BIOASSAYS IN PRODUCT DEVELOPMENT: An Immuno-Oncology Perspective

CASSS Bioassays

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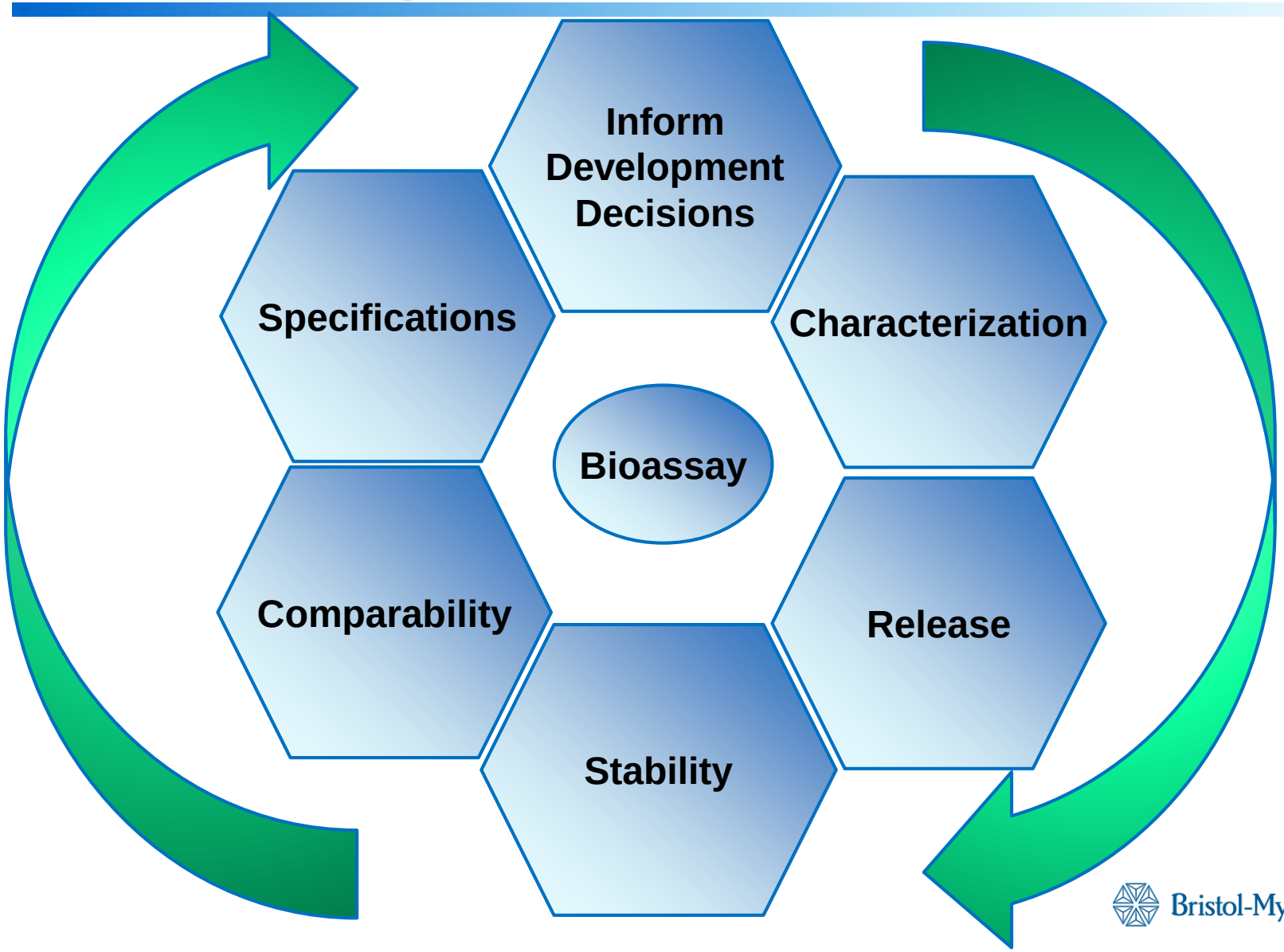
Bristol-Myers Squibb

Overview

- **Introduction:**
  - Cell-based bioassays in IO product development
  - Immuno-Oncology (IO)
  - Monoclonal Antibodies in IO
- **Challenges for potency assay development in IO**
- **Case studies**
  - Late phase optimization to meet performance targets
  - Role of bioassays in determining structure-function relationships
- **Conclusions**

