Challenges and opportunities in developing a sound bioanalytical strategy for PK assessment of Antibody Drug Conjugate Therapeutic

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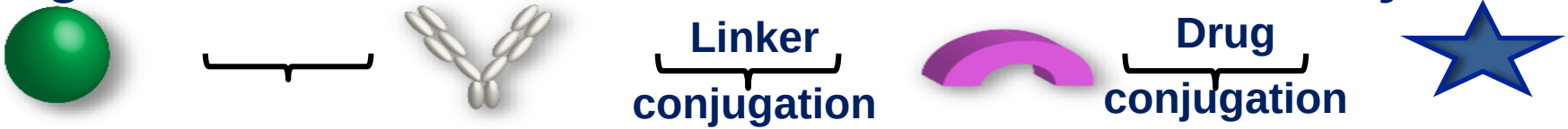
Pfizer WORLDWIDE RESEARCH & DEVELOPMENT

Dissecting the attributes of an ADC

- **Antibody-drug conjugates**: Combine selectivity and antitumor activity of a monoclonal Ab with the potency of a cytotoxin small molecule Drug
- **Goal**: To deliver potent anti-cancer agent to tumor in targeted way with limited systemic exposure
- ADCs are often heterogeneous and contain a mixture of mAb-$(Drug)_n$ conjugates with various loading (n)
- Many conjugation chemistries => variation in linker stability => variation in heterogeneity

Targets **Vehicles** **Linkers** **Payloads**



Antibody

- Targeted recognition
- Abundant target expression and internalization

Linker

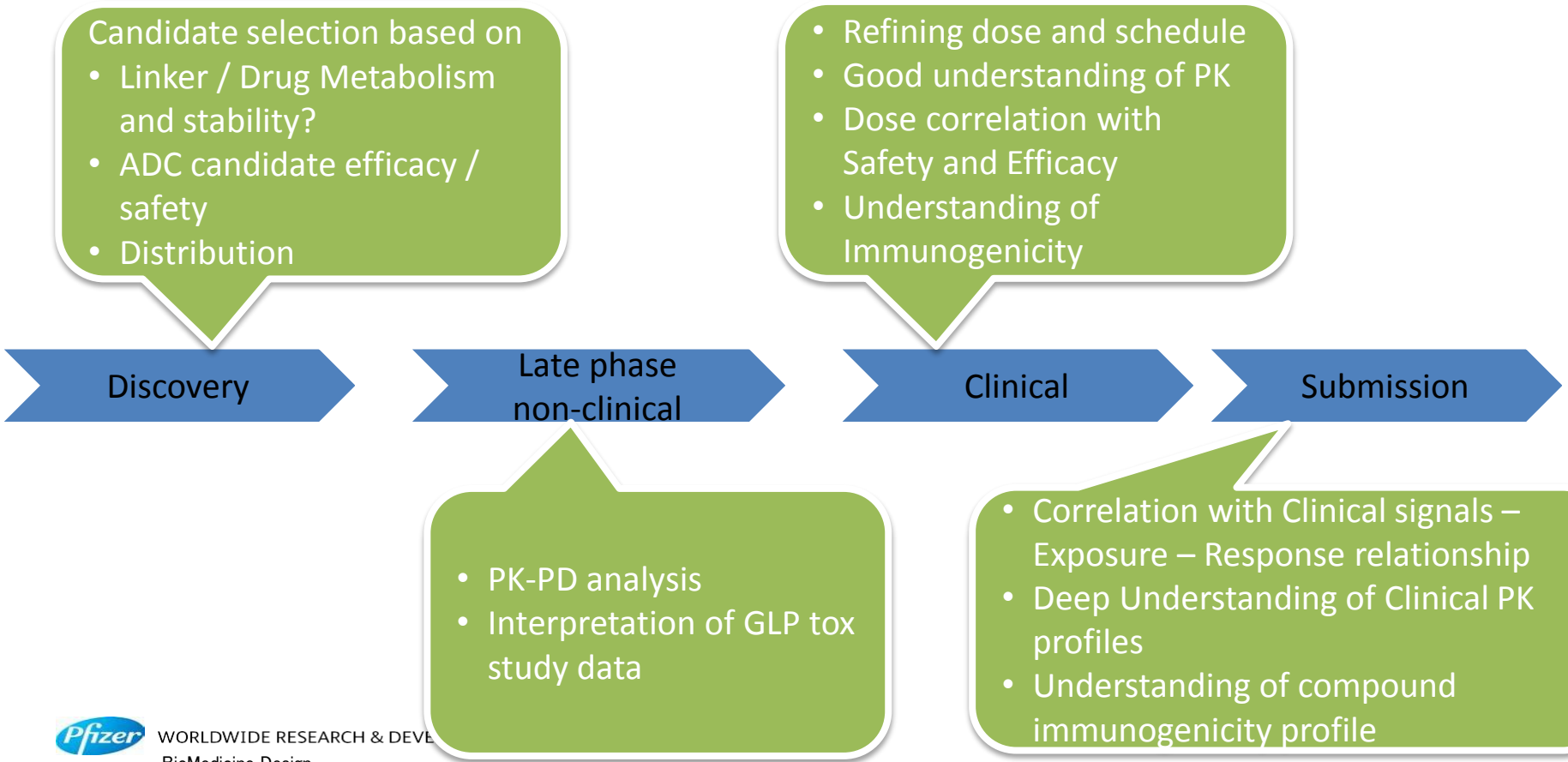
- Stable in plasma
- Linker types: cleavable (cathepsin, pH etc.)
- or non-cleavable (degradation)

