

Antibody-Drug Conjugates (ADC) –A Complex Problem in Regulated Bioanalysis

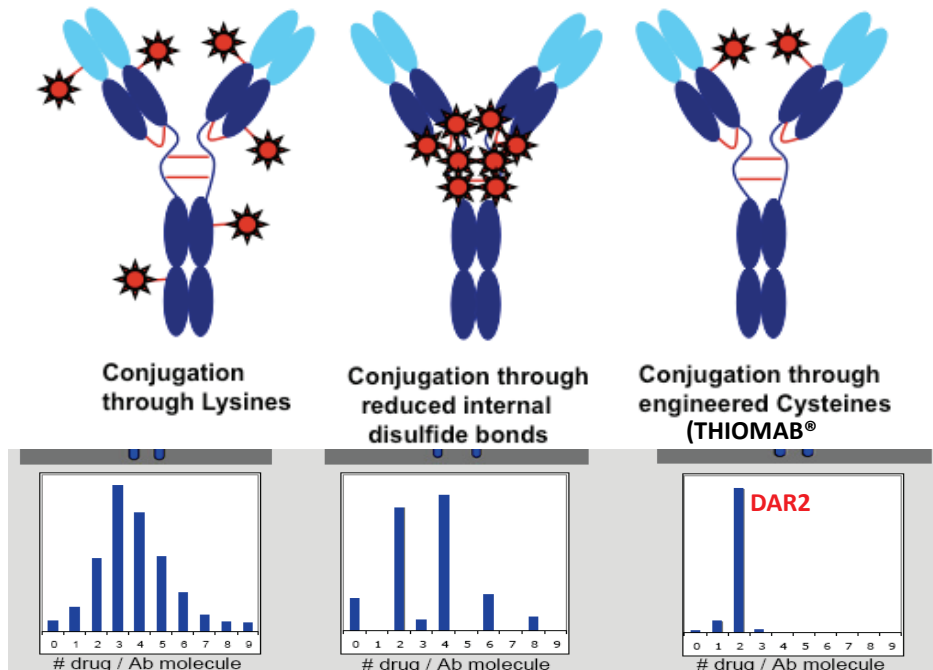
June 17, 2014

- Presiding: Dr. Keyang Xu (Genentech) and Dr. Fabio Garofolo (Algorithme Pharma)
- Attendance: approximately 300 people.
- Panelists: Dr. Keyang Xu (Genentech); Dr. Jian Wang (BMS); Mr. Rand Jenkins (PPD)

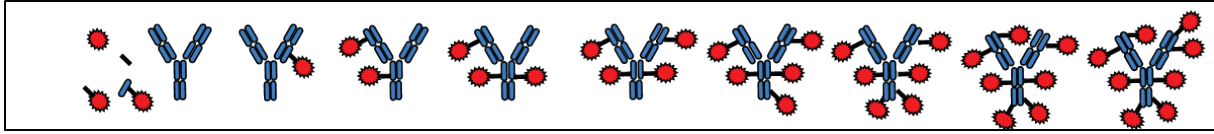
Antibody Drug Conjugate (ADC): A Combination of Small and Large Molecules for Targeted Drug Delivery

ADC are molecules that are developed to specifically deliver cytotoxic payload to tumor cells and minimizes systemic toxicity.

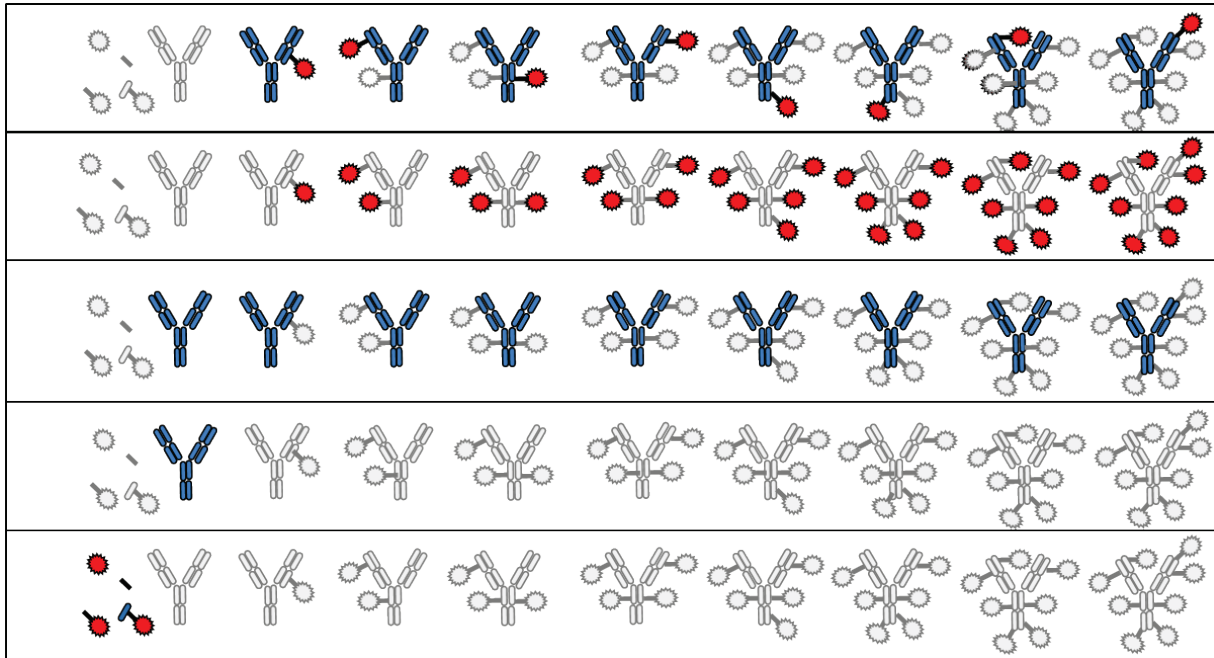
ADC are complex mixtures and require multiple bioanalytical methods for full characterization. Additionally, ADC complexity can also be generated *in vivo*, affecting efficacy and safety, as well as presenting new bioanalytical challenges.