Role of Bioassays in Development of Immunotherapeutic Products



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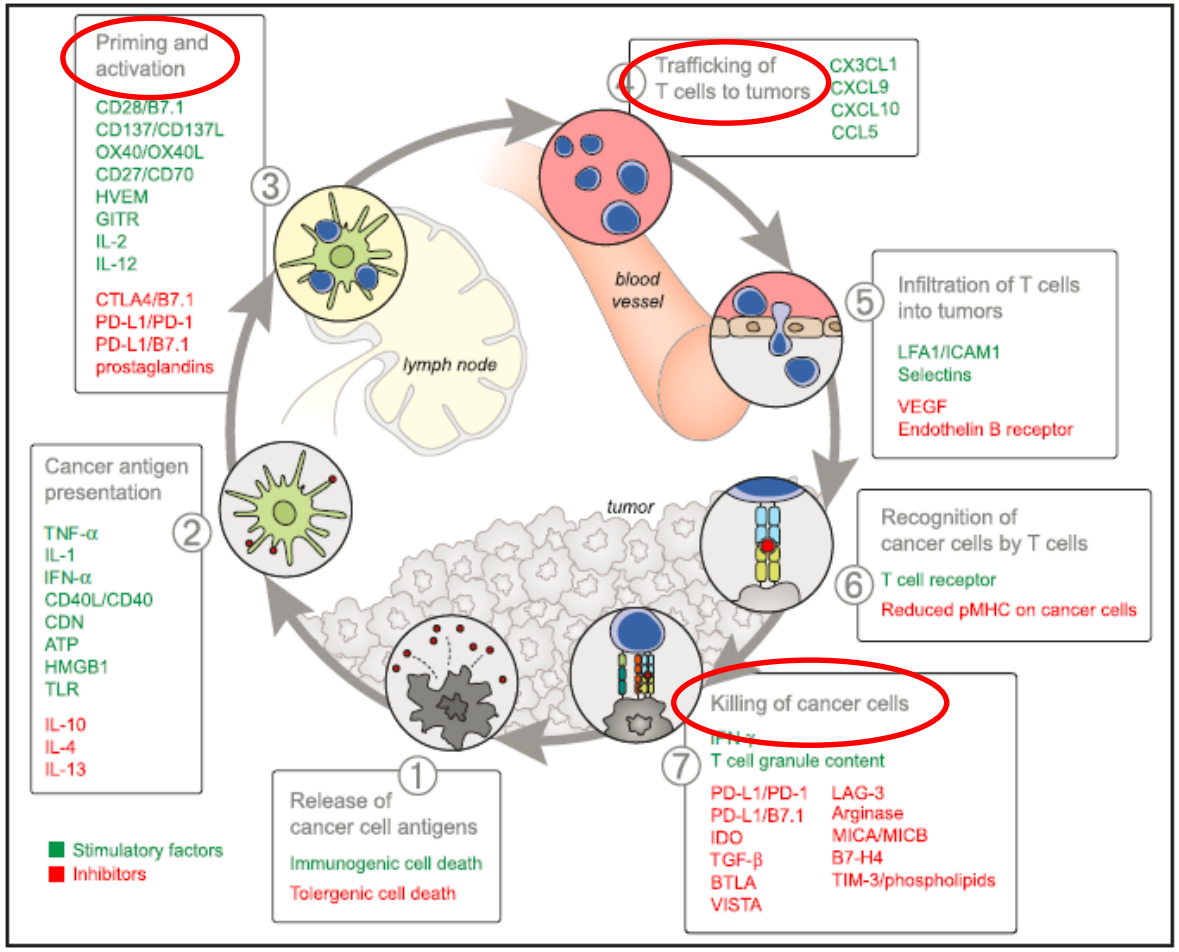
Cell-based Bioassays are Unique Tests

- **Specifically designed for each product**
- **Should inform on higher-order structure and the impact of specific molecular changes**
- **Must represent the mechanism of action for that product**
- **For potency testing of immunotherapeutic products in IO, the bioassay must model the interaction of complex pathways and molecules**

Immuno-Oncology (IO)
Harnessing the Immune System for Cancer Therapy



Mellman, et al., Immunity ***2013*** *39:1-10*

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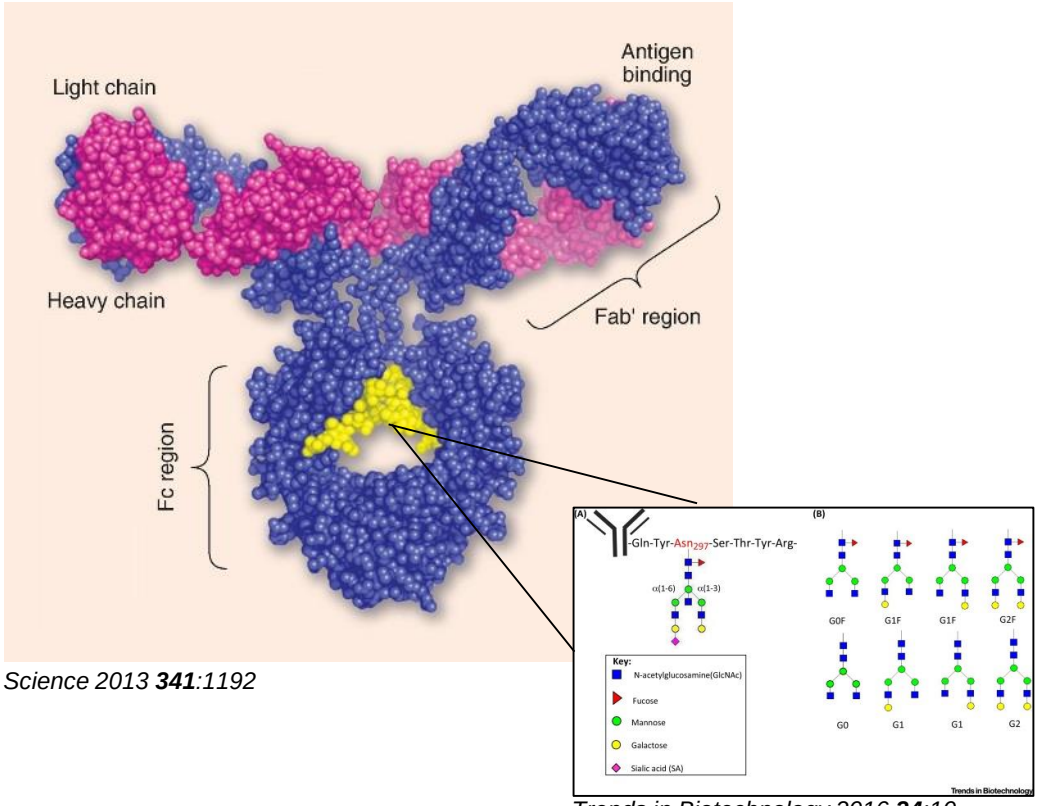
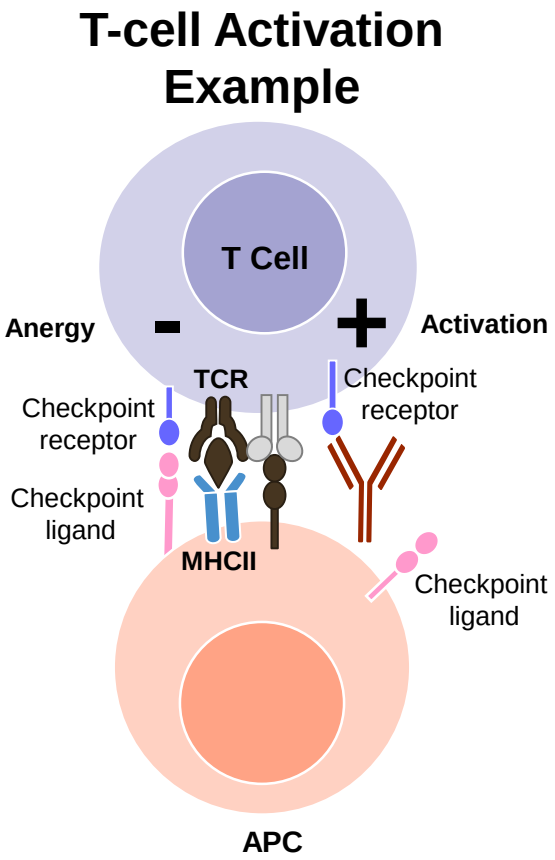
Monoclonal Antibodies in IO

