Development phase bioanalytical strategy approach to
Bioanalysis of ADCs from Discovery
to Late Non-clinical to Clinical support

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Antibody-drug conjugates: Combine selectivity and antitumor activity of a monoclonal Ab with the potency of a cytotoxic small molecule Drug

Goal: To deliver potent anti-cancer agent to tumor in targeted way with limited systemic exposure

Dissecting the attributes of an ADC

**Targets**
- Targeted recognition
- Abundant target expression and internalization

**Vehicles**
- Antibody

**Linkers**
- Stable in plasma
- Linker types: cleavable (cathepsin, pH etc.)
- or noncleavable (degradation)

**Payloads**
- Highly potent
  - Calicheamicin, Binds DNA
  - Maytansin, microtubule inhibitor
  - Auristatin, tubulin polymerization inhibitor

**Conjugation**
- Drug conjugation
- Linker conjugation
- Antibody conjugation
## Analytes Commonly Assessed for ADC Bioanalysis

Considerations for the bioanalysis of antibody drug conjugates (ADCs).
AAPS ADC working group position paper. Bioanalysis 2013

<table>
<thead>
<tr>
<th>Analyte type</th>
<th>Analyte(s) Details</th>
<th>Typical Analytical Method(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugated Antibody</td>
<td>Antibody with minimum of DAR equal or greater &gt; 1</td>
<td>LBA (LCMS)</td>
</tr>
<tr>
<td>Total Antibody</td>
<td>Conjugated, partially unconjugated and fully unconjugated (DAR equal or &gt; 0)</td>
<td>LBA (LCMS)</td>
</tr>
<tr>
<td>Antibody-Conjugated Drug</td>
<td>Total small molecule drug conjugated to antibody</td>
<td>Affinity LC-MS/MS</td>
</tr>
<tr>
<td>Unconjugated Drug</td>
<td>Small molecule drug not conjugated to antibody</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Anti-ADC Antibody</td>
<td>Antibodies directed against antibody component of ADC, linker or drug (binding/neutralizing)</td>
<td>LBA</td>
</tr>
</tbody>
</table>
Analytes Commonly Assessed for ADC PK

- Unconjugated Drug analyte
- Total Antibody analyte
- Conjugated Antibody analyte
- Conjugated Drug analyte

Total Ab LBA

Unconjugated Drug LCMS/MS

Conjugated Ab (ADC) LBA

Conjugated Drug LCMS/MS
Stage based development of ADCs

**Discovery**
- Candidate selection based on
  - Drug Metabolism?
  - Linker-drug stability?
  - ADC candidate efficacy / safety
  - Distribution

**Late phase non-clinical**
- PK-PD analysis
- Interpretation of GLP tox study data

**Clinical**
- Refining dose and schedule
- Good understanding of PK
- Dose correlation with Safety and Efficacy
- Understanding of Immunogenicity

**Submission**
- Correlation with Clinical signals – Exposure – Response relationship
- Deep Understanding of Clinical PK profiles
- Understanding of compound immunogenicity profile
Stage based questions for ADC bioanalysis

**Discovery**
- Limited reagent availability, high number of candidates - Use of generic assays
- Platform limitations – throughput

**Late phase non-clinical**
- Analyte / platform continuity with early Discovery BA / PK
- Type of studies and analytes to measure
- Assay formats – DAR sensitivity
- Reagents availability

**Clinical**
- Analytes to measure. Continuity with non-clinical
- Correlation of PK with Clinical signals - Selection of analytes
- Analytical platform continuity
- Immunogenicity

**Submission**
- Data merging to produce E-R relationship
- Data interpretation
- Deep understanding of Clinical PK profiles
- Compound immunogenicity profile
Assay transitions

- **Generic LBA** → **Specific LBA**
- **Generic LBA** → **Specific LBA**
  - Case-specific, often due to lack of specific reagents early
- **Specific LBA** → **Specific LBA**
- **Hybrid generic LCMS** → **Specific LBA**
- **Hybrid generic LCMS** → **Specific LBA**
  - Case-specific, often due to lack of specific reagents early
- **Hybrid specific LCMS**
Assays and Analyte collection transitions

- Discovery
- Late phase non-clinical
- Clinical
- Submission

Total Ab LBA

ADC LBA

Conjugated Drug

Unconjugated drug

Immuno-genecity
Unique LBA Challenges Posed by ADC

• What material should be used to create reference standard and QCs of the assay?
  – ADC?
  – Naked mAb?

• Based on earlier discussions with industry and regulatory – most appropriate reference material to be used for Total Ab and Conjugated Ab analytes – parental ADC reference material
Unique LBA Challenges Posed by ADC

• What assay format, assay conditions, critical reagents, analytical platform (ELISA, MSD, Gyros etc.) should be used?
  The answer depends on
  – Dose driven desired assay sensitivity
  – Throughput
  – Access to reagents and technology
  – Platform transitions during development
Unique LBA Challenges Posed by ADC

- Are there any pre-existing reactivity against any component of ADC they may interfere in both PK and ADA assays?
  - Be aware of potential anti-payload reactivity in drug naïve samples
  - Prepare for pre-existing compound reactive antibodies when patients had prior exposure to a similar biologic
- Be aware of potential circulating target interference. Although ADCs are mainly anti-cell surface targets, some targets may be shed
Unique LBA Challenges Posed by ADC

- Do assays need to be DAR dependent or independent?
  - The answer depends on the stage of development
  - Several teams have expressed interest in performing DAR sensitive assay early in compound development
Unique LBA Challenges Posed by ADC

- Lower dose - high desired assay sensitivity
- Plasma vs serum as preferred matrix for ADC bioanalysis
- Accumulation of naked Ab – impact on ADC detection?
- Impact of bioanalytical platform transitions
- Impact of changes of the regulatory landscape
- Impact of immunogenicity development

To be discussed later
Early Discovery ADC Assays

• Facilitate selection and ranking of optimal candidates that can progress to lead candidates with best safety and efficacy profile
  • PK comparison of ADC candidates with different linkers, small molecule drugs, antibodies, conjugation chemistry, conjugation site, and DAR
  • Total Ab Vs Conjugated Ab (or average DAR) PK profile- Indicator of in vivo ADC stability
  • Typically includes mouse efficacy, exploratory toxicology studies (ETS) in rodents/NHP, tissue PK and plasma stability
• Limited availability of critical reagents, well characterized reference standard (including DAR standards), lead time, and resources to develop/qualify assays for multitude of candidates
  • flexible “fit-for-purpose” assay strategy to measure what species are present
  • DAR-sensitive LBAs may better describe the changes in conjugated small molecule drug over time and associated PK parameters
Late Stage ADC Assays

- Define exposure-safety relationship of the selected lead candidate; Predict human PK; and Facilitate translation of non-clinical data to clinical outcomes
  - Single well defined ADC as oppose to multiple ADCs in discovery space
  - Typically includes IND-enabling toxicology studies in rodents/NHP
  - ADC-specific reagents and DAR standards are typically available
  - GLP bioanalytical validation must meet regulatory guidance
  - Assay continuity with the clinical assay
- DAR-insensitive LBAs may be needed because of limited current understanding on which DAR species can provide best correlation for exposure-response relationship for safety of ADCs
- Industry recommendations for regulated ADC assays
  - Evaluate DAR sensitivity of the assay using ADC preparations with individual purified (DAR 2, 4, 6, 8) or enriched DAR species
Example: Early Discovery Case Study

- PK measurements of:
  - Total Antibody (Gyros method)
  - Conjugated Antibody (Gyros method)
  - Conjugated Drug (ad hock due to time and resource use)
  - Anti-ADC antibody (ad hock due to value and resource use)
- Generic human IgG assays are often used
- DAR sensitivity is often questioned (asked for?) to understand DAR components impact on efficacy
- Transition from an Early Discovery to later phase method may require assay re-design, platform reconsideration
• DAR-sensitive assay aim to measure ADC concentration based on the number of small molecule drugs attached to the ADC
  • ideally a DAR-sensitive LBA would be equivalent to conjugated assay
• DAR-insensitive assay measures ADC concentration irrespective of the number of small molecule drugs attached to the ADC
  • measure various DAR components of the ADC equally, and are not biased towards the varying DAR values of the ADC
• DAR-sensitivity of LBA is governed by the critical reagents (capture and detection) and assay formats
  • binding of critical reagents to ADC may be hindered by solvent accessibility of conjugation site and/or due to steric hindrance from adjacently located drug
Antibody Drug Conjugate (ADC) Assays

