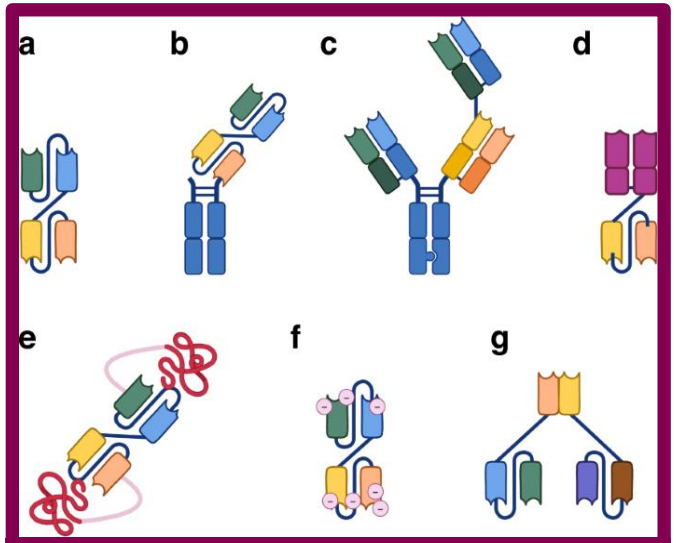
"Tests for potency shall consist of either **in vitro** or in vivo **tests**, or both, which have been specifically designed for each product so as **to indicate its potency** in a manner adequate to satisfy the interpretation of potency given by definition in § 600.3(s) of this chapter."

T-Cell Engager Background & Challenges

TCE Background

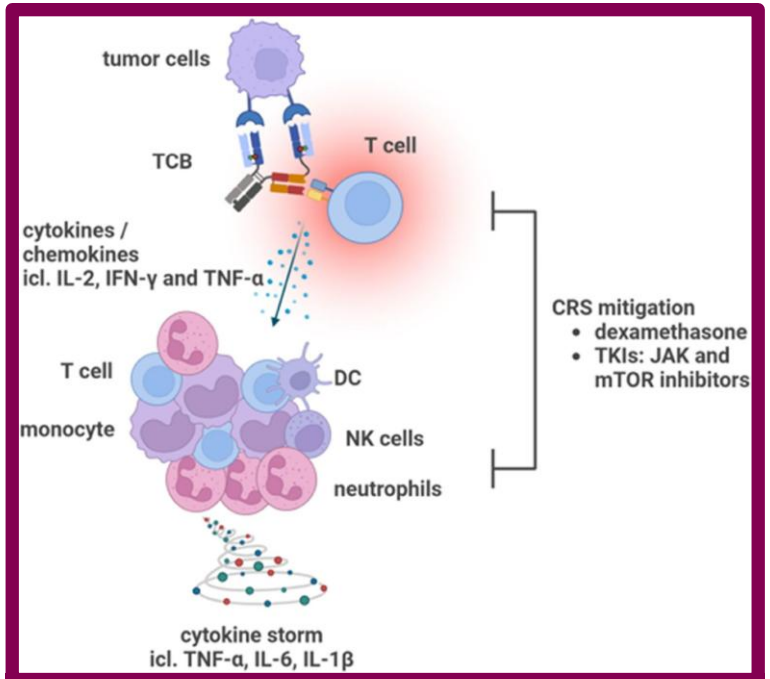


Challenges:



Structural Design:

1. Multiple binding targets
2. Molecule fragility
3. Departure from traditional mAb structure & strategy



Binding Affinity:

Attenuated binding affinity for T-cell markers to reduce CRS risk

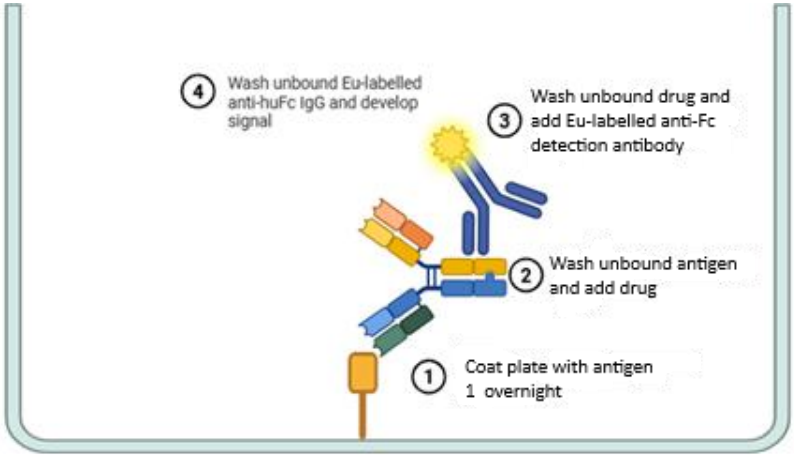
https://www.nature.com/articles/s41416-025-03125-y

https://www.nature.com/articles/s41416-020-01225-5/figures/1

https://jitc.bmj.com/content/12/Suppl_2/A1298

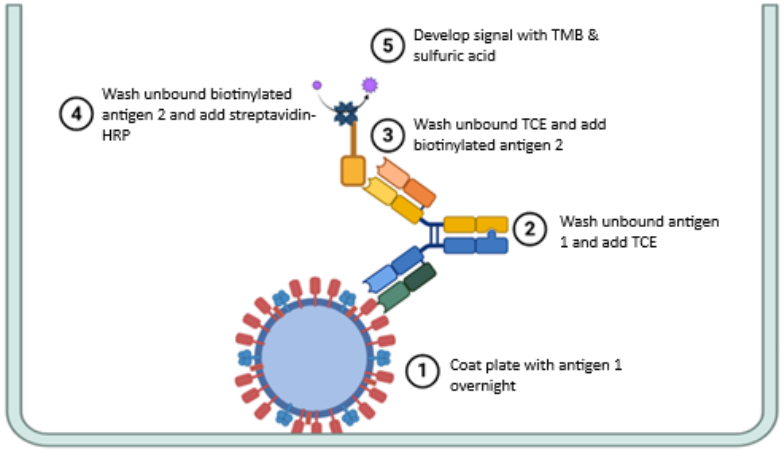
Addressing Structure: T-Cell Engager Drugs Are Inherently Multi-Specific