Well-Characterized Biologics



Science 2013 ***341****:1192*

Trends in Biotechnology 2016 ***34****:10*



Challenge for IO:

Reliably assess a highly complex MoA in vitro

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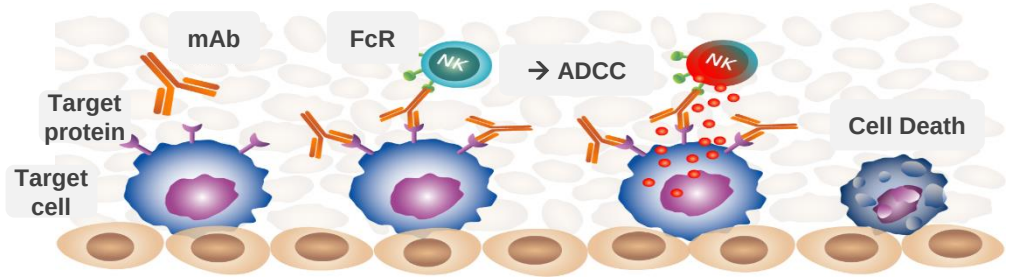
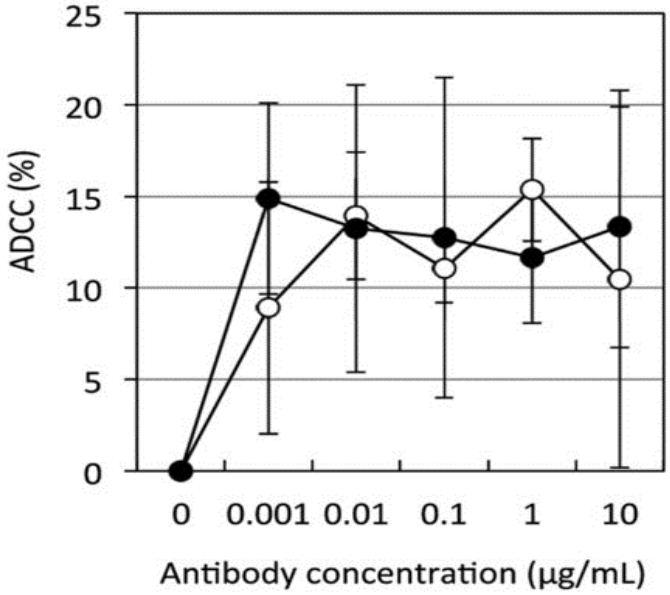
Bioassays for Complex MoAs

Previous "Holy Grail": ADCC Assays Suitable for Product Development

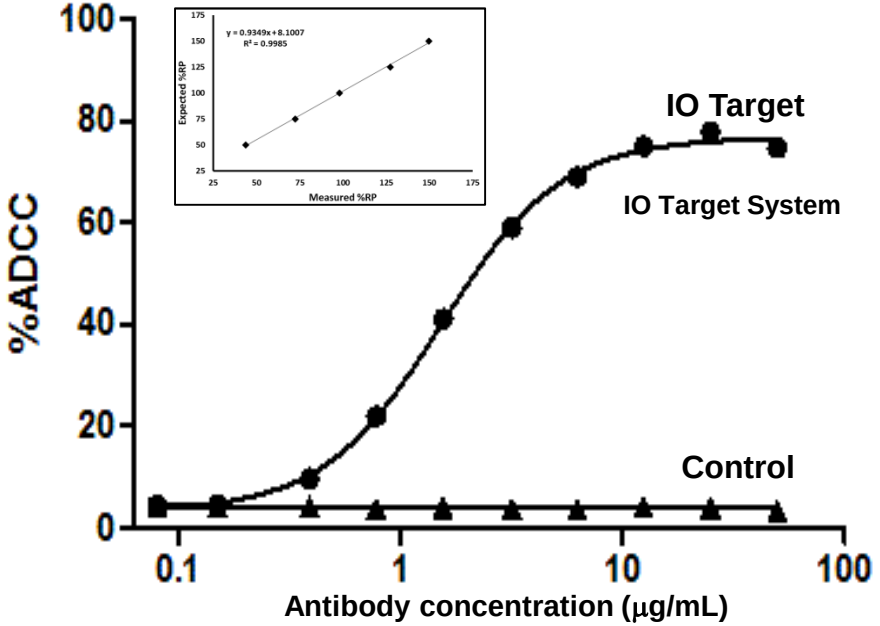




Prior challenges:

Highly Variable

Poorly reproducible



State of the Art:

Highly Precise, Very Accurate

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Mechanistic Options for Assessing Potency of Immuno-Oncology Therapeutic Products

Challenge:

- **Co-stimulation and checkpoint inhibitors inherently complex to model *in vitro***
- **Requires primary signal**
- **Expression of co-stimulatory molecules on lymphocytes tightly regulated**

Solution:

- **Capture function of domains outside true biological activity (binding *via* ELISA, SPR)**
- **Cell-Based Potency Assays**
  - **T-cell activation assays**
  - **Engineered cell lines**
  - **Reporter gene systems**