DAR = Drug Antibody Ratio

Generic Assay
Capture: pAb anti-human IgG
Detect: mAb anti-human Fc

Capture: pAb anti-human IgG
Detect: anti-linker/payload

Detect: anti-antibody (unconjugated)

Total Antibody
DAR sensitive Conjugated Antibody
DAR insensitive Conjugated Antibody
DAR-sensitivity of ADC LBAs

Kumar et al., Bioanalysis 2015

**Total Ab Assay**

- **Detection** (Biotin-anti hlgG mAb/pAb)
- **Capture** (Antigen/anti-CDR mAb)
- ADC (DAR ≥ 0)

**Conjugated Ab Assay**

- **Detection reagent** (Biotin-anti-small molecule mAb or pAb)
- **Capture reagent** (Antigen, anti-id/anti-CDR mAb, antihuman mAb or pAb)
- ADC (DAR ≥ 1)

- **Detection reagent** (Biotin-Antigen, Biotin-anti-id/anti-CDR mAb, Biotin-antihuman mAb or pAb)
- **Capture reagent** (anti-small molecule mAb or pAb)
- ADC (DAR ≥ 1)
ADC-LP1 is composed of a humanized antibody, a hydrazone linker and DNA damaging cytotoxic small-molecule drug

- Conventional random conjugation chemistry; average DAR of ~ 4
- In discovery, non-validated fit-for-purpose LBA based conjugated Ab and total Ab PK assays were used to support mouse efficacy; and rodent and NHP ETS
  - Unconjugated small-molecule drug was measured by LBA and LC/MS
- For regulated toxicology studies (GLP), validated LBA based conjugated Ab and total Ab PK assays were used
- Unconjugated and conjugated small-molecule drug was measured by LC/MS
ADC-LP1: Discovery Vs GLP LBA Assay Formats

**Discovery**
- **Total Ab (DAR ≥ 0)**
  - Biotin anti-human IgG pAb
  - ADC (DAR ≥ 0)
  - Recombinant Target Protein

**Conjugated Ab (DAR ≥ 1)**
- Biotin Rabbit anti-Calicheamicin pAb
  - ADC (DAR ≥ 1)
  - Recombinant Target Protein

**GLP**
- **Biotin anti-human LC mAb**
- ADC (DAR ≥ 0)
- Recombinant Target Protein

- **Anti-Rabbit-Ru**
- Rabbit Anti-Calicheamicin pAb
- ADC (DAR ≥ 1)
- Recombinant Target Protein
ADC-LP1: DAR-sensitivity of Total Ab Assay

- Change in detection reagent from anti-human IgG pAb to anti-human LC specific mAb significantly improved recovery of unconjugated Ab against ADC reference standard curve: DAR-insensitive Total Antibody Assay

<table>
<thead>
<tr>
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<th>Discovery</th>
<th>GLP</th>
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<tbody>
<tr>
<td>QC Samples</td>
<td>Accuracy (%RE) using anti-human IgG pAb detection reagent</td>
<td>Accuracy (%RE) using anti-human LC mAb detection reagent</td>
</tr>
<tr>
<td>HQC - ADC</td>
<td>13 %</td>
<td>8.0 %</td>
</tr>
<tr>
<td>MQC - ADC</td>
<td>7.0 %</td>
<td>-2.0 %</td>
</tr>
<tr>
<td>LQC - ADC</td>
<td>8.0 %</td>
<td>2.0 %</td>
</tr>
<tr>
<td>HQC - Unconjugated Ab</td>
<td>130 %</td>
<td>-5.0 %</td>
</tr>
<tr>
<td>MQC - Unconjugated Ab</td>
<td>70 %</td>
<td>-10 %</td>
</tr>
<tr>
<td>LQC - Unconjugated Ab</td>
<td>17 %</td>
<td>0.5 %</td>
</tr>
</tbody>
</table>
Change in assay format showed no differences in DAR sensitivity
Discovery assay format was thus continued for GLP support but using MSD platform

