Cell-based Binding Assays Using Platform Technology

Mary Hu
Seattle Genetics
CASSS Bioassay Conference
March 4-5th, 2013
Outline

- Antibody drug conjugate (ADC) technology
- Binding ELISA vs cell-based binding assay
- MSD technology
- Development of cell-based binding assay
- Qualification of cell-based binding assay
- Platform assay or platform technology
- Summary
Binding is a Key Component of ADC

- ADC
- Antigen
- Receptor-mediated endocytosis
- Endosome
- Lysosome: drug release from ADC
- Drug escape to cytosol
- Cell death

Cell membrane

Binding is a Key Component of ADC
Binding ELISA vs Cell-based Binding Assay

● Ligand Binding ELISA
  ➢ Pros
    ▪ Easy to perform and transfer
    ▪ Robust
  ➢ Cons
    ▪ Availability of binding ligand
    ▪ Binding ligand reproducibly made
    ▪ Need to demonstrate the recombinant/purified ligand (fusion protein) similar to the native molecule on the cell surface

● Cell-based Binding Assay
  ➢ Target antigen molecule on the cell surface, no need for purified recombinant ligand
  ➢ Less robust
Utilization of Cell-based Binding Assays

● Early phase programs
  ➢ No recombinant ligand available before IND

● Binding assay on the control system
  ➢ Identity test
  ➢ Potency test

● Support process development, formulation development and product characterization
  ➢ Evaluate binding during process/formulation development
  ➢ Define product-related substance or impurity
  ➢ Assess impact of conjugation on binding for ADCs
MSD Technology
(Electrochemiluminescence, ECL)

MSD SULFO-TAG NHS-Ester

Cell Surface Markers
Receptor-Ligand Assays
ECL Technology

Plate-based Electrochemiluminescent Assays

MULTI-ARRAY® Plate

- Electrodes are built into the bottom of the plate and are energized within the instrument.
- **Proximity** – only labels near electrode surface are detected, enabling non-washed or reduced washed assays.
- The electrochemical reaction occurs within the plate and light is measured through a CCD camera or photodiodes.
- **Carbon surface** with hydrophobic dielectric also forms physical and surface tension barrier

Cell Based Assays 2008

SULFO-TAG™

Measured signal is light

Light

\[ \text{Ru(bpy)}_3^{2+} \xrightarrow{\text{TPA}^+} \text{Ru(bpy)}_3^{3+} + \text{TPA}^+ \]

Counter electrode

Working electrode

Dielectric

www.mesoscale.com
Direct Binding vs Competitive Binding Assay Design

SULFO-TAG Anti-Human IgG Detection antibody

SULFO-TAG mAb or ADC

mAb or ADC binding

Cells bound to plate
Representative Competitive Binding Curve
Development of Cell-based Binding Assays

- Optimization of assay conditions
  - Cell density
  - Blocking condition
  - Wash condition
  - Assay incubation time
  - Detection antibody
  - Plate uniformity

- Use DOE extensively
Interesting observation: with cell density increasing, ECL signal first increases, reaches maximal then decreases.
### Assay Qualification: Accuracy, Precision & Repeatability

<table>
<thead>
<tr>
<th></th>
<th>Interim Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>Control %RP range:</td>
<td>91</td>
</tr>
<tr>
<td>Sample %RP range:</td>
<td>92</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
</tr>
<tr>
<td>Control %CV (N=14):</td>
<td>5</td>
</tr>
<tr>
<td>All Samples %CV (N=28):</td>
<td>5</td>
</tr>
<tr>
<td>3SD Range for Control:</td>
<td>86</td>
</tr>
<tr>
<td>3SD Range for Sample:</td>
<td>87</td>
</tr>
<tr>
<td><strong>Repeatability (Same day)</strong></td>
<td></td>
</tr>
<tr>
<td>Control %CV range:</td>
<td>0</td>
</tr>
<tr>
<td>Sample %CV range:</td>
<td>2</td>
</tr>
</tbody>
</table>
### Linearity with Interim Reference