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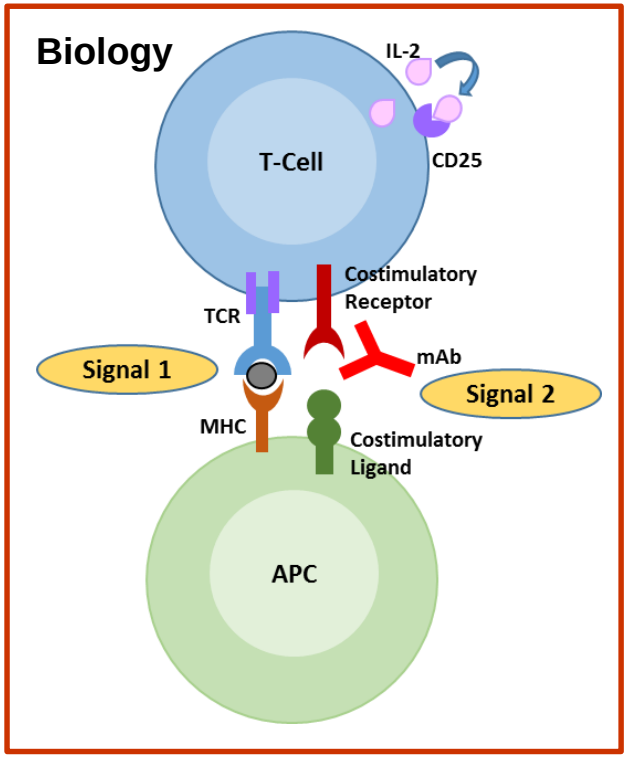
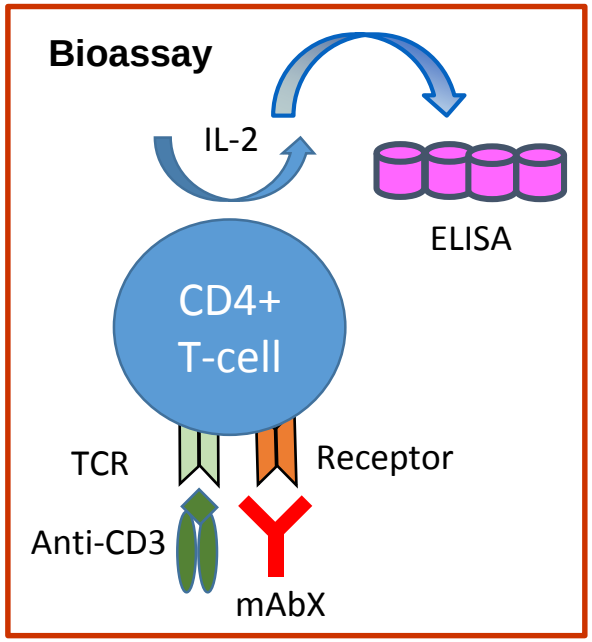
Potency Assays for Immuno-Oncology

Case Study #1: Optimizing Mechanistically Complex Bioassay Models





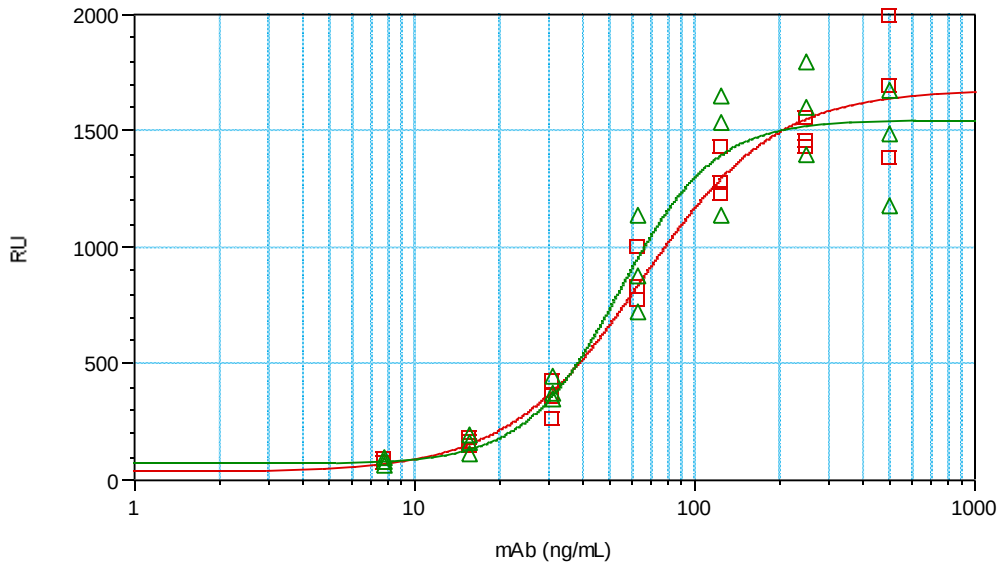
System: Activation of CD4+ T cells

Complex system with primary signal requirement, co-stimulation agonist

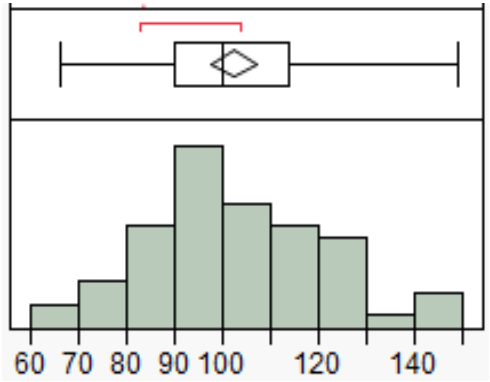
Assay format using anti-CD3 coated plates

Drivers for Late-phase Optimization

Assay Performance and Operational Requirements



Relative Potency Distribution



Proof-of-Concept Assay Parameter	Value
Mean	102.4
SD	18.5
Upper 95% Mean (CI)	107.1
Lower 95% Mean (CI)	97.8

Proof-of-concept assay requires optimization for late-phase implementation

Operational requirements include ELISA readout with multi-source reagent availability

