Bioassay development for complex molecules- Challenges with Bispecific Molecules

Bhavin Parekh, Ph.D.
Group Leader-Bioassay Development
Eli Lilly and Company
Indianapolis, IN 46221
Outline

- Background
  - Bioassay expectations

- Developing a MOA-based strategy for bispecific molecules

- Case Study: Challenges in development, control and analysis of bioassays for a bispecific antibody
Regulatory expectations for Bioassays

- Reflective of the mechanism(s) of action (MoAs) of the product
- Sensitive to structural changes that can impact product safety and efficacy
- Stability indicating (detection of degradation)
- Well controlled (low CV, tight precision, etc.)
- Usable as a QC release assay under GMP quality system
- Binding based assay may be acceptable to support early clinical phase. Need MOA-reflective, functional assay by pivotal.
Bispecific antibodies come in many flavors

c. IgG-based bispecific antibodies

Hybrid hybridoma
Knobs-into-holes with common light chain

IgG–scFv

scFv–IgG

Two-in-one IgG
Dual V domain IgG

IgG–V

V–IgG
Different targets of Bispecific antibody

- Both targets are soluble ligands
- One target is soluble ligand and other is cell surface receptor
- Both targets are cell surface receptors, but on different cells
- Both targets are cell surface receptors, but on the same cell

➢ Influences the strategy of what type/number of potency assay are relevant
BsAbs have the unique potential to mediate two types of biological effects (Additive or synergistic)

- Additive effects
  - Biological outcome with the BsAb is the sum of the two independent target
    - When the targets bind to two cell types, independent two bioassays seem appropriate
    - When the targets bind to the same cell type, a single bioassay is appropriate

\[ \text{BsAb} = \text{mAb A} + \text{mAb B} \]
BsAbs have the potential to mediate two types of biological effects (Additive or synergistic)

- **Synergistic effects**
  - Biological effect of BsAb is greater than the sum of the two independent targets.
  - Can be manifested at different biological levels (physiological and/or cellular)

- **Physiological synergy**
  - Targets can be different cell types (e.g., B-cell and T-cells) with different signaling pathways BUT provide a more synergistic physiological outcome revealed in a preclinical animal model
  - This synergistic outcome cannot be reflected in a unique combined assay. Thus, two separate and distinct bioassays is appropriate
Potency Assay strategy for BsAbs

- Synergistic effects
- Cellular synergy
  - Targets present on same cell population
  - Specific synergy assay is needed

BsAb > mAb A + mAb B
Bioassay strategy for Bispecific antibody: How many assays?

Bioassay Strategy:

- If Receptor 1 and Receptor 2 have similar signaling pathway: Synergistic assay
- If different signaling pathway: Two individual Cell based assay
Case Study: BsAb

- Targets 2 receptors (M and E) involved in cancer
  - Can be expressed on same cells
- Receptors have been targeted independently with specific mAbs
- In cells expressing both receptors, signaling pathways via receptor M and E can cross talk
- Receptor have been shown to hetero-dimerize
Case Study: Bispecific Ab binding to two receptor targets (M and E) on the same cell

Objectives:

- Develop potency assay strategy for BsAb that is reflective of the synergistic MOA
Requirements for a synergy assay for BsAB

- Cell line has to express both receptors
- Readout has to be sensitive to inhibition of both receptors
- BsAb activity greater than
  - Individual mAb M and mAb E
  - Combination of mAb M and mAb E
Synergy assay - Finding the correct cell line

- Cell line G provides response to Receptor M and not Receptor E, thus cannot provide mechanistically relevant synergistic assay.
Cell line F provides response to Receptor E and not Receptor M, thus can not provide mechanistically relevant synergistic assay.
Synergy assay - Finding the correct cell line

In cell line F, BsAb shows superior effect from individual Abs and from Combo.
Potency analysis of typical mAbs vs. BsAb

Synergistic assay profile
- Change in EC\textsubscript{50}, asymptotes
- Not amenable to parallelism test
- How do we adequately capture the biological change?
- How would dose-response curve shapes change upon loss of stability at either sides

\[ y = d + \frac{a - d}{1 + (\text{conc}/c)^b} \]

- a: lower asymptote
- d: upper asymptote
- b: slope
- c: EC\textsubscript{50}

List of mAbs and BsAb:
- mAb M
- mAb E
- BsAb

% viability

Conc.
Analysis of non-parallel curves

- Well documented in the field of pharmacology
- Study of partial agonist or antagonist
- Biological activity is represented by change in EC50 and relative change in asymptote (referred to as Efficacy)
New approach for determining relative potency/efficacy for BsAb with synergistic activity

- BsAb changes two different aspects of the dose response (EC50 and asymptote ratio)

- For Bispecifics with different asymptotes: Efficacy = Proliferation reduction/EC50
  \[
  = \frac{\text{Upper asymptote}}{\text{Lower asymptote}}
  \]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Ligand</th>
<th>Analyte</th>
<th>A (Upper asympt.)</th>
<th>D (Lower asympt.)</th>
<th>Proliferation reduction (PR=A/D)</th>
<th>EC50 (nM)</th>
<th>Efficacy factor (EF=PR/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>No ligand</td>
<td>Recep. E Ab</td>
<td>6370000.00</td>
<td>2200000.00</td>
<td>2.89</td>
<td>0.56</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recep. M Ab</td>
<td>6180000.00</td>
<td>6100000.00</td>
<td>1.01</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Combo-1</td>
<td>6700000.00</td>
<td>1010000.00</td>
<td>6.63</td>
<td>0.72</td>
<td>9.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BsAb B</td>
<td>6290000.00</td>
<td>702000.00</td>
<td>8.96</td>
<td>0.16</td>
<td>56.00</td>
</tr>
</tbody>
</table>
Acknowledgements

Bioassay group
Piyush Vyas
Maroun Beyrouthy
Darren Kamikura
Sara Lally
Denise Lyons
Jane Sterner
Sharon Sibley
Jeanne Helmreich
Claudius Tapia
Jason Lehman
Teresa Ledford
Gretchen Smith

Statistics
Kristi Griffiths
Eric Adamec
Shu Zheng
Kevin Guo