BIOASSAYS IN PRODUCT DEVELOPMENT:
An Immuno-Oncology Perspective

CASSS Bioassays
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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

- Inform Development Decisions
- Specifications
- Characterization
- Comparability
- Release
- Stability

Bioassay

Brassol-Myers Squibb
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
Monoclonal Antibodies in IO
Well-Characterized Biologics

Challenge for IO:
Reliably assess a highly complex MoA in vitro
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
Challenge:

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

Solution:

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

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

Operational requirements include ELISA readout with multi-source reagent availability
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

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

<table>
<thead>
<tr>
<th>[mAb X] ng/ml</th>
<th>Luminescent ELISA</th>
<th>TMB ELISA</th>
<th>Kit-based ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>135.0</td>
<td>130.8</td>
<td>50.7</td>
</tr>
<tr>
<td>250</td>
<td>135.6</td>
<td>130.2</td>
<td>40.4</td>
</tr>
<tr>
<td>125</td>
<td>106.4</td>
<td>98.4</td>
<td>15.7</td>
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<tr>
<td>62.5</td>
<td>78.4</td>
<td>69.8</td>
<td>4.1</td>
</tr>
<tr>
<td>31.25</td>
<td>25.6</td>
<td>29.5</td>
<td>ND</td>
</tr>
<tr>
<td>15.6</td>
<td>10.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Signal: Bkgd

- 53
- 16
- 49
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

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

*multiple vendor availability*
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.
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
Case Study #1 highlights how rigorously designed, MoA-reflective assays can be used for 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed Changes in Physicochemical Properties</th>
<th>Relative Potency</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N/A</td>
<td>91%</td>
<td>112%</td>
</tr>
<tr>
<td>Low pH (3.0)</td>
<td>Increase in HMW species (0.3% - 56%)</td>
<td>&gt;175%</td>
<td>41%</td>
</tr>
</tbody>
</table>

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
Enriched Size Variants from Drug Substance

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

SPR used to further assess binding

<table>
<thead>
<tr>
<th>Fraction</th>
<th>HMW%</th>
<th>Monomer %</th>
<th>LMW%</th>
<th>Bioassay</th>
<th>Competition ELISA</th>
<th>(K_a) (M(^{-1})s(^{-1}))</th>
<th>(K_d) (S(^{-1}))</th>
<th>(K_D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>99.3</td>
<td>0.2</td>
<td>94%</td>
<td>92%</td>
<td>3.59E+05</td>
<td>2.59E-05</td>
<td>72 pM</td>
</tr>
<tr>
<td>Dimer Enriched</td>
<td>76.0</td>
<td>24.0</td>
<td>ND</td>
<td>&gt;175%</td>
<td>72%</td>
<td>3.41E+05 (105%)</td>
<td>3.34E-05 (78%)</td>
<td>98 pM</td>
</tr>
<tr>
<td>Monomer Enriched</td>
<td>0.2</td>
<td>99.8</td>
<td>ND</td>
<td>101%</td>
<td>91%</td>
<td>3.66E+05 (98%)</td>
<td>2.19E-05 (119%)</td>
<td>60 pM</td>
</tr>
</tbody>
</table>

Dimeric species lead to increased potency in bioassay
Case Study #2: Assay Format Influences Data Interpretation

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Competition ELISA (% Relative Potency)</th>
<th>T-Cell Activation Bioassay (% Relative Potency)</th>
<th>$K_D$ (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92</td>
<td>94</td>
<td>72 pM</td>
</tr>
<tr>
<td>Dimer</td>
<td>72</td>
<td>&gt;175%</td>
<td>98 pM</td>
</tr>
</tbody>
</table>
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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