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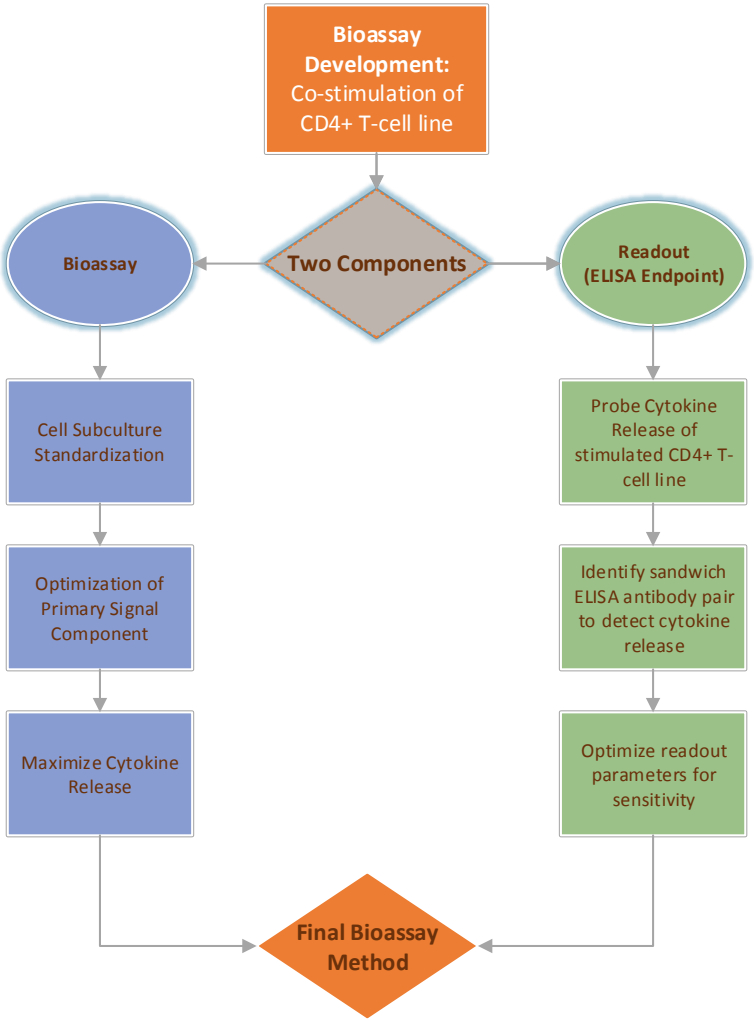
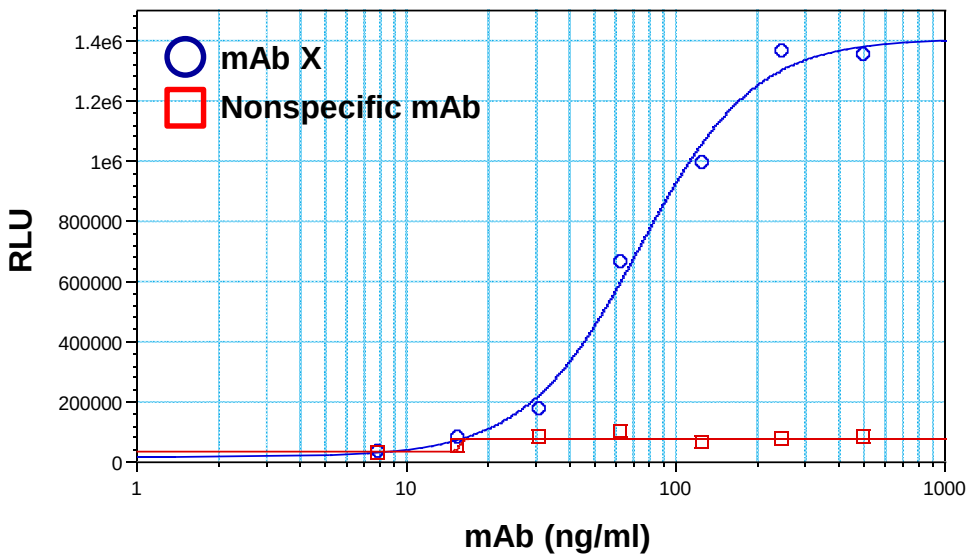
Design Criteria for Assay Optimization



- **State-of-the art System Suitability and Sample Acceptance Criteria**
- **Performance criteria must be met**
- **Transferable to a QC environment**
- **Assay readout must meet operational requirements**

Operational Bioassay Challenge:

Solution: Optimize ELISA Detection System and Maximize Signal



[mAb X] ng/ml	IL-2 Detected (pg/ml)		
	Luminescent ELISA	TMB ELISA	Kit-based ELISA
500	135.0	130.8	50.7
250	135.6	130.2	40.4
125	106.4	98.4	15.7
62.5	78.4	69.8	4.1
31.25	25.6	29.5	ND
15.6	10.2	ND	ND
7.8	ND	ND	ND
0	ND	ND	ND
Signal: Bkgd	53	16	49

- **No kit**
- **Reagent Control**
- **Simplified transfer**
- **Any luminescent reader**

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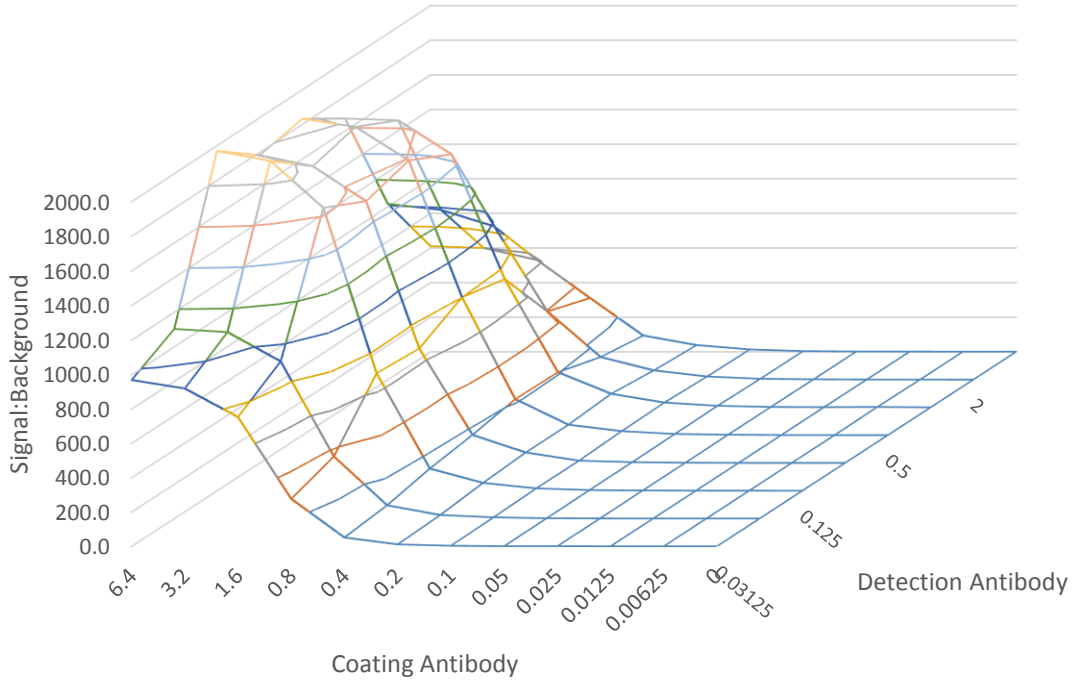
Optimizing Assay Readout:
ELISA Development Approach