Drug

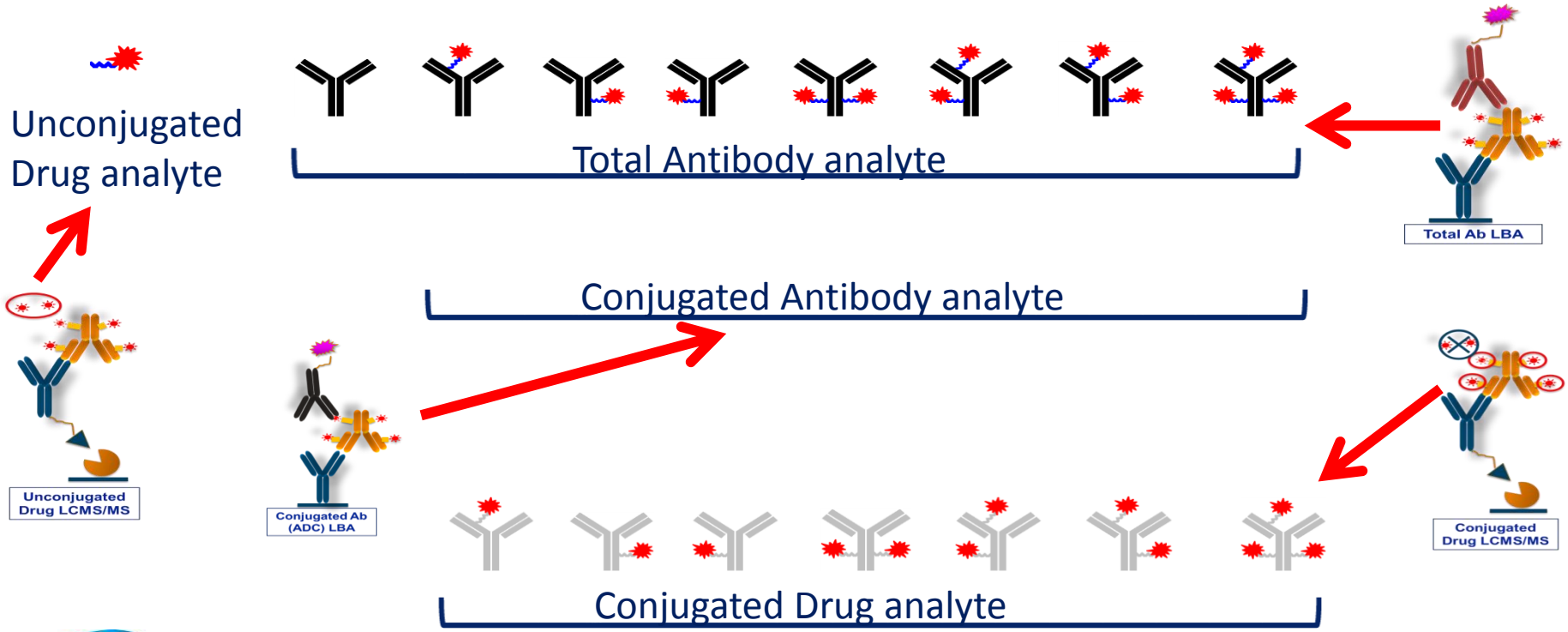
- Highly potent

Calicheamicin, Binds DNA
Maytansin, microtubule inhibitor
Auristatin, tubulin polymerization inhibitor