Approach 1: Single Target Binding



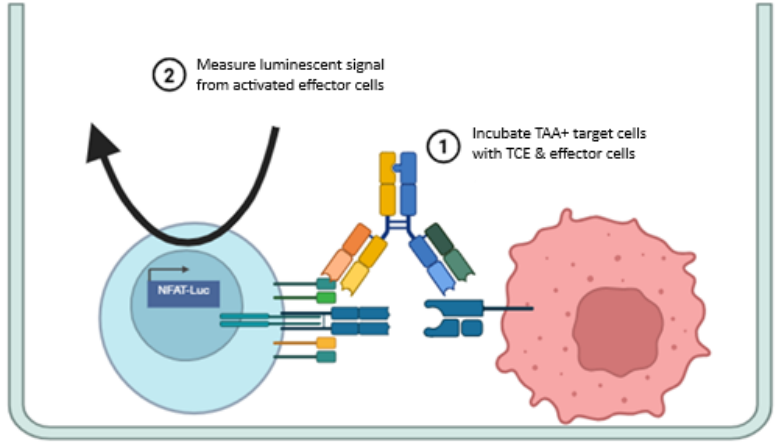
- Each binding arm of the T-cell engager is assayed individually using ELISA or RIA
- **>2** potency readouts for each sample

Approach 2: Dual Target Binding



- Binding arms of the T-cell engager assayed together
- Acceptable for phase 0-1

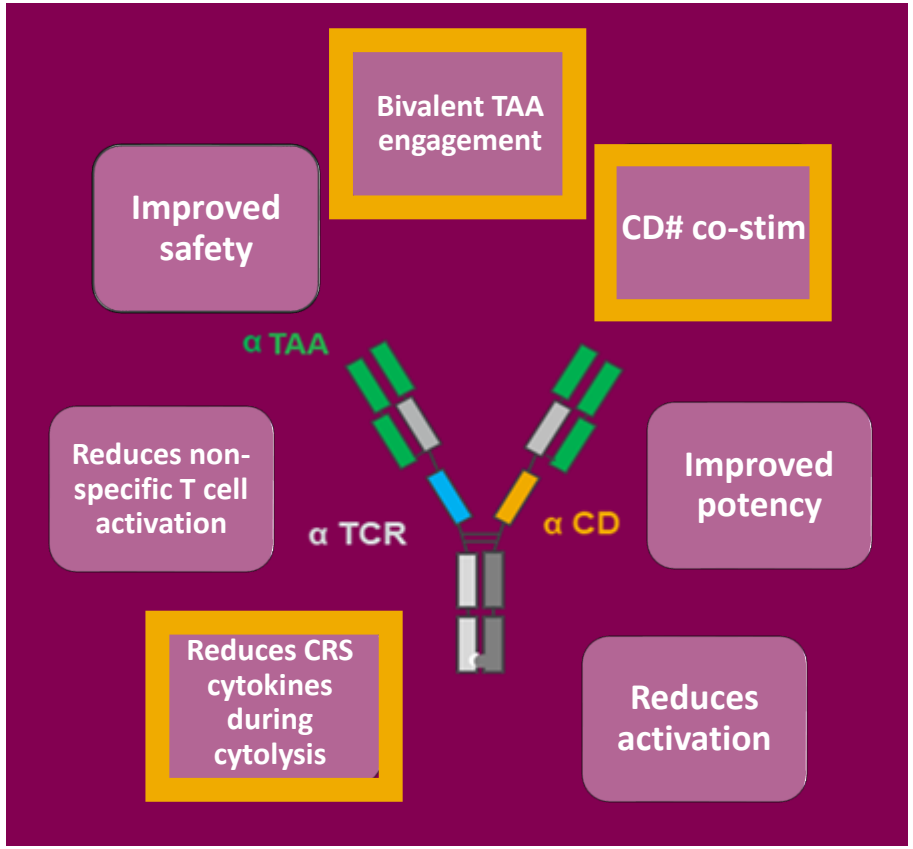
Approach 3: Cell Based Reporter Gene



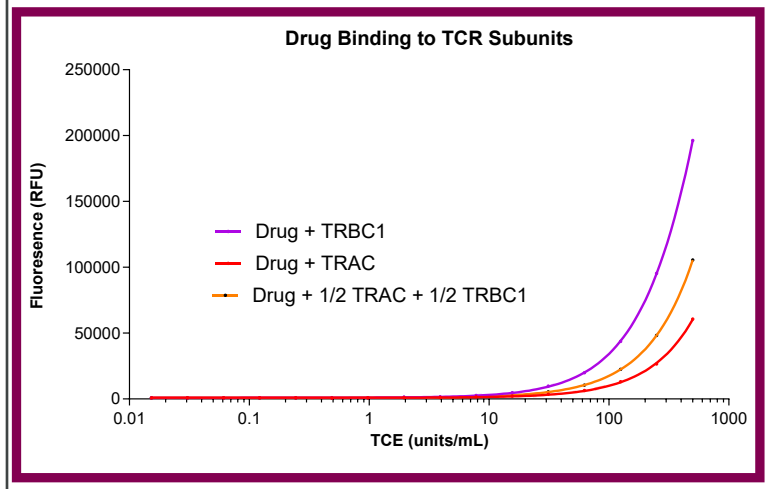
- Binding arms of the T-cell engager assayed together; one to two step assay
- Acceptable for all development phases

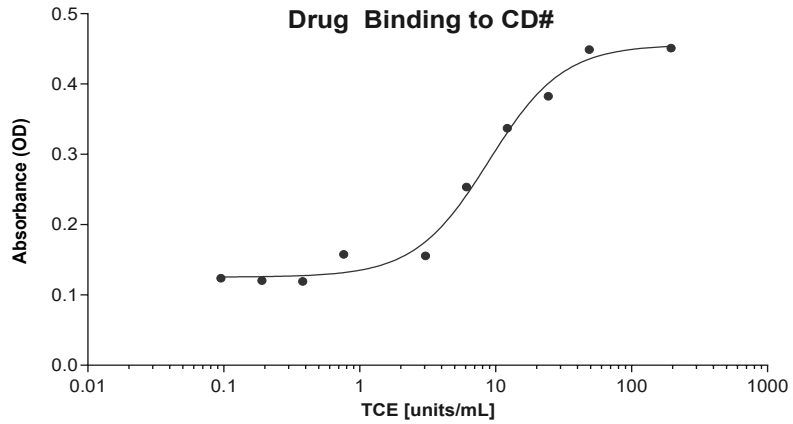
Affinity Challenges: Intentional Attenuation to Reduce CRS Risk Also Reduces Binding

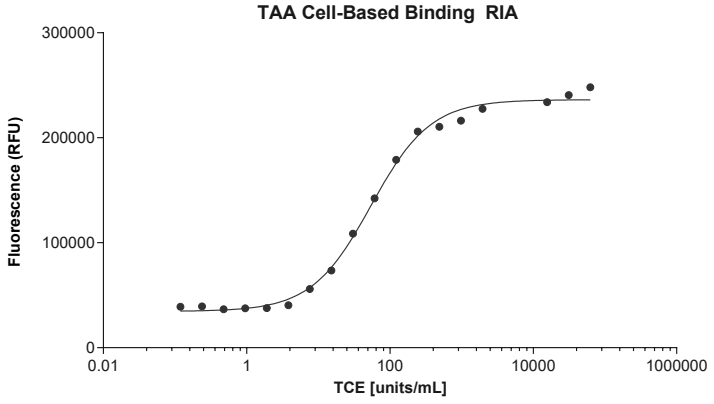
Drug Design & Rationale



Affinity Challenges







- Decreased affinity for T-cell associated proteins poses challenge to characterize this interaction

Early leverage of a cell-based assay addresses many of these challenges!