- **Selected IL-2 antibody combinations**
- **Examined combinations of unlabeled coating and biotinylated detection antibodies using purified IL-2 protein**



- **Coating conditions***
- **Blocking conditions**
- **Detection antibody concentration**
- **SA-HRP concentration***
- **Luminol/peroxide substrate***
- **Incubation lengths**
- **Optimal ELISA plate type***
- **Signal detection parameters**

**multiple vendor availability*

Parameters assessed by effect on overall signal, signal:background at various IL-2 concentrations, availability of replacements/impact if required

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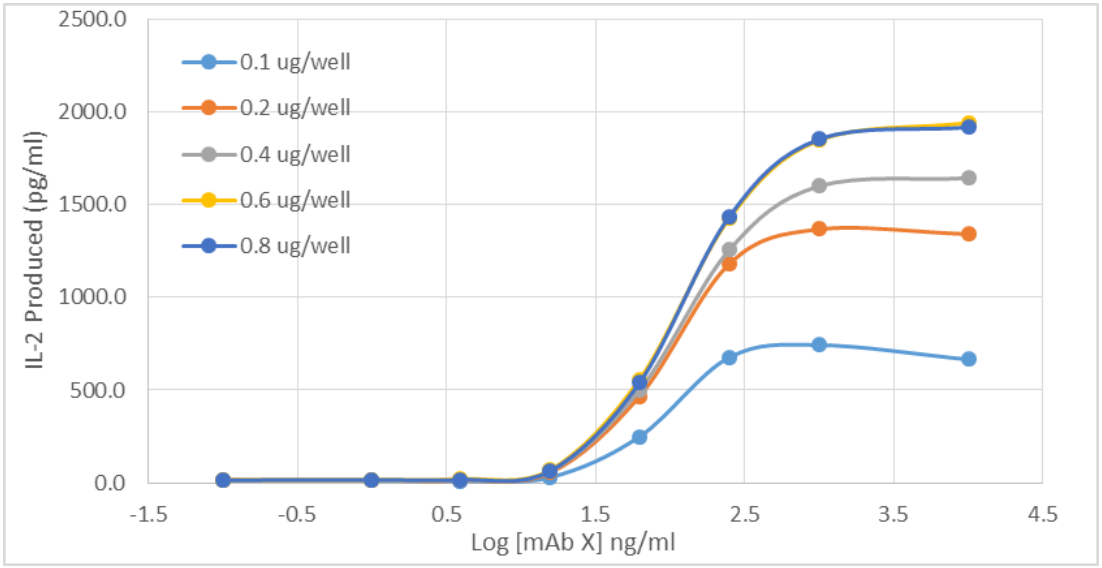
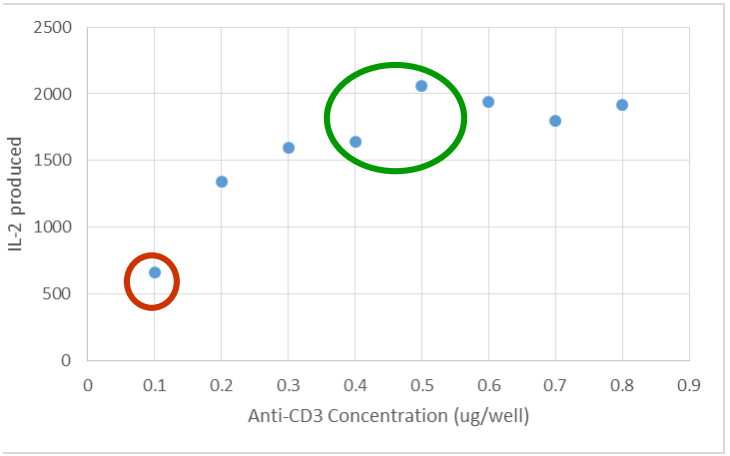
Maximizing Co-Stimulatory Response

Effect of Anti-CD3 concentration





Additional optimization parameters included cell handling, stimulation time and cell density effects

Individual components of T-cell activation response were optimized for maximum IL-2 release

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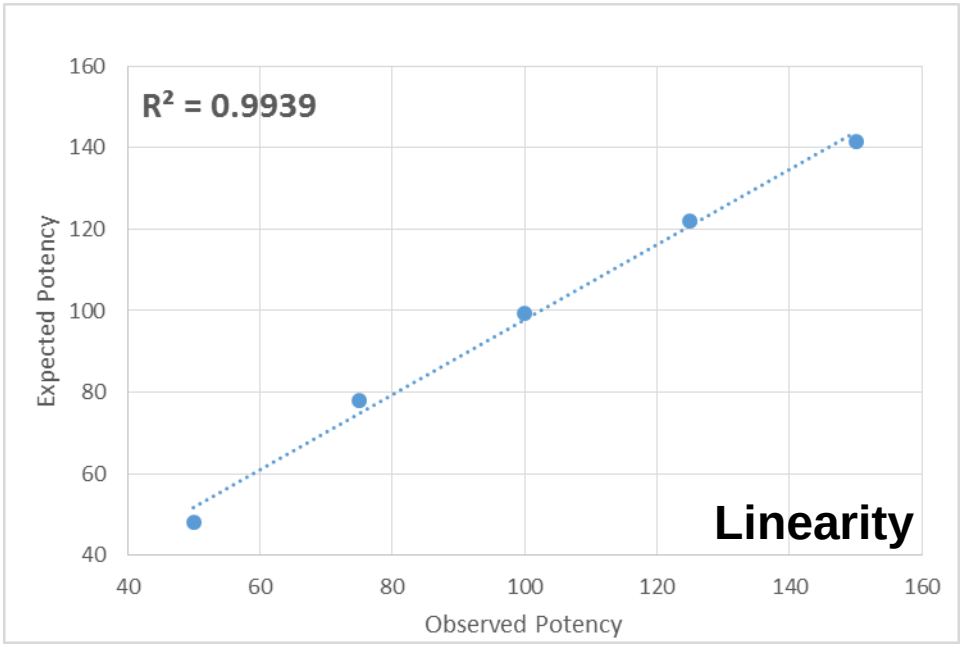
Potency Assays for Immuno-Oncology

