Bioassay Strategies for Assessment of Co-stimulation Inhibitors in Immuno-Oncology:
Considerations from Development to Commercialization

Cynthia A. Inzano
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Outline

- Background of Immunomodulatory Antibodies
- Targeting Checkpoint proteins
- Inhibitory pathway
- Immunomodulatory Antibodies
- Bioassay Development and Complexities
- Considerations in Bioassay Development
- Example Bioassay
- Summary
Immune System and Tumors

- Immune response to tumors involves:
  - Foreign protein or antigen
  - Antigen Presenting Cell (APC) recognition and processing
  - APC present via Major Histocompatibility Complex (MHC) to T-cell
  - T-cell receptor recognition and activation
  - Co-Stimulation required
  - Balance of T-cell regulation through inhibitory and co-stimulatory pathways

- Tumor mechanism for immune resistance:
  - Dysregulation of immune checkpoints of effector cells:
    - T-cell and NK cell
  - Immunomodulatory antibodies
    - Agonist/antagonist activities
Approaches to Immunotherapy in Immuno-Oncology

Mechanisms of Immunomodulation

1. Immunomodulatory
   - Cancer
   - Autoimmunity

2. Properties
   - Bi-functional
     - F(ab) domain
     - Fc domain – therapeutic function
   - Target effector functions
   - FcγRs (ADCC and ADCP)
   - Inhibitory/Co-stimulatory pathways of T-cells

Inhibitory and Co-Stimulatory Checkpoints

- Targeting immune checkpoints
  - Target effector cells: T-cells stimulatory and inhibitory pathways
  - Modulate agonist and antagonist response
  - T-cell activation requires co-stimulatory interaction

Inhibitory Pathway

Inhibitory Signaling
- Block CD3/CD28 mediated activation
- Results in decreased
  - Proliferation
  - Cytokine production
  - Migration

Strategies Bioassay Development
- Understand MOA
- Cell Line
Examples of Targets in Immuno-Oncology

Approved
- Opdivo® (Nivolumab)
- Yervoy® (Ipilimumab)
- Nulojix® (Belatacept)
- Orencia® (Abatacept)

Pipeline
- Urelumab (anti-CD137)
- Lirilumab (Anti-KIR)
- Lulizumab (Anti-CD28)
- BMS-9860616 (Anti-LAG3)
- BMS-936559 (Anti-PD-L1)
- BMS-936561 (CD70)
- BMS-986178 (anti-OX-40)

Bioassay Development Complexities

- **Complex Signaling System**
  - Multiple Receptors involved
    - CD3 /anti-CD3 (primary)
    - CD28 /CD80/86 (co-stimulatory)
    - PD-1/PDL-1 (inhibitory)

- **Mechanism of Action**
  - In Vitro
  - In Vivo

- **Multiple Cellular Events**
  - Proliferation
  - Cytokine production
  - Migration
Immunomodulatory Antibodies

Yervoy® (Ipilimumab) Mechanism

T-cell Activation

T-cell Inhibition

T cell Remains Active

APC: antigen-presenting cell; CTLA-4: cytotoxic T-lymphocyte antigen-4; MHC: major histocompatibility complex; TCR: T-cell receptor.
Immunomodulatory Antibodies

Opdivo® (Nivolumab) Mechanism

Recognition of tumor by T cell through MHC/antigen interaction mediates IFNγ release and PD-L1 upregulation on tumor

Priming and activation of T cells through MHC/antigen and CD28/B7 interactions with antigen-presenting cells

Blockade of PD-1 and PD-L1 results in reactivation of T-cell–mediated tumor cell killing
Bioassay Development Considerations

- **Cell Line Selection**
  - Single or Multiple
  - Transfection
  - Beads

- **Example Signaling Pathway**
  - PI3K /Akt

- **Example Readouts**
  - Cytokine → ELISA = Absorbance
  - Reporter → Luciferase = Chemiluminescence
Example Development

Cell based Bioassay using a cytokine ELISA Kit
- Day 1 Bioassay = Cell prep, antibody dilution, incubation
- Day 2 ELISA

Evaluation of Bioassay
- Characterization of cell lines (one or two)
- Components in Bioassay
- Components in ELISA

Points to Consider in Development
- Cell Passage
- Cell Bank Quality
- Serum
Considerations for Pre-Commercial: Cell Passage

- Considerations for
  - Identification of Cell line(s)
  - Characterization and Trending
    - Passage
    - Receptor level overtime (flow cytometry)
    - Viability
    - Doubling time
    - Performance in the bioassay

**Percent Viability of Two different Cell Lines over passaging**

**Signal-to-Noise Consistency over passaging**

**Receptor Density in Flow Cytometry**

Antibodies per cell (ABC) vs. Passage Number

Viability vs. Passage Number

Quality Control

BMS People Strategy | People Helping Patients Prevail | Bristol-Myers Squibb
Considerations for Pre-Commercial: Cell Bank

- Developed In-House or Purchased
  - Cost
  - Speed
  - Technical expertise
  - Control/Experience

- Single use vials or Continuous Culture
  - Cost to Produce
  - Manufacturing capacity
  - QC friendly
  - Passaging hands-on-time
  - Cell Changes over time
Considerations for Pre-Commercial: Serum

Points to Consider

– Vendor to Vendor Differences
– Lot to Lot Variation
– Components within Serum
– Serum treatments
  • Heat treated/no-heat treatment
  • Gamma irradiated
– Serum alternatives

Qualification

– Passaging
– Trending
– System Suitability

http://www.saawinternational.org/cows.htm
Considerations for Pre-Commercial: Serum Vendor Differences

- Shape of Curve
  - Lower Asymptote/Upper Asymptote, Slope, EC50
Considerations for Pre-Commercial: Serum Lot Differences

- Shape of Curve
  - Lower Asymptote/Upper Asymptote, Slope, EC50
Considerations for Pre-Commercial: Serum Treatment

- Shape of Curve
  - Upper Asymptote, Slope, EC50
Example of Bioassay Accuracy from Immuno-Oncology

### Summary of Bioassay Accuracy and Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nominal Potency (%)</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
<th>RSD (%)</th>
<th>Acc. Crit. Mean % Recovery</th>
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Example of Bioassay Linearity from Immuno-Oncology

\[ y = 0.9645x + 2.0017 \]
\[ R^2 = 0.9958 \]
Control Trending of Commercial Bioassay in Immuno-Oncology
Summary

- Immunomodulatory Antibodies
  - Complex co-stimulatory systems
- Bioassay Development
  - Impact of Particular Components
    - Cell Passaging
    - Cell Banking
    - Serum
- Exploring co-stimulatory systems
  - Relation to other mechanisms of action
    - ADCC
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