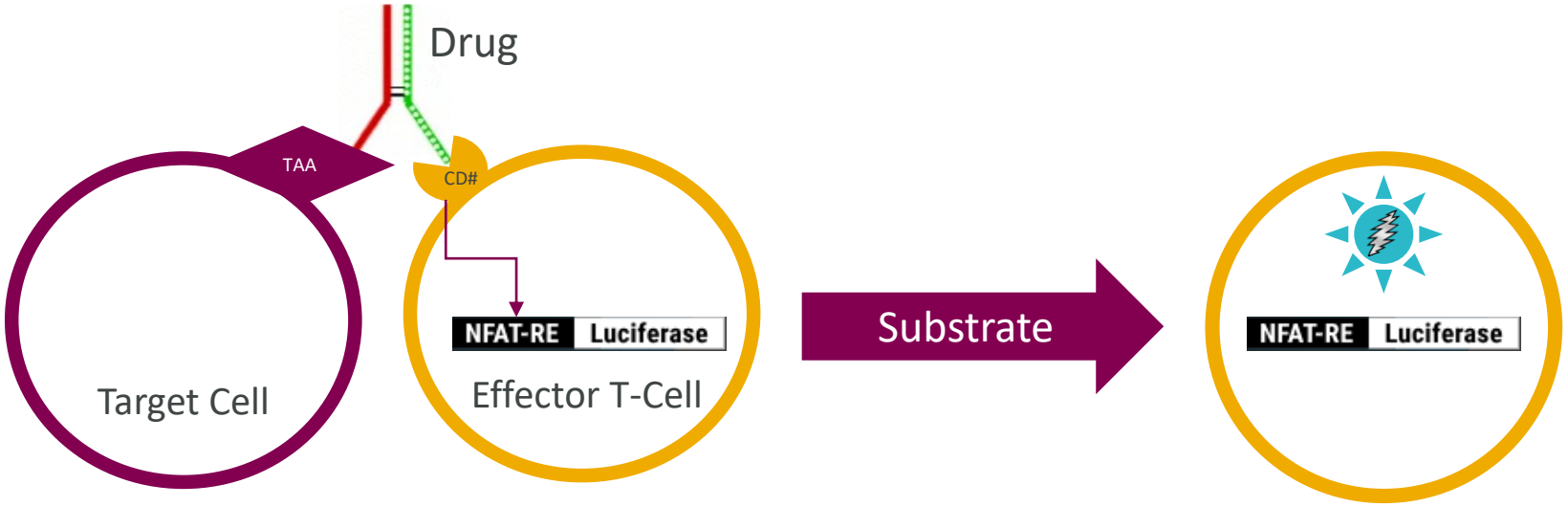
T-Cell Activation Reporter Gene Assay Overview

Engineered TAA expressing target cells & Jurkat NFAT-Luc effector cells are mixed & plated

TCE drug serial dilutions are added to plate

Reaction incubated for several hours to allow for expression of luciferase by stimulated Jurkat cells

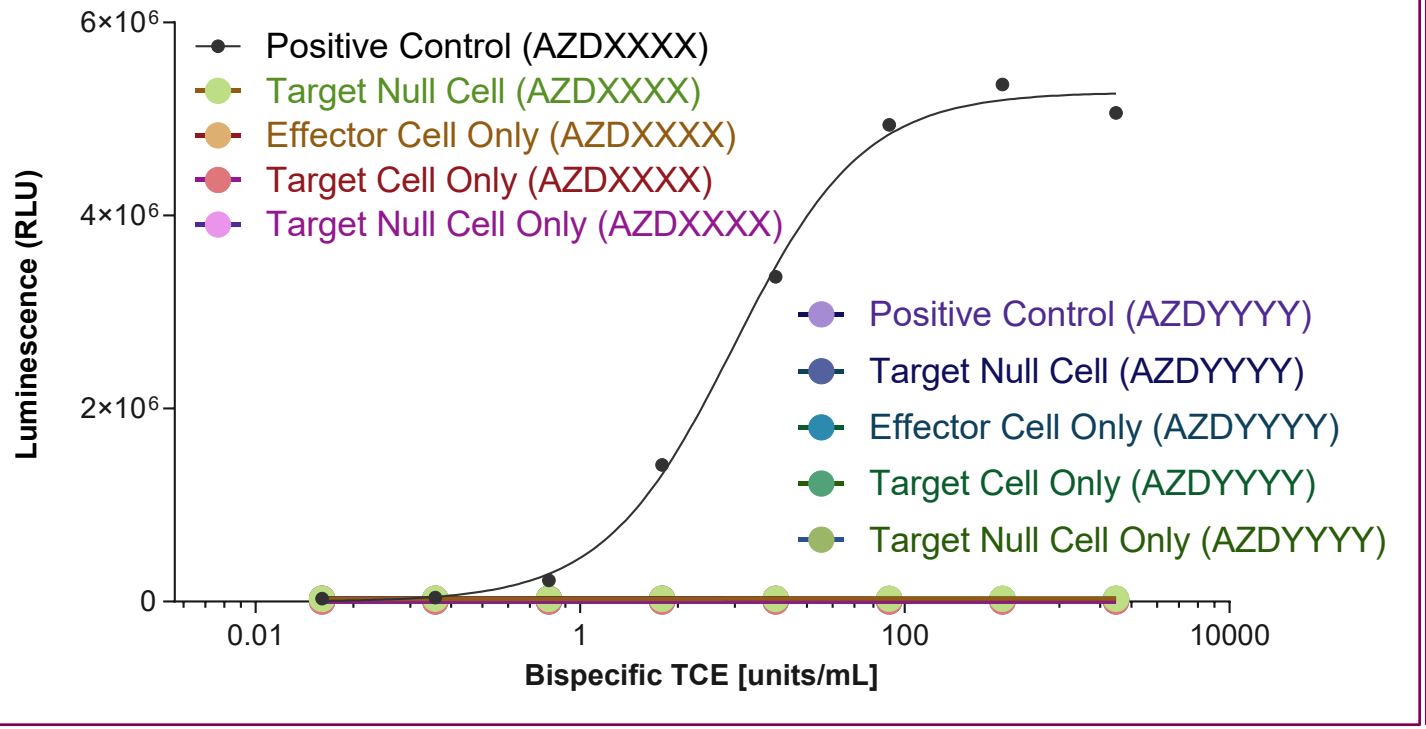
Substrate is added & luminescence is read