Rigorously Developed Systems Can Meet Performance Expectations



Nominal % Potency		Mean % Recovery
Sample	50	96.3
	75	104.0
	100	99.4
	125	97.7
	150	94.3
	Mean	98.3
	% CV	3.7

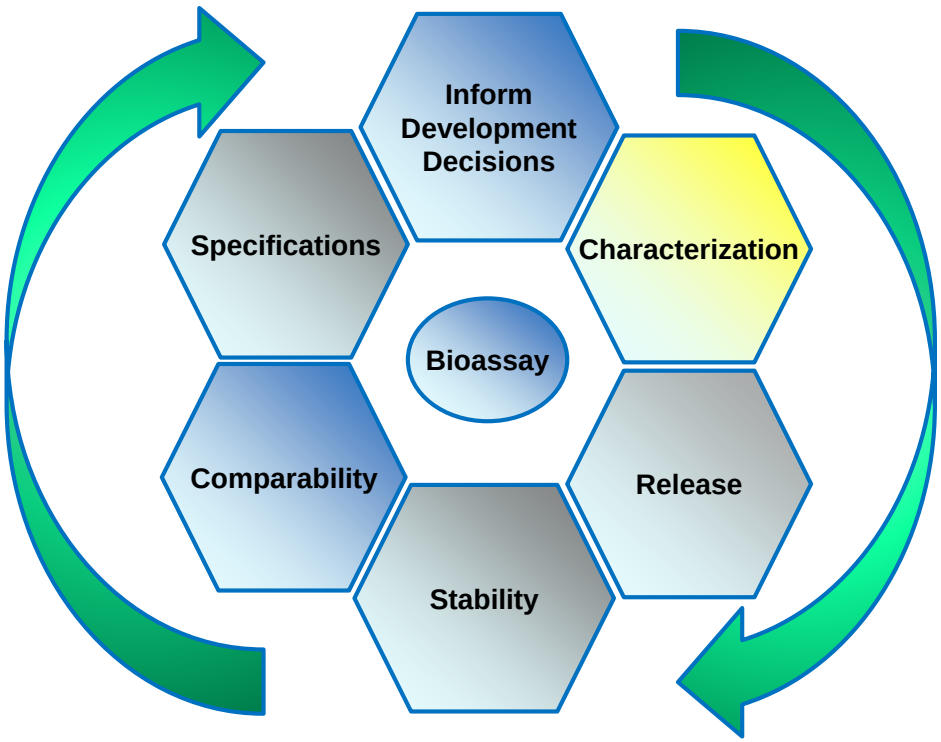
Accuracy

Optimized assay meets performance expectations for accuracy, linearity, precision, specificity and range

Case Study #1 Conclusions

- **Bioassays must be designed for intended use and for the product lifecycle**
- **Both assay performance and operational drivers for late-phase programs need to be considered**
- **Complex MoAs can be modeled *in vitro* with rigorous assay development**
- **Rigorously developed bioassays can meet performance expectations**

Role of Bioassays in Development of Immunotherapeutic Products



- Case Study #1 highlights how rigorously designed, MoA-reflective assays can be used release and stability
- How else might bioassays be employed in product development?
- What can be learned from the bioassay that orthogonal methods may not inform?

Bioassay in Structure-Function Relationships

- Ideal bioassay is MoA-reflective and stability-indicating
- Bioassays play a role in ensuring higher-order structure is consistent throughout manufacturing
- Are integral in determining CQAs, can assist with setting CQA specifications
- Correlate molecular changes with bioactivity
- As such, bioassay can elucidate impact of these changes on a mechanistic level in conjunction with other analytical tests

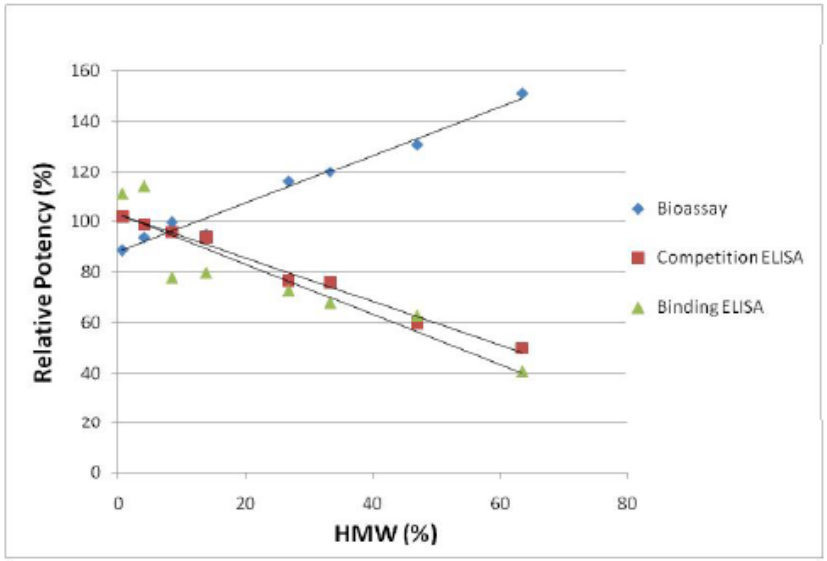
Case Study #2:
Impact of High Molecular Weight Formation on Bioactivity

