Antibody-Mediated Effector Function: Learning and Challenges from Early Discovery to Development

Payal Mehta, PhD
Research Scientist
Eli Lilly & Company
CaSSS Bioassay Conference, Silver Spring, MD
9th May 2017
Agenda

♦ Overview of Antibody (Ab)-Mediated Effector Function
♦ Ab-Effector Function Testing During Life Cycle Development of Drug
♦ Transition Challenges from Discovery to Development
  • Mouse to Human transition
  • Choice of Appropriate Assay Format When Multiple Receptors Are Part of MOA
  • Finding Appropriate Target Cell Lines
    – Representative of Physiological Target Expression
    – Stability Over Assay Runs
♦ Summary
Bimodal Nature of Antibodies: Implications for Safety and Efficacy

Table 1  Key features of the four IgG isotypes

<table>
<thead>
<tr>
<th></th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FcRn&lt;sup&gt;e&lt;/sup&gt;</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Effector functions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>++</td>
<td>–</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>FcgRI&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+++</td>
<td>–</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>FcgRII&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>FcgRIIIa/b&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>
Is Effector Function Always Non-Desirable?

**IMMUNO-ONCOLOGY AUTOIMMUNITY**

- $\gamma_2 \alpha$
- Inhibitory $\text{Fc}yR$
- Activating $\text{Fc}yR$
- ITIM

**ONCOLOGY Depleting antibodies**

- $\gamma_2 \alpha$
- Inhibitory $\text{Fc}yR$
- Activating $\text{Fc}yR$
- ITIM

- IgG 1
- IgG 3
- IgG 2
- IgG 4

- High effector function

- Low effector function

---

- Beneficial where target cell depletion is **NOT** desired- Ex. autoimmunity targets, immuno-oncology targets

- Fc-engineering of antibody to ablate effector function

- Beneficial where target cell depletion is **desired** MOA

- Fc-engineering of antibody to enhance effector function
Effector Function Potential: Interplay with MOA

Advances in the assessment and control of the effector functions of therapeutic antibodies

Xu-Rong Jiang, An Song, Svetlana Bergelson, Thomas Arroll, Bhavin Parekh, Kimberly May, Shan Chung, Robert Strouse, Anthony Mire-Sluis and Mark Schenerman

Nature Reviews Drug Discovery, 2011 (10), 101

Target
- Localization: membrane bound or soluble
- Cellular density
- Rate of internalization

MOA

Antibody
- Isoforms
- Glycosylation
- Mutations that impact Fc binding

FcγR’s
- Allelic variants
- Glycosylation

Class I MOA:
Cell-bound antigen with depletion

Class II MOA:
Cell-bound antigen with blocking

Class III MOA:
Soluble antigen with blocking

Apoptosis
CDC
NK cell
ADCC
Effector Function Testing: Continuous Monitoring

Effector Function Status is impacted by isotype, Fc engineering or Glyco-engineering

Jian et al; Nature Reviews Drug Discovery, 2011 (10), 101
Transitioning Effector Function Cellular Assays from Discovery to Development

**Discovery**
- **Purpose**: Establish the presence or absence of effector function and establish link to MOA.
- **Assay Format**: Target cells could either be primary cells or cell lines with endogenous or overexpression of antigen.
- **In vivo** studies to establish link of effector function to MOA.
- **Species**: Human or mouse.

**Development**
- **Purpose**: An assay representative of MOA that could be used to monitor CQA.
- **Assay Format**: Highly sensitive, reproducible, amenable to TQM.
- **Assay Format**: Cell lines preferably with endogenous expression of target.
- **Species**: Human.

Lead | CS | FTD | FHD
--- | --- | --- | ---
Case Study #1: Transitioning Effector Function Data from Mouse to Human

Understanding of putative MOA of therapeutic antibody within the context of a disease comes from *in vivo* mouse studies

- Mouse and human FcγR repertoire is very different
- Mouse IgG isotypes different than humans
- Human and mouse FcγR (s) exhibit differences in IgG binding pattern’s
- Human and mouse FcγR (s) differ in lineage expression profiles
- Reporter cell-based assays use cell lines expressing individual FcγR’s

These differences add to the complexity of crossing over experimental data between the two species.
Transitioning *in vivo* Results to Design Cell-based Effector Function Assays

- Effector function independent trans-co engagement of agonist CD40 Ab with FcγRIIb is MOA.
- PD-L1 blocking antibody requires trans engagement with activating receptor for optimal *in vivo* efficacy.
- On the contrary interaction with FcγR’s reduces efficacy of PD-1 blocking antibody in *in vivo* mouse tumor models.
- Therefore, choice of backbone is dictated by a better understanding of MOA of antibody and a thorough understanding of biology (QoD)

Using mice surrogate antibody data to understand relevant human FcγR-engagement and its link to MOA is a challenge

Dahan R et al, Cancer Cell, 2015, 285
Case Study #2: Choosing a Reporter Assay When Multiple FcγR’s Are Involved in MOA

Evidence for FcγRIIa


Evidence for FcγRIIa


Should QC Assay be Selected Based on Its Ability to Monitor CQA?
Case Study #3: Choosing an Appropriate Target Cell Line with Endogenous Receptor Expression

♦ None of the IgG-fusion constructs gave a response in FcγRIIIa activity assays.
♦ Is it the Cell Line issue? Expression issue?
Selecting a Target Cell Line with a Second Positive Marker for Effector Function

- The Biological function of the receptor was confirmed via Signaling experiments on the new Target Cell Line.

- If IgG1-Fc functionality does not respond in the FcγRIIIa activity assays do we need to confirm this with multiple target cell lines?

- However, it is not always easy to find cell lines with physiologically relevant target expression.
Data with overexpression systems tend to be exaggerated and may not be representative of true physiological scenario’s.

It is important to put the data obtained within appropriate context.

Clinical data from and knowledge from previous efforts may be helpful in this regard.
Summary

♦ Effector function is a critical component of life cycle management of therapeutic Mab. It is reflective of
  • Therapeutic Efficacy
  • Safety/risk
  • Lot to Lot variation (CQA)
♦ The goals of discovery work on ab-mediated effector function testing is very different than development work
♦ Transition Challenges include
  • Mouse to human transition
  • Multiple receptors involved in MOA
  • Finding suitable target cell lines with physiologically relevant cell lines
♦ It is important to integrate the knowledge of FcγR-MOA into an appropriate cell-based assay as early as possible and use previously available clinical data to drive testing some of the molecules
Acknowledgments

Eli Lilly & Company

Derrick Witcher
Bhavin Parekh
Robert Benschop
Uma Kuchibhotla