Stage based development of ADCs



Analytes Commonly Assessed for ADC PK



Analytes Commonly Assessed for ADC Bioanalysis

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

Analyte type	**Analyte(s) Details**	**Typical Analytical Method(s)**
Conjugated Antibody	Antibody with minimum of DAR equal or greater > 1	LBA (LCMS)
Total Antibody	Conjugated, partially unconjugated and fully unconjugated (DAR equal or > 0)	LBA (LCMS)
Antibody-Conjugated Drug	Total small molecule drug conjugated to antibody	Affinity LC-MS/MS
Unconjugated Drug	Small molecule drug not conjugated to antibody	LC-MS/MS
Anti-ADC Antibody (immunogenicity)	Antibodies directed against antibody component of ADC, linker or drug (binding/neutralizing)	LBA

Assays and Analyte collection transitions

Total Ab LBA

ADC LBA

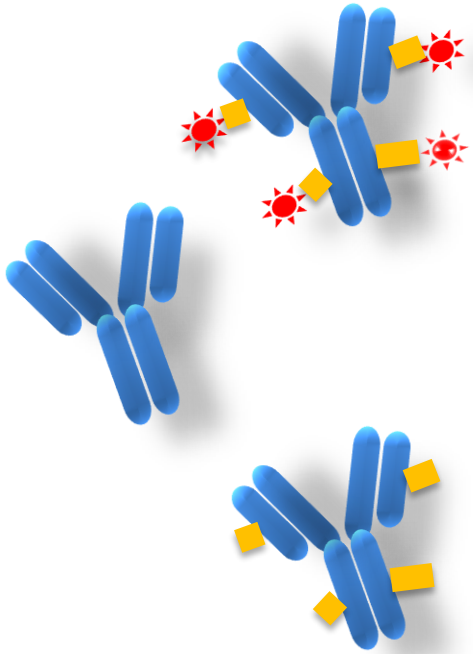
Conjugated Drug (Hybrid LCMS)

Unconjugated drug (LCMS)

Immunogenicity

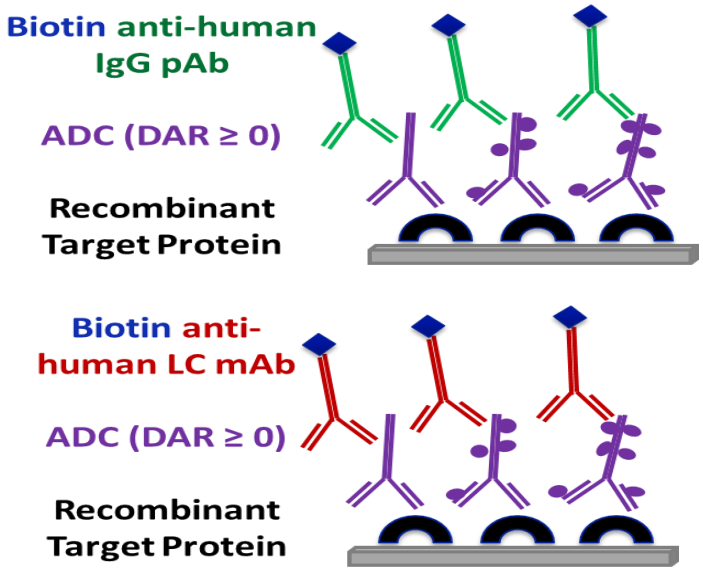
Unique LBA Challenges Posed by ADC

- What **reference material** should be used to create reference standard and QCs of the assay?
  - ADC? Naked mAb?
- Based on industry and regulatory feedback – **parental ADC reference material** is most appropriate for Total Ab **and** Conjugated Ab analytes
- What assay format, assay conditions, critical reagents, analytical platform (ELISA, MSD, Gyros etc.) to use?
- The answer depends on
  - Dose driven desired assay sensitivity
  - Throughput
  - Access to reagents and technology
  - Expected platform transitions during development