Parameters Assessed

Parameter	Range Tested
Cells Per Well	e5 – e6
Effector to Target Ratio	• 4:1 • 3:1 • 2:1 • 1:1 • 1:2
Primary Incubation Period	180-360 minutes
Substrate Incubation Period	5-60 minutes
Pre-Incubation Options	• Overnight Drug + Target Cells • One Hour Drug + Effector Cells
Plate Layout	3 & 4 Sample Layout (1 plate)
Target Cell Type	• CHO TAA+ • HEK293 TAA+
Assay Media Composition	DMEM vs RPMI vs RPMI Amended
Plate Reader	SpectraMax i3x & Envision
Substrate	Substrate 1 & Substrate 2
Cell Passage Limits	HEK293 TAA+ Jurkat NFAT-Luc
Assay Read vs Continuous Culture Cells	All ARCB or all CC

Q: Is there any non-specific T-cell activation?
A: Negligible – all three components required for dose-response curve



Effector Cell	Target Cell	BsTCE	Max Signal
Jurkat NFAT-Luc	HEK293 TAA+	AZDXXXX	5,062,610
Jurkat NFAT-Luc	HEK293 TAA-	AZDXXXX	30,150
Jurkat NFAT-Luc	Null	AZDXXXX	28,910
Null	HEK293 TAA+	AZDXXXX	180
Null	HEK293 TAA-	AZDXXXX	140
Jurkat NFAT-Luc	HEK293 TAA+	AZDYYYY	29,210
Jurkat NFAT-Luc	HEK293 TAA-	AZDYYYY	24,640
Jurkat NFAT-Luc	Null	AZDYYYY	27,970
Null	HEK293 TAA+	AZDYYYY	130
Null	HEK293 TAA-	AZDYYYY	120

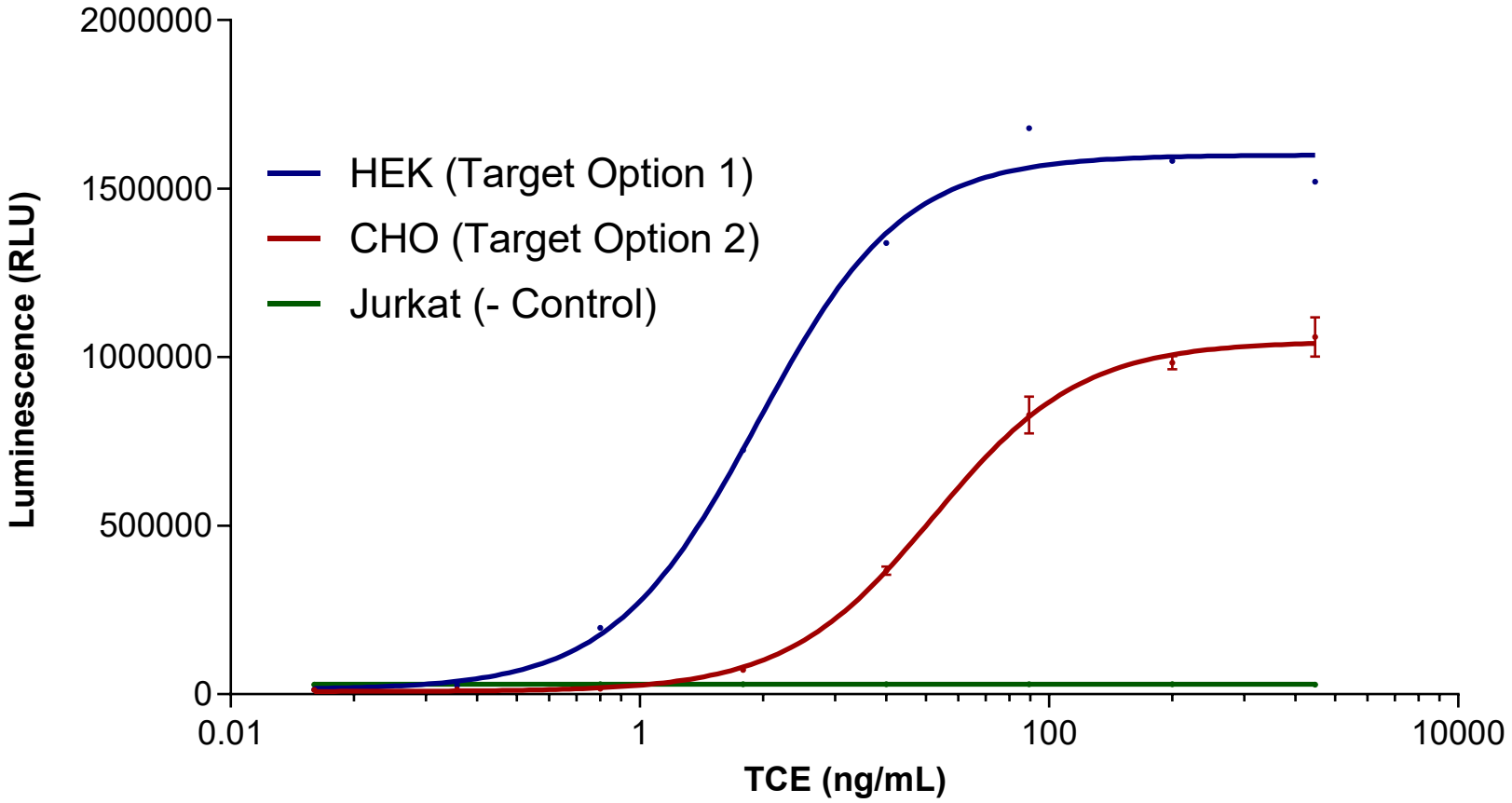
AZDXXXX is specific for TAA+ cells

AZDYYYY is not specific for TAA+ cells but does interact with Jurkat NFAT-Luc

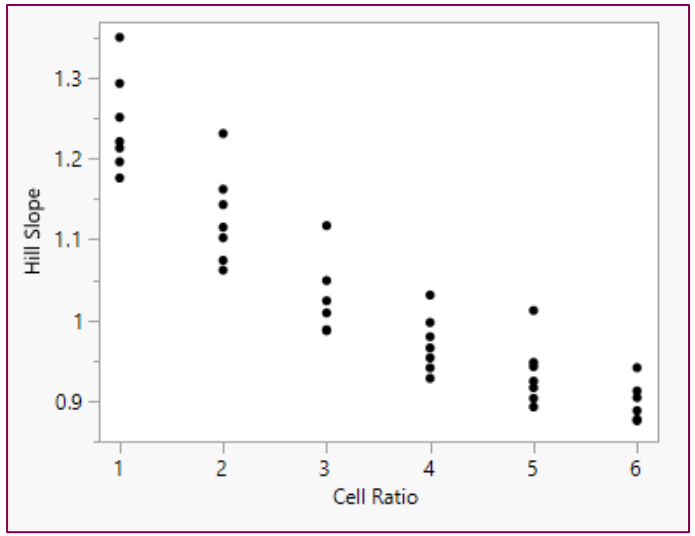
Q: Which TAA expressing target cell is optimal?

