Technology or ‘how do I measure ADCs?’
ADC Bioanalysis: Challenges and opportunities when using Ligand Binding Assay platform, including assay development and validation

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Overview

- Ligand Binding Assay Platforms
- Assay requirements
- Method development
- Validation
- Challenges
ADC Development to Clinic

• Stage
  • Discovery
  • Preclinical
  • FTIH
  • Late clinical

• Considerations for bioanalysis and method development
  • Species
  • Antibody backbone
  • Matrix
    • Circulating target
    • Anticoagulants
  • Reagent availability
  • Analytical assay range
  • Validation strategy
## Ligand Binding Assay Platforms

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th>MesoScale Discovery (MSD)</th>
<th>GyroLab Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Traditional plate based immunoassay</td>
<td>Electro-chemiluminescence plate based immunoassay</td>
<td>High-throughput, microfluidic immunoassay</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>ng</td>
<td>ng - pg</td>
<td>ng - pg</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>Good</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>Sample Volume</strong></td>
<td>50-100 μL</td>
<td>10-25 μL</td>
<td>&lt;5 μL</td>
</tr>
<tr>
<td><strong>Dynamic Range</strong></td>
<td>1 – 2 logs</td>
<td>3 – 4 logs</td>
<td>3 – 4 logs</td>
</tr>
<tr>
<td><strong>Automated</strong></td>
<td>Automatable by standard systems</td>
<td>Automatable by standard systems</td>
<td>Semi-automated</td>
</tr>
<tr>
<td><strong>Multiplex Capability</strong></td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
| **Weakness**              | Poor sensitivity  
Significant analyst time | Single vendor  
Expensive (compared to ELISA)  | Single vendor  
Expensive reagent costs                           |

- **Other technologies**
  - Singulex Erenna System, ultra sensitive single molecule counting
  - Luminex high-throughput improved speed and dynamic range, good for multiplexing
  - Real time immune quantitative PCR (Immuno-PCR Imperacer)
Assay Requirements

Multiple assays required to define PK of an ADC antibody:

- **Total antibody assay** (LBA)

- **ADC conjugated antibody assays**
  - **Conjugated-antibody assay** (LBA)
    - Concentration of mAb with one or more drug
  - Antibody-conjugated drug assay (LC/MS)
    - Concentration of drug attached to antibody (LC/MS)

- **Anti-Drug Antibody (ADA) assay** (LBA)
  - Abnormal PK profile

- Unconjugated drug (LC/MS)
- Total drug and metabolites (LC/MS)
- Naked antibody (LBA)
– Bioanalytical strategy to support ADC development to clinic

– Ligand binding assay
  – Total antibody assay
  – ADC antibody assay

– LC/MS
  – Antibody-conjugated drug assay (Payload)
Total Antibody Assay

- Measures conjugated and unconjugated antibody
- Confirms mAb like PK

Labelled detection reagent
- Anti-human IgG mAb (or relevant species)
- Anti-Idiotypic mAb
- Antigen

ADC conjugated or unconjugated mAb

Capture reagent
- Antigen
- Anti-Idiotypic mAb
- Anti-human IgG mAb (or relevant species)

- Should use ADC material as reference standard
  - Could use unconjugated material but need to show data to support
ADC Conjugated Antibody Assay

- Measures antibody with at least one drug
  - LBA does not distinguish changes in drug load (Response for DAR1=DAR>1)

Labelled detection reagent
- Anti-Idiotypic mAb
- Anti-human IgG mAb (or relevant species)
- Antigen

Labelled detection reagent
- Anti-drug mAb

Capture reagent
- Anti-drug mAb

Capture reagent
- Anti-Idiotypic mAb
- Anti-human IgG mAb (or relevant species)
- Antigen

- ADC material as reference standard
Method Development

- Consider
  - LBA platforms
  - Capture and detection reagents
  - Species, matrix and antibody backbone
  - Homogeneous assay formats with overnight incubation
  - Assay buffer, blocking and wash composition

- Method development
  - 4-point curve in buffer with ADC material as standard
    - Determine optimal capture and detection combinations
    - Matrix effect (serum, plasma, blood)
  - 4-point curve in matrix with ADC material as standard
    - Determine optimal capture and detection concentrations (S:B at LOQ of ~2)
    - MRD (minimum required dilution)

- Reference standard may not be the same as the ADC from in vivo samples at later PK time points

- Impact of circulating target
ADC Conjugated Antibody Mean PK Profile

- Increase in unconjugated mAb or lower DAR mAb at later time points
Method Development

• Use reference material with varying DAR
  – Purified DAR1, DAR2, DAR3...
  – Different DAR distribution (low, mid, high)
  – Select reagents which bind comparably to full range of DAR

• Look at effect of DAR in optimised assays
  – Generic assay formats may be less susceptible to varying DAR
  – Conjugation may interfere with reagent binding, particularly at high DAR
    – Use reagents which do not bind near conjugation site

• Impact of circulating target
mAb and ADC Assays For FTIH (Patients)

What are the Individual Assays Measuring?