Unique LBA Challenges Posed by ADC

- Do assays need to be Drug Antibody Ratio (DAR) sensitive or insensetive?
  - The answer may depend on the stage of development
  - Several teams have expressed interest in performing *DAR sensitive* assay early in compound development



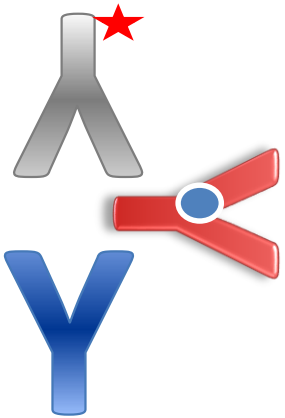
DAR Sensitivity of LBA for ADCs

- **DAR-sensitive** assay aims to measure ADC concentration based on the number of small molecule drug moieties attached to the ADC
  - ideally a DAR-sensitive LBA would be equivalent to conjugated drug assay
- **DAR-insensitive** assay measures ADC concentration irrespective of the number of small molecule drug moieties attached to the ADC
  - measures various DAR components of the ADC equally, not biased to the changing DAR value of the ADC while in circulation
- DAR-sensitivity of LBA is **governed by the critical reagents** (capture and detection) and assay format
  - binding of critical reagents to ADC may be hindered by solvent accessibility of conjugation site and/or due to a steric hindrance from adjacently located drug moiety

Antibody Drug Conjugate (ADC) Assays

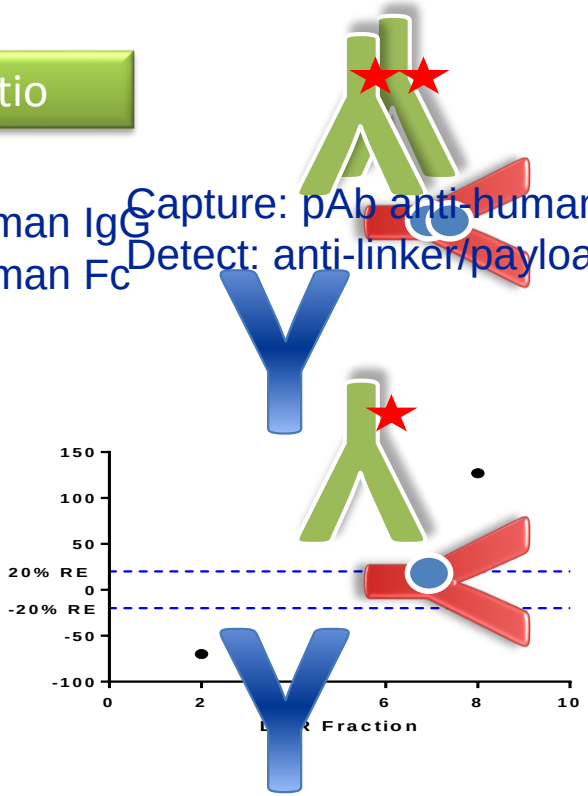
DAR = Drug Antibody Ratio

Generic Assay
Capture: poly-Ab anti-human IgG
Detect: mono-Ab anti-human Fc



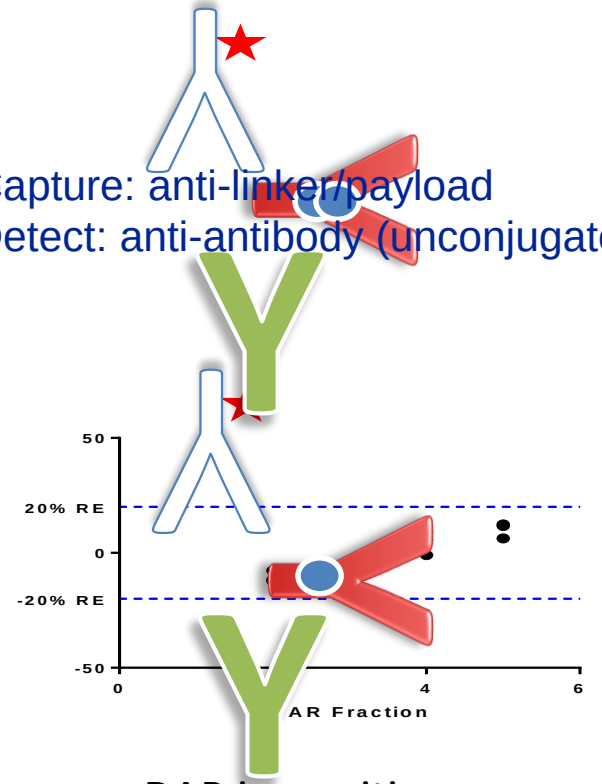
Total Antibody

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



DAR sensitive
Conjugated Antibody

Capture: anti-linker/payload
Detect: anti-antibody (unconjugated)