- **Monoclonal antibody to immune checkpoint molecule expressed on surface of T-cells**
  - **mAb drug substance exposed to pH 3.0**
  - **HMW species were identified**

- **Potency assays used for characterization:**
  - **ELISA to detect binding of mAb to receptor**
  - **Competition ELISA to detect mAb disruption of receptor binding to ligand**
  - **Surface Plasmon Resonance for further binding evaluation**
  - **Cell-based bioassay for T-cell activation**

Case Study #2:
Impact of Low-pH Induced HMW Species on Potency

Sample	Observed Changes in Physicochemical Properties	Relative Potency		
		Bioassay	Competition ELISA	Binding ELISA
Control	N/A	91%	112%	101%
Low pH (3.0)	Increase in HMW species (0.3% - 56%)	>175%	41%	24%



Relative potency as a function of HMW formation

All assay formats indicate potency changes, but opposite trends

Cell-based bioassay and ELISA yield inverse responses

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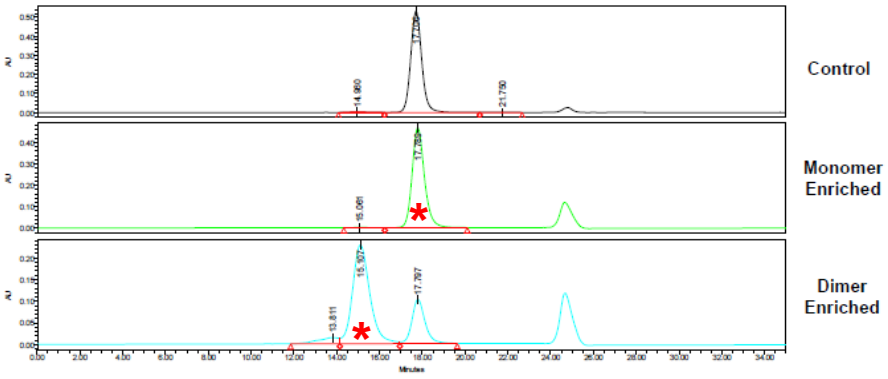
Enriched Size Variants from Drug Substance



SEC used to enrich HMW and monomer species from drug substance.

SPR used to further assess binding

	SE-HPLC			Relative Potency		SPR		
Fraction	**HMW%**	**Monomer %**	**LMW%**	**Bioassay**	**Competition ELISA**	**K_a ($M^{-1}s^{-1}$)**	**K_d (S^{-1})**	**K_D**
Control	0.5	99.3	0.2	94%	92%	3.59E+05	2.59E-05	72 pM
Dimer Enriched	76.0	24.0	ND	>175%	72%	3.41E+05 (105%)	3.34E-05 (78%)	98 pM
Monomer Enriched	0.2	99.8	ND	101%	91%	3.66E+05 (98%)	2.19E-05 (119%)	60 pM

Dimeric species lead to increased potency in bioassay

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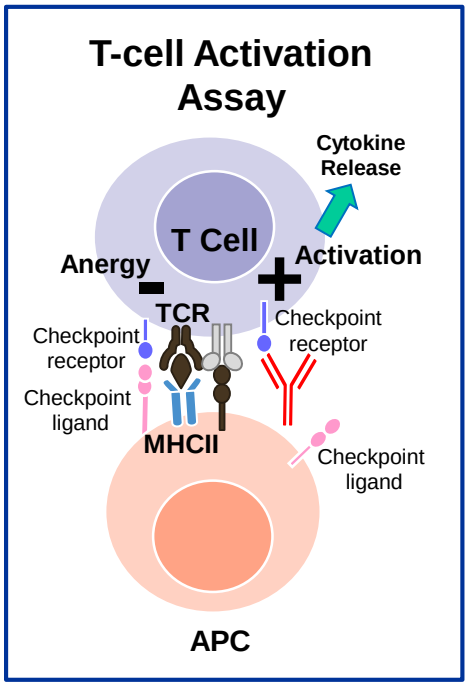
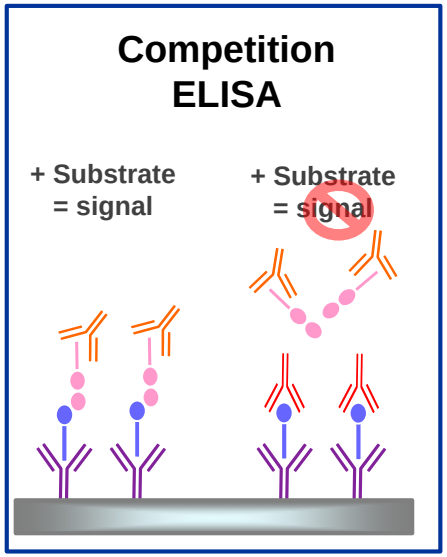
Case Study #2:
Assay Format Influences Data Interpretation





Sample	Competition ELISA (% Relative Potency)	T-Cell Activation Bioassay (% Relative Potency)	K_D
Control	92	94	72 pM
Dimer	72	>175%	98 pM

- ***Both formats are designed to measure disruption of target-ligand binding***
- ***Bioassay informs as to mechanism of mAb dimer on T-cell activation***

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Case Study #2 Conclusions

- Low pH induces HMW formation for this molecule
- Bioassay and binding assays demonstrate inverse responses for relative potency of HMW species
- Isolated dimer leads to increased potency in a cell-based assay, but decreased potency in a binding assay
- Decreased binding activity is consistent with lower affinity (K_D) of the dimer
- Enhanced T-cell response measured by cell-based bioassay indicates increased avidity of the dimer
- Bioassay data indicate that clustering of the mAb may play a role in enhancing T-cell activation

Summary

- **Significant challenge for IO mAb product development is modeling the complexity *in vitro***
- **Well-developed bioassays for therapeutic products in IO can be accurate and precise, and therefore suitable for release and stability testing**
- **Mechanistically-relevant bioassays can provide information on the impact of structure-function modifications in conjunction with other analytical tests**

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Acknowledgements

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