A:

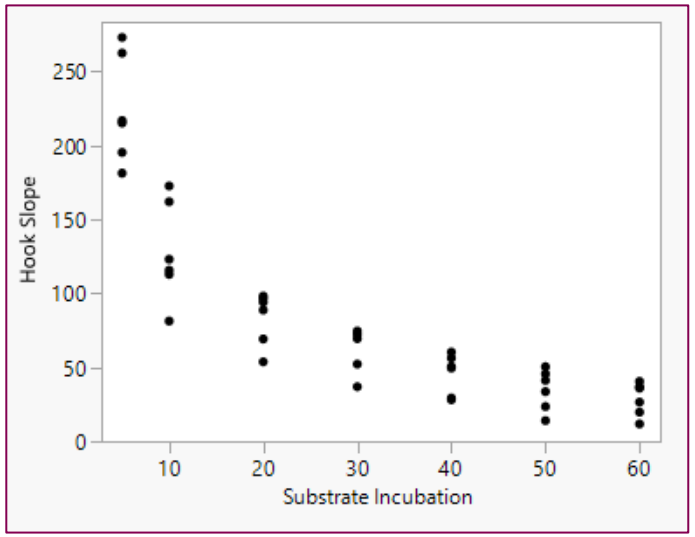
Higher overall response & lower EC50 with HEK cells vs CHO



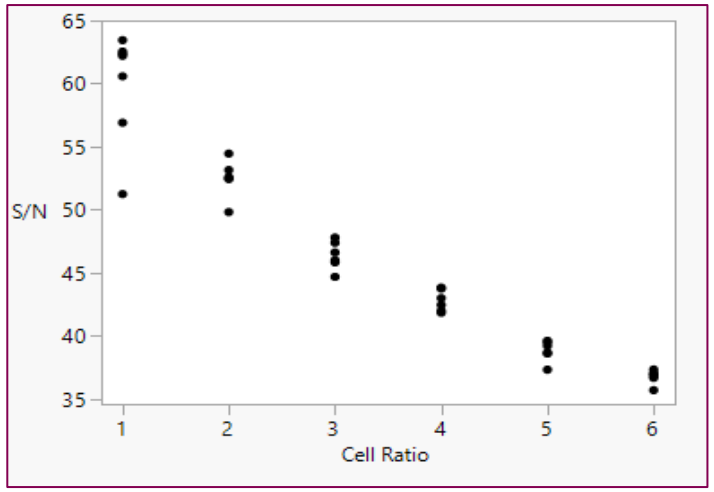
Curve Profile Shaping & Relationships



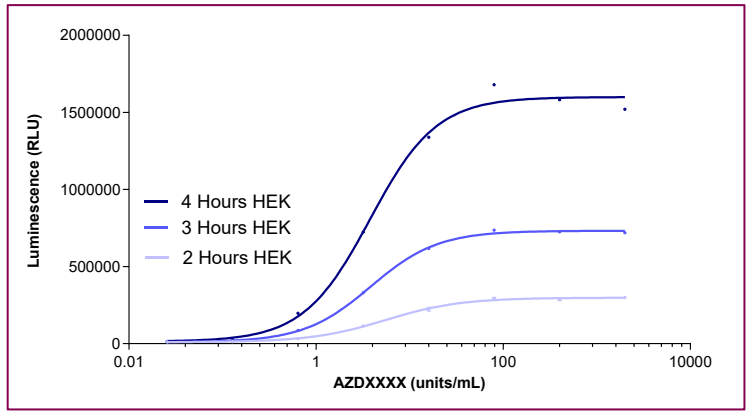
$$\text{Hill Slope} \propto \frac{1}{Cell\ Ratio\ (E{:}T)}$$



$$\text{Hook Slope} \propto \frac{1}{OneGlo\ Incubation}$$



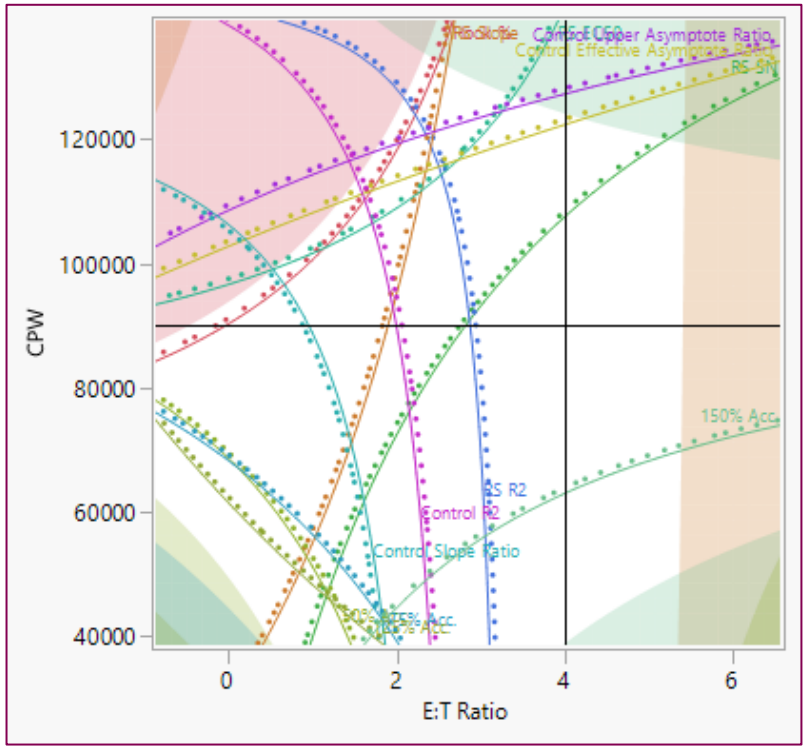
$$\text{S/N} \propto \frac{1}{Cell\ Ratio\ (E{:}T)}$$