DAR insensitive
Conjugated Antibody

DAR-sensitivity of ADC LBAs

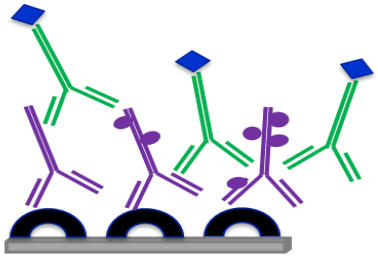
Kumar *et al*., Bioanalysis 2015

Total Ab Assay

Detection
(Biotin-anti hIgG mAb/pAb)

ADC (DAR≥0)

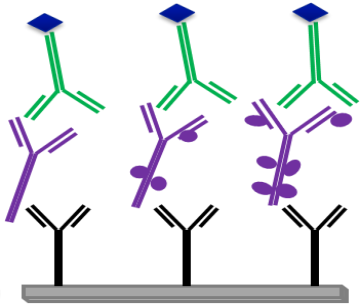
Capture
(Antigen/anti-CDR mAb)



Detection
(Biotin-anti hIgG mAb/ pAb)

ADC (DAR ≥ 0)

Capture
(Anti hIgG mAb/pAb)

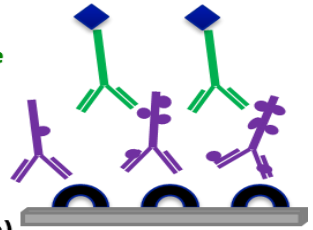


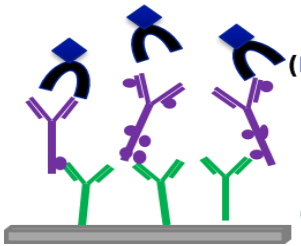
Conjugated Ab Assay

Detection reagent
(Biotin- anti-small molecule mAb or pAb)

ADC (DAR≥1)

Capture reagent
(Antigen, anti-Id/anti-CDR mAb, antihuman mAb or pAb)





Detection reagent
(Biotin-Antigen, Biotin-anti-Id/anti-CDR mAb, Biotin-antihuman mAb or pAb)

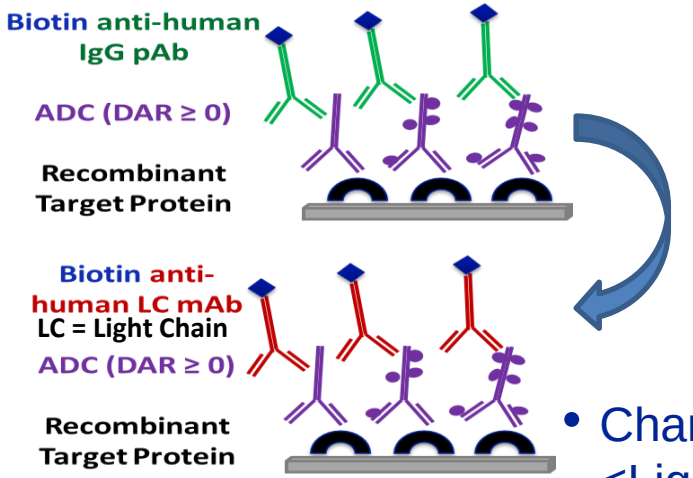
ADC (DAR≥1)

Capture reagent
(anti-small molecule mAb or pAb)

Case Study 1: ADC-LP1

- ADC-LP1: composed of humanized antibody, hydrazone based linker and DNA damaging cytotoxic small-molecule drug
- Conventional random conjugation chemistry; average DAR of ~ 4
- In discovery: fit-for-purpose LBA based conjugated Ab and total Ab PK assays were used to support mouse efficacy and rodent and NHP exploratory tox (ETS)
  - Unconjugated small-molecule drug was measured by 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

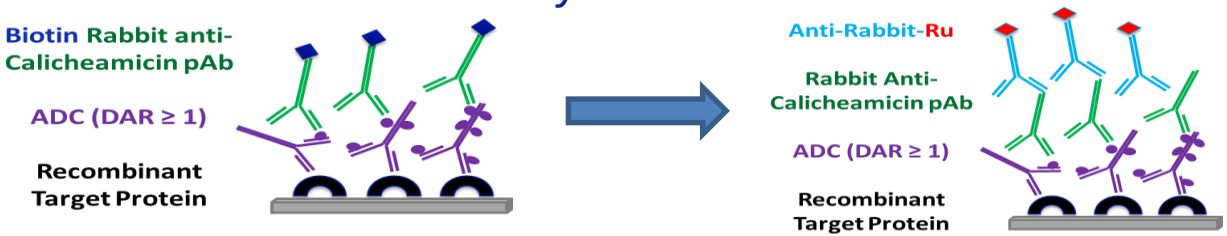
ADC-LP1: Discovery vs. GLP LBA Assay Formats
DAR-sensitivity of Total Ab Assay



	Discovery	GLP
QC Samples against ADC reference standard	Accuracy (%RE) using anti-human IgG pAb detection reagent	Accuracy (%RE) using anti-human LC mAb detection reagent
HQC - ADC	13 %	8.0 %
MQC - ADC	7.0 %	- 2.0 %
LQC - ADC	8.0 %	2.0 %
HQC - Unconjugated Ab	130 %	- 5.0 %
MQC - Unconjugated Ab	70 %	- 10 %
LQC - Unconjugated Ab	17 %	0.5 %

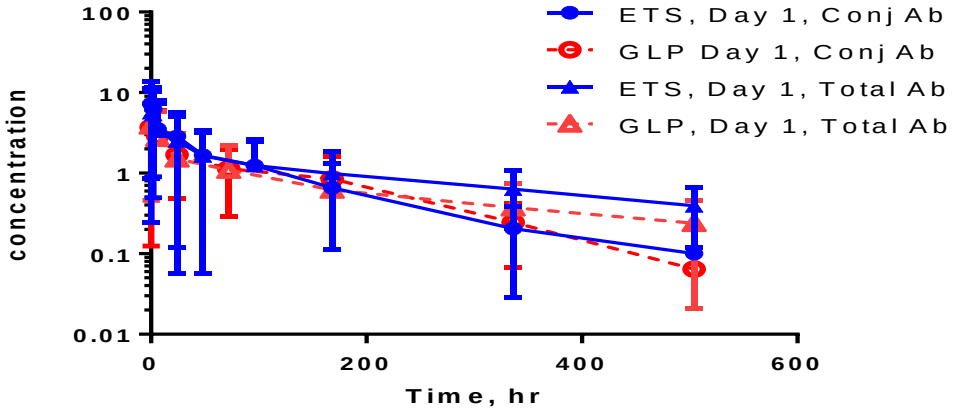
- Change in detection reagent (anti-huIgG pAb to anti-hu LC <Light Chain> specific mAb) significantly improved recovery of DAR=0 Ab vs. ADC standard curve: DAR-insensitive Total Ab Assay

Similar considerations for the Conjugated Ab (ADC, DAR≥1) analyte assay



Kumar *et al.*, Bioanalysis 2015 7(13), 1605-1617

ADC-LP1: Discovery (ETS) vs. GLP TK Data



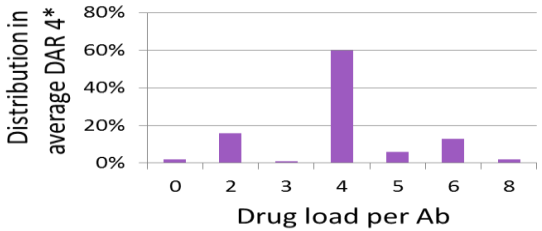
	Cmax (µg/mL)	AUC (µg•h/mL)
ETS TAb	9.6 ± 2.0	820 ± 150
ETS ADC	12 ± 3.0	660 ± 92.0
GLP TAb	6.4 ± 1.3	590 ± 70.0
GLP ADC	6.2 ± 1.2	530 ± 120

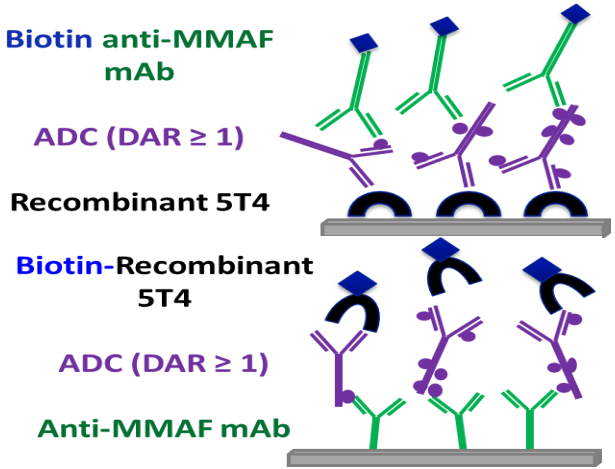
- Assay formats / assay reagents provided relative consistency in ETS vs. GLP PK profile and PK parameters

Courtesy: Frank Barletta

ADC LP2: DAR sensitivity depends on reagent choice

- Humanized IgG1 antibody conjugated via **cysteine residues** to tubulin inhibitor drug (MMAF)
- Average DAR of ~ 4
- Discovery: fit-for-purpose LBA Conjugated Ab and Total Ab PK assays to support mouse efficacy, rat/cynomolgus monkey ETS
- Unconjugated cys-mc-MMAF was measured by LC/MS
- GLP tox studies: validated LBA Conjugated Ab and Total Ab PK assays

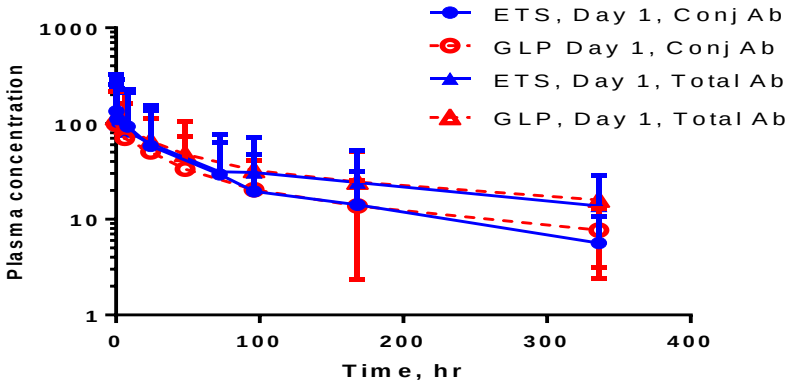




	Discovery	GLP
Purified DAR species	Accuracy (%RE) using anti-MMAF detection reagent	Accuracy (%RE) using anti-MMAF capture reagent
DAR 2	-70 %	-48%
DAR 4*	2.5 %	-4.0 %
DAR 6	100 %	13 %
DAR 8	127 %	-24 %

Change in the assay format results in significantly improved recovery of DAR species against ADC ref. standard curve: **DAR-insensitive** Conjugated Ab Assay

ADC LP2: Limited overall impact on ETS vs. GLP PK



	Cmax (µg/mL)	AUC (µg•h/mL)
ETS TAb	280 ± 44.0	25000 ± 3400
ETS ADC	370 ± 29.0	20000 ± 1300
GLP TAb	280 ± 50.0	25000 ± 2900
GLP ADC	240 ± 45.0	15000 ± 1800

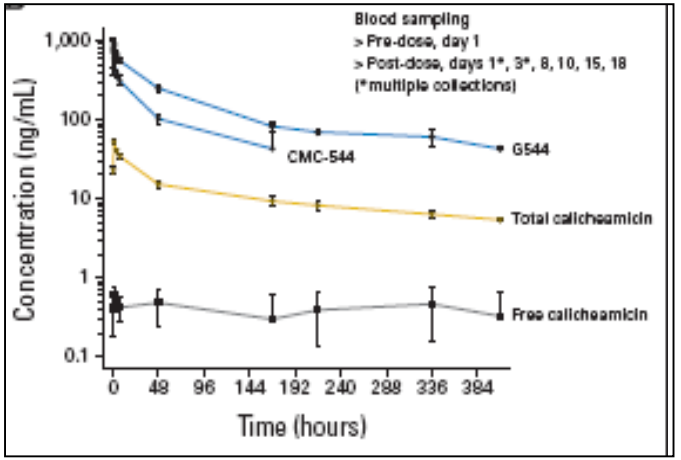
- Different assay format with relatively different DAR sensitivity
- Limited impact on the observed ETS vs GLP PK profile and PK parameters
  - In ETS (DAR sensitive) assay format, under-recovery of low DAR species is potentially compensated by over-recovery of high DAR species