#### Nominal %RP vs. Measured %RP

<table>
<thead>
<tr>
<th>Nominal %RP</th>
<th>Measured %RP</th>
<th>%Diff from Nominal %RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>48</td>
<td>-4</td>
</tr>
<tr>
<td>75</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>104</td>
<td>4</td>
</tr>
<tr>
<td>133</td>
<td>135</td>
<td>1</td>
</tr>
<tr>
<td>200</td>
<td>201</td>
<td>1</td>
</tr>
</tbody>
</table>

#### Graphical Representation

- **Nominal %RP vs. Measured %RP**
- **R² = 0.9993**
Specificity Demonstrated for ADC-1

Antigen positive cells
- Specificity demonstrated for the mAb and ADC
- No competition observed for the unrelated mAbs and ADCs

Antigen negative cells
- No signal observed
# Qualification Summary

<table>
<thead>
<tr>
<th>Interim Reference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td></td>
</tr>
<tr>
<td>Control %RP range:</td>
<td>91</td>
</tr>
<tr>
<td>Sample %RP range:</td>
<td>92</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
</tr>
<tr>
<td>Control %CV (N=14):</td>
<td>5</td>
</tr>
<tr>
<td>All Samples %CV (N=28):</td>
<td>5</td>
</tr>
<tr>
<td>3SD Range for Control:</td>
<td>86</td>
</tr>
<tr>
<td>3SD Range for Sample:</td>
<td>87</td>
</tr>
<tr>
<td><strong>Repeatability</strong> (Same day)</td>
<td></td>
</tr>
<tr>
<td>Control %CV range:</td>
<td>0</td>
</tr>
<tr>
<td>Sample %CV range:</td>
<td>2</td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
<td></td>
</tr>
<tr>
<td>%Diff from Nominal %RP</td>
<td>0</td>
</tr>
<tr>
<td>Trend Line R² Value</td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
</tr>
<tr>
<td>Other unrelated products</td>
<td>Curves not similar to reference</td>
</tr>
</tbody>
</table>
## Stability Indicating: Correlation of Binding Activity with Oxidation Level

<table>
<thead>
<tr>
<th>%Total Oxidized $F_{ab}$</th>
<th>%RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>94</td>
</tr>
<tr>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>7.2</td>
<td>96</td>
</tr>
<tr>
<td>14</td>
<td>92</td>
</tr>
<tr>
<td>20</td>
<td>73</td>
</tr>
<tr>
<td>22</td>
<td>73</td>
</tr>
</tbody>
</table>

$R^2 = 0.8831$

![Graph showing correlation between %Total Ox Fab and %RP, with $R^2 = 0.8831$.](image)
Continued Assay Performance Monitoring

- **Statistical Process Control (SPC)**
  - 4 parameters for reference curves
  - %RP for controls
  - Work closely with statistician

- **Close communication between testers and assay developers**
  - Address assay issues quickly
  - Develop assay optimization plan for late stage
Platform Assay or Platform Technology

- Platform approach for process and assay
  - Efficiency and productivity – “do more with less”
    - Less resources needed
    - More predictable timeline
    - Easier assay transfer
    - More effective use of instruments

- Platform bioassays
  - Impossible for product specific MOA-based bioassay
  - Effector function assays
    - Binding assays
  - Utilize platform technology (MSD, AlphaScreen…)
Challenges in Platforming Bioassay

● Each therapeutic has unique MOA
  - Ligand binding
  - Effector functions – ADCC, CDC and ADCP
  - Cytotoxic signalling
  - Cytotoxic drug delivery

● Cell lines have different characteristics
  - Target surface antigen/receptor number varying in a wide range
  - Suspension or adherence
  - Growth rate
  - Growth condition
  - Passage number
Summary

- We have developed robust and reliable cell-based binding assays for early phase programs.
- MSD can be utilized as a platform technology for cell-based binding assays.
- Continued assay performance monitoring is more important than a snapshot of assay qualification.
Thanks

Bioassay group members at Seattle Genetics