Many Potential Analyte Choices for ADCs



Conjugated Ab, Ab-conjugated Drug, Total Ab, Naked Ab or Unconjugated Linker, Drug



S. Kaur et al., *Bioanalysis* (2013)

Bioanalytical Strategy for ADC PK:

- Immunoassays: measure antibodies (total Ab, conjugated Ab, immunogenicity)
- LC-MS/MS: measure payloads (conjugated drug, unconjugated drug, SM catabolite/metabolite)
- Other assays: structural characterization (DAR)

The biggest challenges -

- Heterogeneity of the ADC reference material containing molecules with different DAR's (Drug-to-Antibody Ratios)
- Changes in ADC composition in biological samples *in vitro*, and especially, *in vivo*
- Assessment of the ELISA performance, e.g., equivalent recovery of all DAR species
- Availability and characterization of the DAR reference materials

Some Specific Challenges (Examples)

Payload assays related -

- Co-existing ADCs (also representing conjugated payloads) and unconjugated payloads *in vivo* are vastly different (3-4 order of magnitude or even higher)

- Significant challenge for *in vivo* sample collection and preparation
- Acceptance criteria for payload stability should be broader than for SM's? ISR criteria?

ADC assays related -

- Measuring ADC conjugates by ELISA or LCMS?
- Selectivity of LBAs for ADC conjugates may be DAR dependent
 - Biased recovery with different DAR's
 - Some DAR reference materials may be structurally different from that formed *in vivo*

General discussion -

- Analyzing serum, plasma or both?
- Any changes in assay strategy when analyzing ADCs at different stages of drug discovery vs. development (preclinical vs. clinical)?

Panel Discussion:

Some people in the room already started working on regulated bioanalysis for ADC.

Question period:

What are the differences between an ADC and a monoclonal antibody (mAb)?

For the ADC vs the mAb, you have to consider the small molecule conjugated payload attached to the mAb. For the unconjugated payload present in matrix, a quantitation using a separation from the mAb by protein precipitation or any other type of sample preparation, filtration or immunocapture is usually done. However, it is important to avoid breaking the ADC during the sample preparation to avoid any bias during the quantitation of the unconjugated payload.

The concentration of the unconjugated payload is 3-4 orders of magnitude lower than the ADC. Therefore, the stability evaluation could be challenging due to impurities present in the reference material or a slight unconjugation process occurring during the sample preparation which could lead to stability failure.

Some pharma such as BMS and Seattle Genetics which are assaying ADC are currently including two sets of QCs in their assay for the unconjugated payload; one with the payload only and one with the payload+ the total ADC at the cmax concentration.

What are the challenges for sample processing and stabilities during processing?

The stability will be depending on the analyte that is conjugated with the mAb.

For unconjugated payload, protein precipitation is usually used. For total antibody, enzymatic digestion followed by quantifying based on a signature peptide is usually done. Need to understand what should be measure to evaluate appropriate stabilities.

As of now, limited knowledge on ADC is available on the stability and how the sample collection should be done to ensure stability. It is important to know exactly what needs to be measured when developing assays for ADC.

How the ratio of free drug should be evaluated?

It should be evaluated in molar ratio. It seems that Seattle Genetics may be using the rule that up to 2% of the free drug can be present for specific drugs.

As the some toxicity may be mediated by the unconjugated payload, reporting the accurate concentration of the free payload is very important for regulatory agencies.

Also, the stability of the ADC will be dependant of the linker. Some linker can be more sensitive to different condition such a pH modification.

Is there a consensus on the methodology to use for each analyte quantification?

-Total antibody: Concentration of the circulation antibody: ELISA or LC-MS/MS

-Conjugated antibody/: Critical analyte to measure the efficacy: ELISA (some DAR species can be more difficult to detected. Recommend to check if anti-drug Ab is capable of recognising all DARs)

- Conjugated payload: typically LC-MS/MS

-Unconjugated payload: LC-MS/MS.

Hybrid assays can be used also.

What kind of standard curve is used?

-RSM material: ADC in plasma or serum

Some pharma are using absolute concentration in ng/mL and some are using molar ratio. Molar ratio should be better. The DAR ratio needs to be taken into consideration for the concentration determination.

Can MALDI/TOF be used for DAR determination?

Usually ESI/TOF is used. Some preliminary data suggests that MALDI/TOF may be used for the DAR distribution profile, but resolution can be a concern.

Can we use the orbitrap to evaluate the DAR distribution? In spectra, you can look at the heavy and light chain. Thermo is using the orbitrap to quantitate ADC.

What mass range should you expect to detect the charge envelopes of the ADC?

Intact - m/z: 2000-3500

Heavy chain- m/z 800 – 2000

Light chain: 300-800