Max Signal ∝ Drug Incubation

Design of Experiment for Accuracy Optimization & Robustness

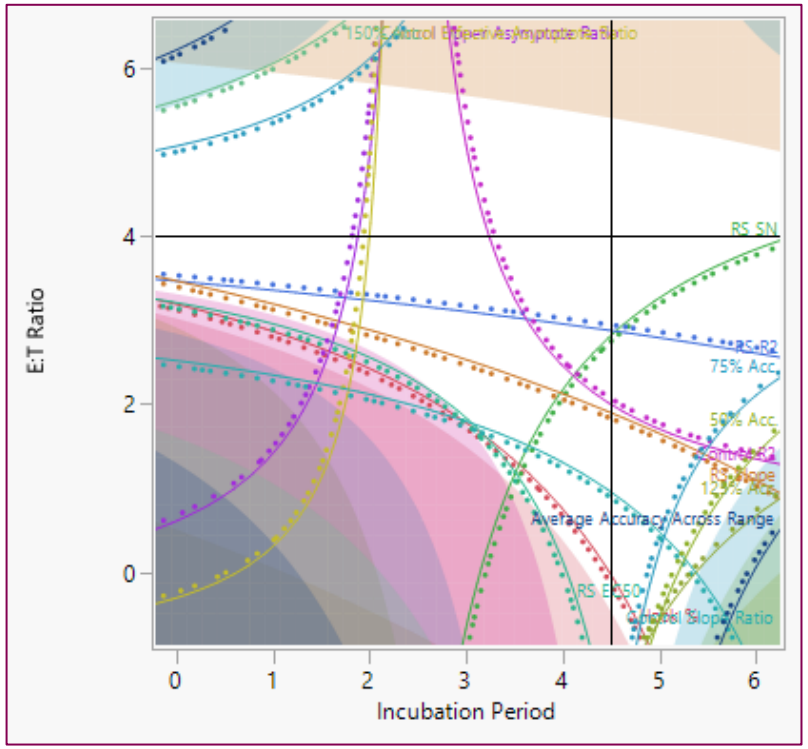
CPW vs E:T Ratio



Response Surface Design

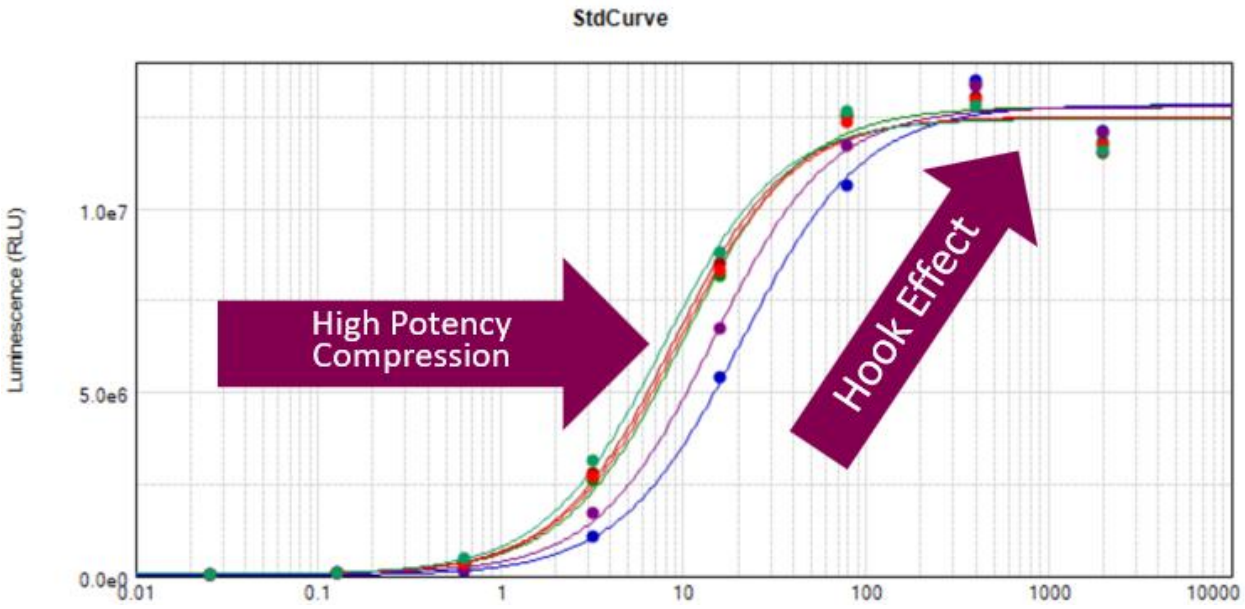
- 20 linearity assays in 5 blocks with a variety of conditions
- Accuracy response was analyzed both per potency point as well as overall
- Curve profile e.g. RS & Control R2, asymptote ratios, slope, S/N also included in model

E:T Ratio vs Incubation Time

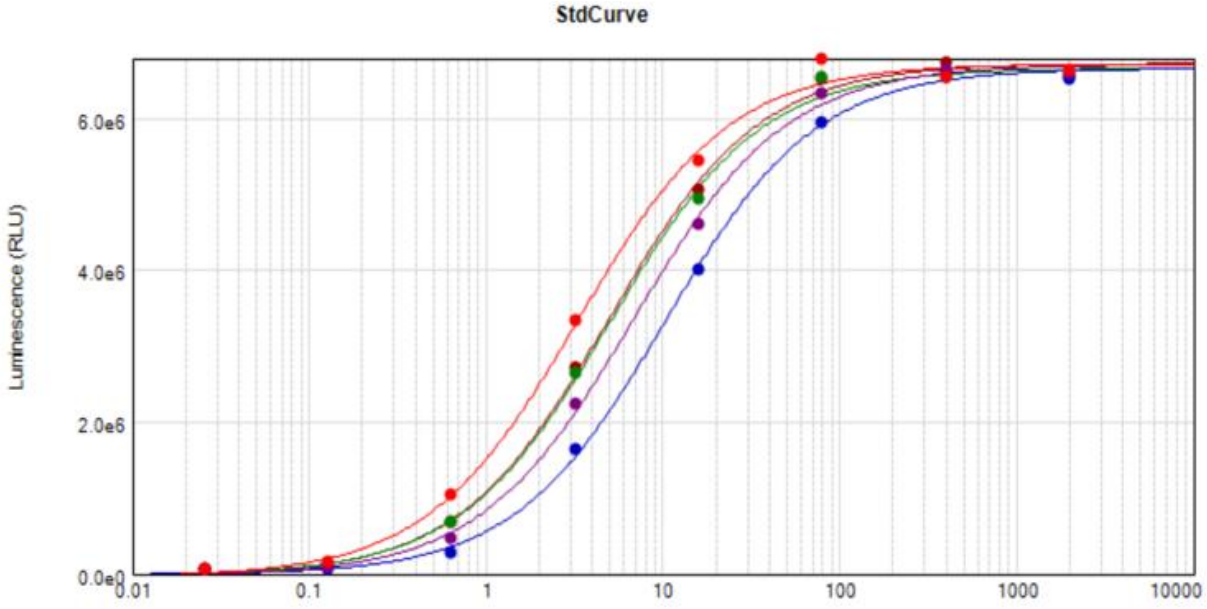


From Proof of Concept to Finished Product

Proof of Concept (Month 1)