**mAb Assay**

- Detection mAb
- mAb Drug
- Antigen capture

**ADC Assay (Conjugated Antibody)**

- Detection mAb
- mAb Drug
- Anti-linker capture

(DAR 0-8)

(DAR 1-8)
Validated Assays for Analysis of Study Samples

Preclinical Quantitation of ADC, mAb, and cys-mc-MMAF

- Assays measured ADC species, mAb species, and free cys-mc-MMAF in preclinical species
- Rat and Monkey GLP studies supported
- Systemic exposure for ADC and mAb were similar with mAb concentrations slightly higher than ADC
Circulating target concentrations have been reported to vary in disease-state (2-1000 ng/mL).
- mAb assay utilizes target based capture reagent
- Suitable selectivity achieved in disease-state plasma during method validations

Validated Assays for Analysis of Study Samples

Unexpected PK variability observed between ADC and mAb assays (FTIH)

Validated Assays for Analysis of Study Samples
ADC and mAb Assay Considerations

- What is the target?

- Soluble therapeutic targets can result in free, partially bound, and fully-bound antibodies in circulation

**ADC Assay (Conjugated Antibody)**

- DAR = 1 – DAR = 8
- Free, partially bound, and bound species

**mAb Assay**

- DAR = 0 – DAR = 8
- Free and partially bound species
Impact of Circulating Target

Conjugated/ Unconjugated and Free/ Bound

- Drug can circulate bound or free to the target
- ADC spiked into assay buffer with Target at 100 ng/mL or 1000 ng/mL
- Back spiked patient predose with ADC at two levels

ADC assay measures **total** conjugated Ab

mAb assay measures **free** Ab
Impact of Circulating Target
Conjugated/ Unconjugated and Free/ Bound

- Drug can circulate bound or free to the target
- ADC spiked into assay buffer with Target at 100 ng/mL or 1000 ng/mL
- Back spiked patient predose with ADC at two levels

- ADC assay measures total conjugated Ab
- mAb assay measures free Ab

### mAb Assay

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>% Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC (ng/mL)</td>
<td>300</td>
</tr>
<tr>
<td>1000 ng/mL antigen</td>
<td>-84.0</td>
</tr>
<tr>
<td>100 ng/mL antigen</td>
<td>-41.0</td>
</tr>
<tr>
<td>Back – Spike Predose</td>
<td>-55.0</td>
</tr>
</tbody>
</table>
Impact of Circulating Target
Conjugated/ Unconjugated and Free/ Bound

- Drug can circulate bound or free to the target
- ADC spiked into assay buffer with Target at 100 ng/mL or 1000 ng/mL
- Back spiked patient predose with ADC at two levels

ADC assay measures total conjugated Ab
mAb assay measures free Ab
Need total mAb

mAb Assay

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>% Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC (ng/mL)</td>
<td>300 4000</td>
</tr>
<tr>
<td>1000 ng/mL antigen</td>
<td>-84.0 -86.3</td>
</tr>
<tr>
<td>100 ng/mL antigen</td>
<td>-41.0 -32.0</td>
</tr>
<tr>
<td>Back – Spike Predose</td>
<td>-55.0 -43.8</td>
</tr>
</tbody>
</table>
New Reagent for the mAb assay

Quality of Reagents directly related to quality of data

- Contracted with CRO for creation of new antibody
- Screened for assay suitability and resistance to Target
- Increase in capture concentration & Increase in plasma dilution:
  - Tolerance of circulating Target
  - Assay Performance
ADC Conjugated Antibody PK Profile – ADA?

Compound Y (hIgG-ADC) 3mg/kg IV in Mouse

- Confirm if increased clearance is due to ADA
Anti-Drug Antibody Assay

• Measures anti-drug (ADC) antibodies (ADA)

- Capture reagent
  ADC conjugated mAb

- Labelled detection reagent
  ADC conjugated mAb

• Positive control
  – Surrogate positive control (anti-species or anti-Idiotypic mAb)
Method Development

- Anti-drug antibody (ADA) Assay
  - ADC mAb is already conjugated
    - Efficiency of labelling for capture/detect reagent may be compromised
  - Homogeneous or heterogeneous bridging assay
    - Potential to use generic reagents
      - hIgG in rodent species: anti-human capture with anti-species detect
  - Understand tolerance to ADC conjugated antibody
  - Acid dissociation?
  - Understand tolerance to antigen/target
  - Define ADA positive cut point by screening naive serum samples (50-100)
    - 5% false positive rate
  - Further characterisation of ADA positive samples required
Validation

• Discovery (non-GLP)
  – Scientific (tiered) approach
  
  – Consider broader acceptance criteria (20-30%)
  
  – At least 5 QC samples (LOQ, 20-30% LOQ, mid-point, 80% HLQ, HLQ) over analytical range
    – Precision & Accuracy, 1 run
    – Specificity (Blank and Spiked)
    – Stability
      – Bench top, 3x Freeze Thaw
      – Dilutional linearity
  
  – Reference material with varying DAR, if available
  
  – Matrix stability at 37°C
Validation

- Preclinical to Clinic
  (later session)
  - Feedback from submissions and regulatory aspects
    - The regulatory space
    - Speaker: Matthew Barfield (on behalf of the EBF)
Challenges

• Multiple assays are required to define PK

• Multiple LBA platforms

• Heterogeneous reference material
  – DAR changes in vivo
  – Reference standard may not be the same as the ADC from in vivo samples at later PK time points

• Assay development involves screening multiple assay formats, conditions and reagents for DAR sensitivity
  – Demonstrate assays are DAR insensitive
    – Not always possible
    – Interpret data with knowledge that assays are DAR sensitive
  – Understand what you are measuring
    – Appropriate interpretation of results
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The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals