Characterization of Pharmacokinetic Properties for a Site-Specific Antibody Drug Conjugate (ADC) Using Multiple-Platform Bioanalysis Assays

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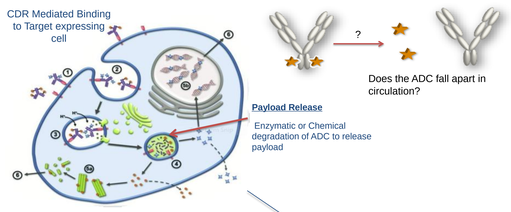
Introduction

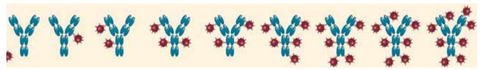
ADC: Mechanism of Action and PK Questions



Modified from Thudium et al., Mabs 2012.

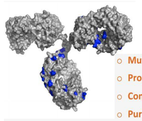
Site Specific Conjugation

Standard conjugation techniques yield a heterogeneous mix of ADCs



Adapted from Kaur et al. (2014)

Site-specific conjugation Advantages:
- Specific location of payloads
- Precise drug/antibody ratio (DAR)

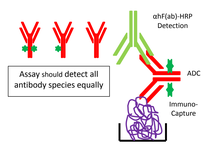


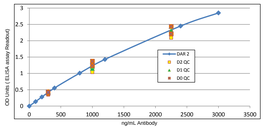
- Mutate Sites in IgG
- Produce nnAA IgGs
- Conjugate
- Purify
- Test

Methods

Multiple Platform Bioanalytical Assays to Assess Different PK Properties of ADC

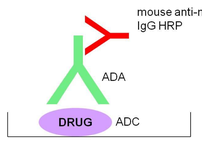
Total Antibody Assay by ELISA





Range: 100-3000 ng/mL; in Monkey Serum

Antidrug Antibody (ADA) Assay by ELISA

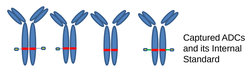


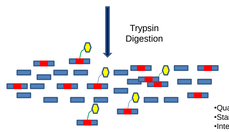
- Capture reagent: ADC
- Testing sample: predose and last sample(2% serum)
- Detection reagent: mouse anti-monkey IgG HRP (1:12000 dilution)
- Substrate: TMB (3,3', 5,5''-tetramethylbenzidine)

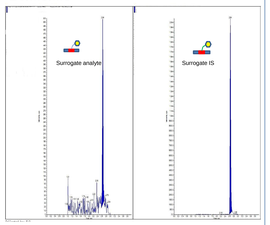
Methods (cont'd)

Total Conjugated Drug Assay by Hybrid IP-LC/MS/MS

Immuno-capture of human antibody from monkey serum samples using biotinylated anti-Human Fc antibody; then pulled down by Streptavidin coated magnetic beads







- •Quantitation of peptide containing the tryptic peptide-linker-toxin complex
- •Standard curve range 100 to 25000 ng/mL of ADC (DAR-2) in monkey serum
- •Internal Standard , an analog ADC with a slightly different linker, was spiked at the beginning of Immunocapture

Automated Sample Preparation for Total-Conjugated Drug Assay

Hamilton STAR on Day 1
- •STD/QC preparation
- •STD/QC/ sample aliquot
- •IS addition
- •Addition of immuno - capture reagent

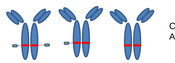
→ •Incubate overnight at 4°C

Hamilton STAR on Day 2
- •Add beads
- •Incubate 1hr at RT on deck
- •Wash beads multiple times (3X)
- •Shake plate between washes
- • Elute from beads
- • Reduction and Alkylation
- • Add Trypsin

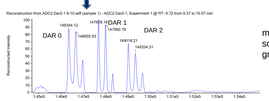
→ Incubate overnight at 37°C → LC-MS/MS

High Resolution MS for Drug-Antibody Ratio (DAR) Analysis

Immuno-capture of human antibody from monkey serum samples using biotinylated anti-Human Fc antibody; then pulled down by Streptavidin coated magnetic beads



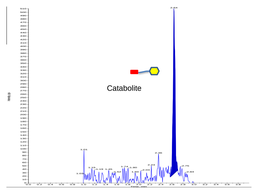
Drug-Antibody Ratio (DAR) measurement was conducted using high-resolution mass spectrometry (LC/HR-MS) on a Shimadzu HPLC coupled with Sciex 5600 Q-TOF.



m/z spectrum of each sample was de-convoluted by software (BioPharmaView) to produced mass distribution graphs of intact ADC showing DAR distribution

Catabolite Assay by LC-MS/MS

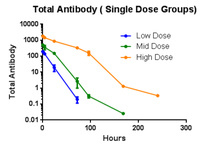
- •Catabolite is defined as the active species that is released from the ADC, upon internalization
- •The Catabolite generated from our ADC (non-Cleavable linker) is : NN-aa + Linker + Toxin
- • Requires very low LLOQ due to low catabolite levels, but highly toxic
- •LLOQ=1 ng/mL

