2015 ASMS Regulated Bioanalysis Interest Group (RBIG) Workshop Report

Topic: Ligand Binding Assays (LBA) and LC-MS/MS Integrated Antibody-Drug Conjugate (ADC) Bioanalysis - Immuno-capture LC-MS/MS Hybrid Assays: Challenges, Solutions, and Complementarity with LBA

June 3rd, Wednesday, 2015
Presiding: Jian Wang (BMS)
Panelists: Keyang Xu (Genentech), Shawna Hengel (Seattle Genetics), Moucun Yuan (PPD), Aihua Liu (Tandem), Ryan Gu (BMS)
Attendance: Approximately 100

Outline of the workshop:

Antibody-drug conjugates (ADCs) consist of a cytotoxic drug covalently bound to an antibody (mAb) via a linker. The complex structure of ADCs presents unique bioanalytical challenges and requires novel strategies. Multiple analytes in the heterogeneous mixture may contribute to the efficacy and safety of ADCs. Four quantitative assays are considered essential, industry-wide, in ADC PK bioanalysis, (1) total-antibody, (2) conjugated-antibody, (3) conjugated-payload, and (4) unconjugated-payload. Immuno-capture LC-MS/MS hybrid assays are required for the analysis of conjugated-payload and are viable alternatives or complementary to ligand binding assays (LBA) for the analysis of total-antibody and conjugated-antibody.

Each hybrid assay involves three essential experimental steps: capture, enzymatic cleavage or digestion, and LC-MS/MS detection. After the initial immuno-capture of the ADC, the conjugated-payload assays proceed with the cleavage of the payload using Cathepsin B enzyme and LC-MS/MS measurement of the released payload, while the conjugated-antibody and total-antibody assays measure the signature peptides generated by trypsin digestion of the mAb. Immuno-capture with either anti-id or anti-payload capture reagents could be conducted using magnetic beads or cartridges formats.

This workshop focused on current hybrid assay strategies, applications, and their complementarity to ligand binding assays. Technical details of hybrid assay development and validation were discussed. The capability of hybrid assays to appropriately quantify mixtures of analytes with different Drug to Analyte Ratio (DAR) was addressed as well.

Topics discussed at the workshop:

1. What assays/analytes are required in preclinical and clinical PK studies?
2. How do you use the *in vivo/ex vivo* DAR distribution information to guide the PK assays development?
3. What hybrid assays are you conducting and how are the results used in PK modeling?
4. What is your experience about correlation between LBA and Hybrid assays for ADC PKs?
5. What regulatory guidelines do you follow for the hybrid assays?
6. DAR sensitive conjugated-antibody vs. conjugated-payload assays?
7. Comparison of magnetic beads vs. cartridges for immuno-capture?
8. Comparison of generic vs. specific capture reagents?

9. How will you construct a new ADC bioanalytical group in Discovery and Development if you have the opportunity?
10. Should we continue the topic on ADC in next year's Regulated Bioanalysis Workshop at ASMS?

Summary of panel discussion:

Both conjugated-antibody and conjugated-payload represent the active ADC. They describe the ADCs from two different perspectives, antibody or payload. Because of few ADCs are on the market and there is limited clinical information on ADCs, it is unclear whether conjugated-antibody or conjugated-payload correlates more closely to efficacy or toxicity or is ADC dependent. The conjugated-antibody assay measures the antibody conjugated to at least one payload and, by definition, it should be independent of the number of payloads, i.e., DAR independent. The conjugated-payload assay on the other hand provides direct information of drug load and, by definition, is proportional to the number of payload or DAR.

Historically, the primary measurement of total-antibody and conjugated-antibody use ligand binding assay (LBA) technologies while the unconjugated-payload is LC-MS/MS based. The conjugated-payload is predominately developed as a ligand binding and LC-MS/MS hybrid assay (hybrid assay). Meanwhile, based on the collective industry experience total-antibody and conjugated-antibody can also be measured by immuno-capture LC-MS/MS hybrid assays as the orthogonal methods or the alternatives to the LBAs. Hybrid assays are capable of supporting ADC PK studies. The industry-wide ADC bioanalysis strategy continues to evolve and will need to be refined as more is understood and new generations of ADCs are coming through the pipeline.

There was intensive discussion on reducing the number of assays and whether both conjugated-antibody and conjugated-drug/payload need to be analyzed. Both panelists and audience expressed that there is a trend to use conjugated-drug/payload assay to characterize the active ADC component. It is worth of bringing this topic to wider combined audiences of LBA and LC-MS scientists.

Perspective for future RBIG workshops at ASMS:

The Pharmaceutical Interest Group held the workshop "MS analysis of Antibody-Drug Conjugates" at the 2015 ASMS conference on Tuesday June 2nd. Pharmaceutical analysis of ADC is focusing on characterization of the drug substances and products such as determining nominal DARs and stability of ADCs in neat and dosing solutions. ADC bioanalysis handles *in vivo* PK samples and faces the challenges of *in vivo* DAR distribution change of ADCs. Though there are no extensive overlaps between ADC pharmaceutical analysis and ADC (PK) bioanalysis task wise combining workshops from Pharmaceutical and Regulated Bioanalysis Interest Groups on ADCs to include both analysis and bioanalysis of ADC in future ASMS conferences will be considered.