Pre-Qual Assay #3 (Month 2)



Pre-Qualification Data

- **98.5%± 5.3% Accuracy**
- 5 Assays over 4 days

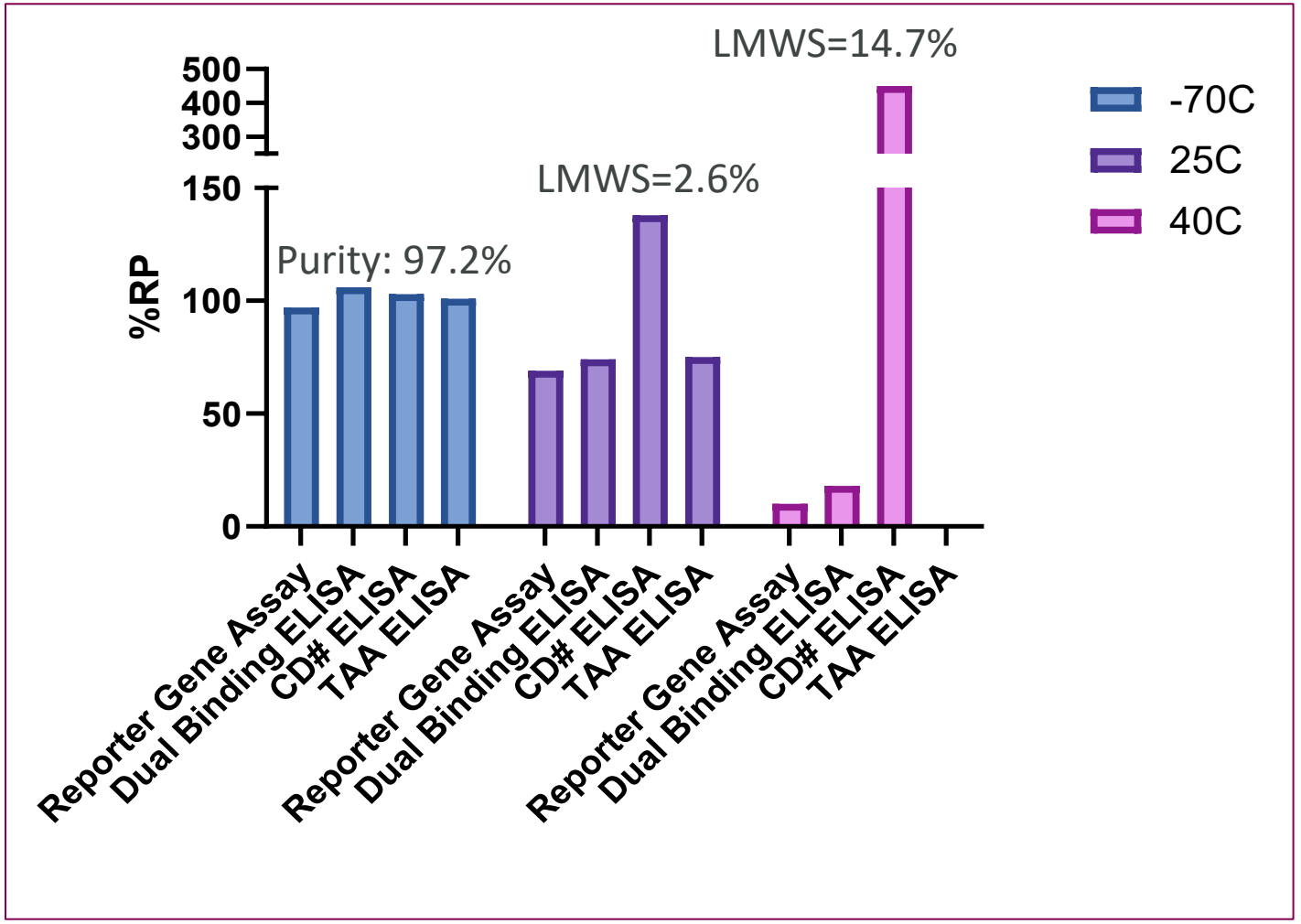
Linearity	50%	75%	100%	125%	150%
Replicate 1	49.4	77.4	94.0	125.3	133.2
Replicate 2	48.2	74.2	100.6	130.3	141.1
Replicate 3	53.1	73.3	107.5	117.8	139.6
Average	**50**	**75**	**101**	**124**	**138**
% CV	5.1	2.9	6.7	5.1	3.0

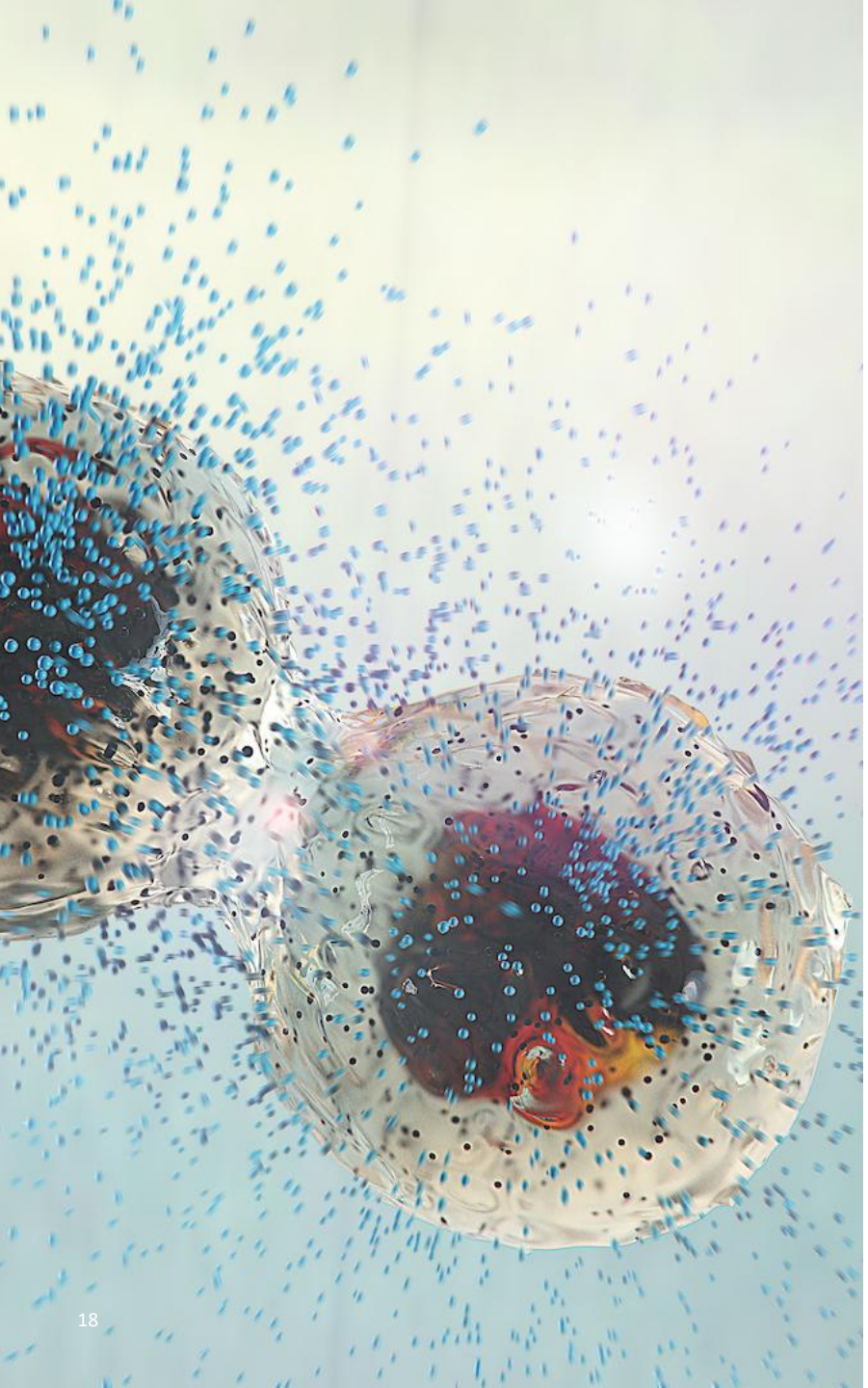
Accuracy	50%	75%	100%	125%	150%
Replicate 1	98.8	103.2	94.0	100.2	88.8
Replicate 2	96.4	98.9	100.6	104.2	94.1
Replicate 3	106.2	97.7	107.5	94.2	93.1
Average	**100**	**100**	**101**	**100**	**92**