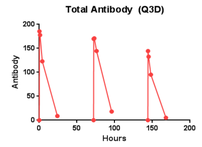


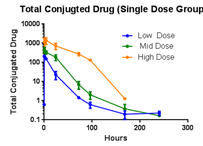
Results

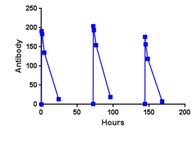
Exploratory Toxicity Study in Monkey

- • Study includes single dose and multi-dose arms
- • Approximately 230 serum samples need to be analyze for
  - Total Antibody (Elisa)
  - Total Conjugate drug
  - DAR
  - Catabolite
  - ADA (Elisa)
- •Quick turn around time to support program decision



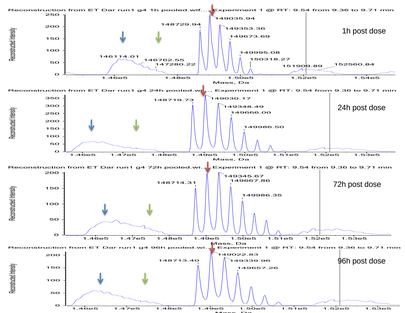






Conjugated drug is defined as drug that is still linked to the antibody. Concentrations of conjugated drug were much higher (over 1000 fold) than concentrations of released catabolite. Conjugated drug is typically a driver of efficacy, while free catabolite or toxin in circulation is a driver of non-target mediated toxicities.

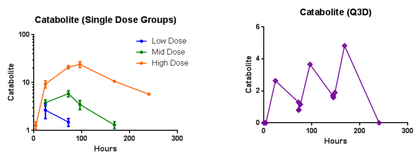
Drug-Antibody Ratio Analysis



All human antibodies circulating in monkey serum were DAR 2 at all time-points tested. Samples collected from high dose animals were tested up to 96 hours post dose

Each DAR species is indicated by the colored arrows

Dar0
Dar1
Dar2



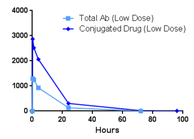
Catabolite (released by cells via Ab digestion) concentrations are in the low ng/mL range in circulation. These concentrations are in the range of highly stable ADCs, and expected to be relatively 'safe'
No free toxin was detected in circulation (data not shown). Free toxin is expected only if the toxin 'falls-off' during circulation, due to linker instability, or if there was free toxin contamination in the dosing material

Antidrug Antibody (ADA)

Pre-dose samples were ADA negative; No ADA was detected in samples collected prior to Day 10
Animals dosed with ADC were ADA positive on Day 29. The Presence of ADA may have an impact PK parameters

Discussion

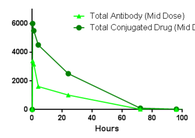
Comparing Total-conjugated Drug and Total Antibody in Circulation

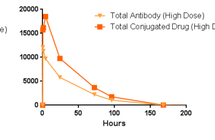


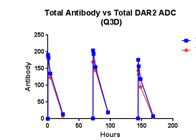
Molar ratio of

$$\frac{\text{Total-conjugated-drug}}{\text{Total-antibody}} \approx 2$$

at all time-points on the PK curve and at all doses in the monkey study, indicating a DAR 2 is maintained.







DAR-adjusted to show overlapping of total conjugate and total antibody in multiday arm

We have demonstrated that the ADC is stable in circulation, and the drug : antibody ratio (DAR) of 2 is maintained throughout. There is no detectable de-conjugated ADC in circulation.

Conclusions

Concentration – time profiles for total conjugated drug correlated well with those from total antibody assays, suggesting that this site-specific ADC candidate is stable in circulation.

DAR measurement confirmed in vivo linker stability and maintenance of drug antibody ratio (DAR) of 2 - Both ELISA- and MS-based assays performed reliably during sample analysis, with the consistent results among multiple assays (from multiple analytical platforms).
The ADC drug compound was stable in circulation for at least up to the 10 days tested.

Concentrations of free catabolite in circulation were minimal, less than 5 ng/mL at low or mid dose and less than 25 ng/mL at high dose. No detectable free drug/toxin, or other drug containing moieties in circulation.

All animals that survived past 2 weeks were identified as positive for the presence of anti-drug antibodies

The total conjugated antibody assay utilizes robust tryptic digestion and provides a highly reproducible DAR-bias-free assay. It's internal standard undergoes all steps including immunocapture and tryptic digestion which increases reproducibility and robustness of the assay

Total antibody, total conjugated drug, DAR, catabolite and immunogenicity assays collectively provided comprehensive PK data to support the multi-phased exploratory monkey tox study.

Acknowledgments

Joseph Piccotti and Nate Collins : toxicology study design and conduct
Eric Schwartz, Xiao-Ping Dai for reagents. Jason Kahana for anti-toxin antibody.
Jason Kahana and the ADC team
Steve Maxwell, and Gondi Kumar for discussions and resources

‡ : Author contributed independently for each of the five assays