Kumar et al., Bioanalysis 2015 7(13), 1605-1617
ADC-LP1: Discovery (ETS) vs GLP TK Data

- Similar assay formats/assay reagents provided relative consistency in ETS Vs GLP PK profile and PK parameters

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<thead>
<tr>
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<th>Cmax (µg/mL)</th>
<th>AUC (µg•h/mL)</th>
</tr>
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<tbody>
<tr>
<td>ETS TAb</td>
<td>9.6 ± 2.0</td>
<td>820 ± 150</td>
</tr>
<tr>
<td>ETS ADC</td>
<td>12 ± 3.0</td>
<td>660 ± 92.0</td>
</tr>
<tr>
<td>GLP TAb</td>
<td>6.4 ± 1.3</td>
<td>590 ± 70.0</td>
</tr>
<tr>
<td>GLP ADC</td>
<td>6.2 ± 1.2</td>
<td>530 ± 120</td>
</tr>
</tbody>
</table>

Courtesy: Frank Barletta
Case Study 2: ADC LP2

- Humanized IgG1 antibody conjugated via cysteine residues to a tubulin inhibitor with a maleimidocaproyl (mc) linker
- Average DAR of ~ 4
- In discovery fit-for-purpose LBA based Conjugated Ab and Total Ab PK assays used to support mouse efficacy, rat/cynomolgus monkey ETS
- Unconjugated cys-mc-MMAF was measured by LC/MS
- In Regulated toxicology studies, used validated LBA based Conjugated Ab and Total Ab PK assays
Change in assay format showed significantly improved recovery of individually purified DAR species against ADC reference standard curve: DAR-insensitive Conjugated Antibody Assay
Case Study 2: ADC LP2

- Different assay format with relatively different DAR sensitivity.
- Limited impact on the observed ETS vs GLP PK profile and PK parameters
  - In ETS (DAR sensitive) assay format, under-recovery of low DAR species is potentially compensated by over-recovery of high DAR species

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<th>Cmax (µg/mL)</th>
<th>AUC (µg•h/mL)</th>
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<tbody>
<tr>
<td>ETS TAb</td>
<td>280 ± 44.0</td>
<td>25000 ± 3400</td>
</tr>
<tr>
<td>ETS ADC</td>
<td>370 ± 29.0</td>
<td>20000 ± 1300</td>
</tr>
<tr>
<td>GLP TAb</td>
<td>280 ± 50.0</td>
<td>25000 ± 2900</td>
</tr>
<tr>
<td>GLP ADC</td>
<td>240 ± 45.0</td>
<td>15000 ± 1800</td>
</tr>
</tbody>
</table>
Case Study 3: Discovery and Regulated Assays: Impact on PK

- Different elimination slopes observed depending on the assay format
- Potential interference by shed target in specific assay format.
- Target or ADA interference may not be visible in the generic assay?

Courtesy: Eugenia Kraynov
Case Study 3: Discovery and Regulated Assays: Impact on PK

- Specific capture assay format is desirable for consistency between GLP and clinical study
- Different assay format and assay reagents provided some observed differences in non-reg vs GLP PK profiles but no significant change in PK parameters

Courtesy: Eugenia Kraynov
PK measurements of:
- ADC – brentuximab vedotin antibody-drug conjugate
- MMAE – monomethyl auristatin E (released small molecule)
- TAb – total antibody (ADC plus unconjugated cAC10 antibody)
- Anti-ADC antibody response

Analysis was performed to determine the relationship between ADC and MMAE exposure and response

“Anti-ADC antibody titer by treatment cycle was a statistically significant covariate for brentuximab vedotin clearance, but the difference was small”


Using trough concentrations, safety and efficacy were correlated to the exposure of the Conjugated Antibody
- Probability of overall response rate
  - increases with increasing ADC Ctrough (left)
  - decreases or flattens with increasing MMAE Ctrough (right)
Thank you!