Application of methods and their outcomes… One last example…

Forced Degradation Assay Impact & Methods Comparison: Does Fragmentation Decrease Steric Hindrance?

Assay	Sample	%RP
Reporter Gene	-70°C	97%
Dual Binding ELISA		106%
CD# ELISA		103%
TAA ELISA		101%
Reporter Gene	25°C	69%
Dual Binding ELISA		74%
CD# ELISA		137%
TAA ELISA		75%
Reporter Gene	40°C	10%
Dual Binding ELISA		18%
CD# ELISA		409%
TAA ELISA		0.2%





Take Home Messages

1. Early implementation of a cell-based assay addresses many challenges posed by TCE drugs.

2. A single readout assay reduces analytical resource cost in comparison to multiple binding assay strategy and avoids arduous bridging process.

3. Use of single target binding assays can provide granularity at expense of broader MOA picture.

Acknowledgements

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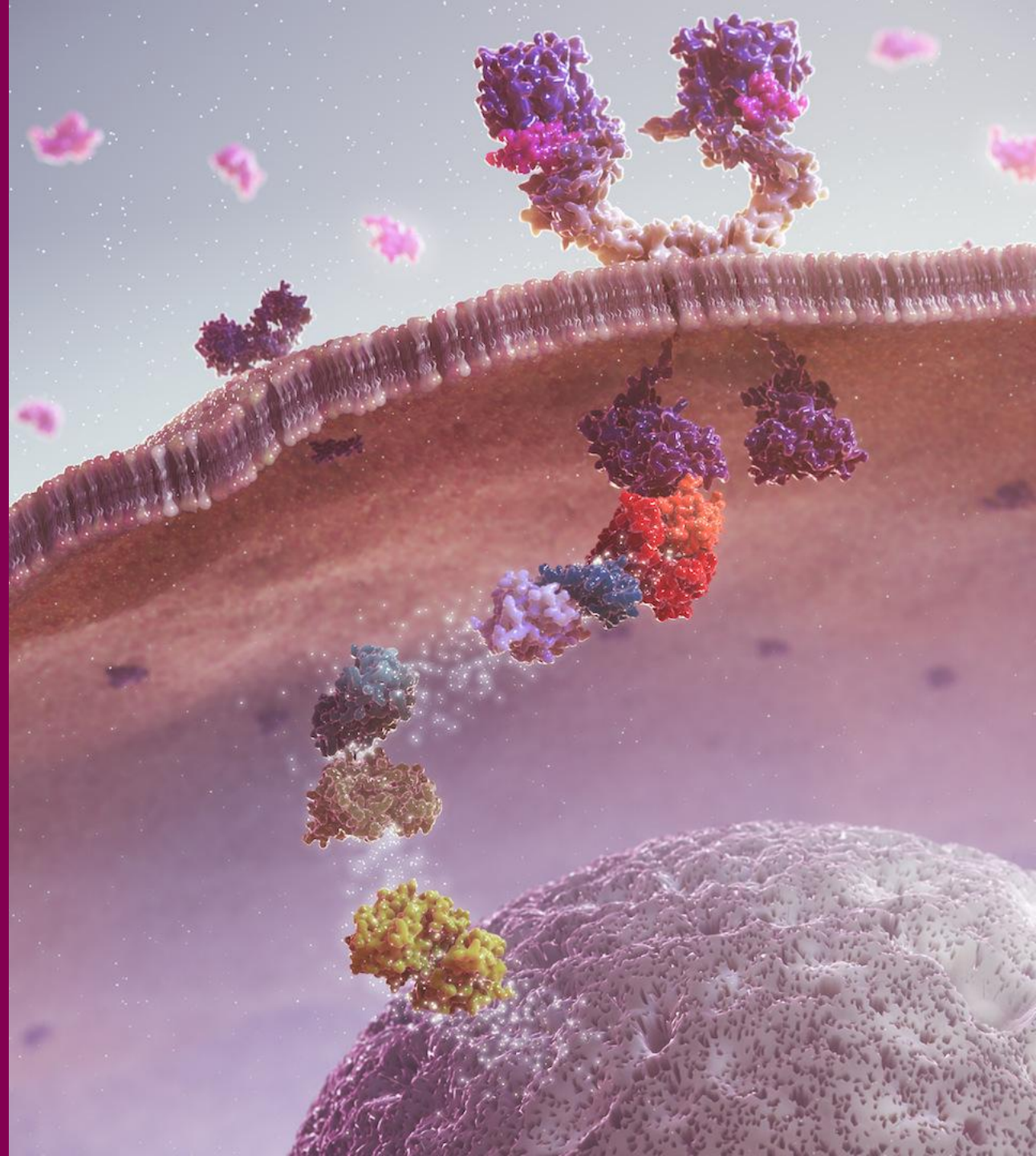
Q&A



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