Courtesy: Mauricio Leal

Total Ab to ADC accumulation and impact

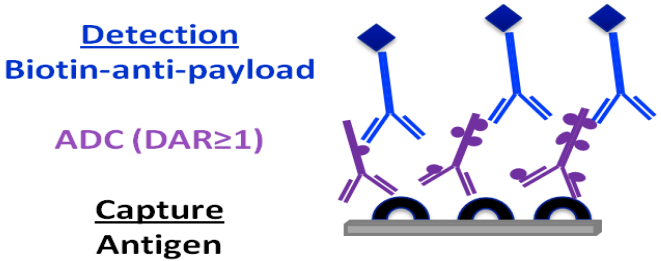
A Advani et al J Clin Onc v28 2010

- Typically half-life for Total Ab is expected to be longer vs. half-life of conjugated antibody (ADC)
- Assessment of Total Ab or low DAR ADC fraction accumulation on performance of the ADC assay(s) is needed



Spike recovery (%RE) in presence of naked mAb

Target Protein Capture Conc.	2 ug/mL		5 ug/mL		10 ug/mL	
Naked mAb (ng/mL)	LQC	HQC	LQC	HQC	LQC	HQC
0	-19%	-22%	-25%	-24%	-27%	-28%
2500	2.0%	-18%	-2.0%	-19%	0.4%	-22%
5000	-11%	-27%	-5.0%	-23%	2.0%	-19%
25000	-65%	-79%	-52%	-73%	-53%	-70%

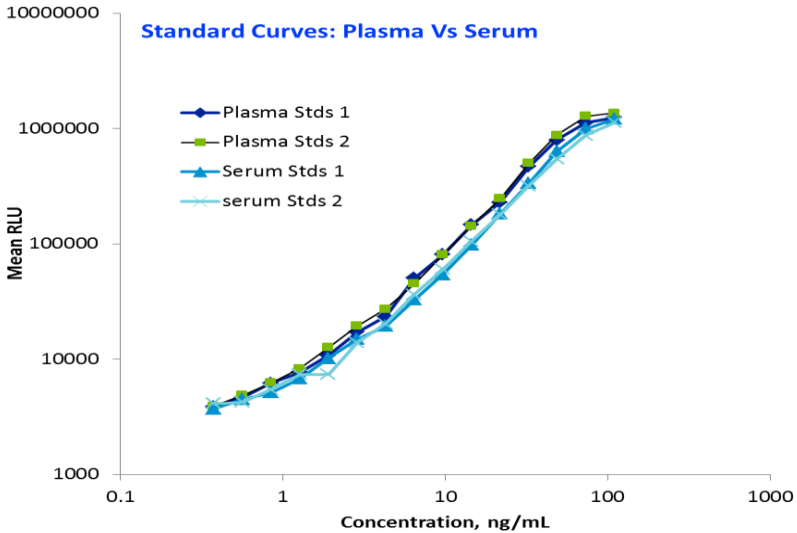


Assay can tolerate < 5 μg/ml naked mAb

Assay performance in Plasma vs Serum

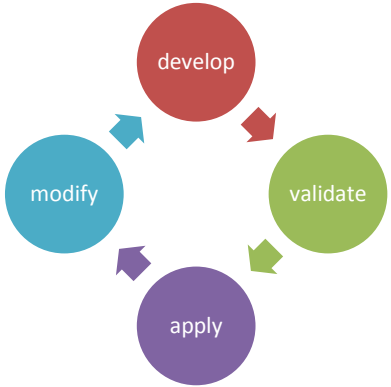
- Why?
  - LCMS assays prefer plasma
  - LBA prefer serum
- Additional assessment:
  - Transition from existing method (e.g. in serum)
  - Stability
    - Freeze / Thaw
    - Bench top (RT)
  - Selectivity

Remember that there is one serum and many types of plasma!



Life cycle. Analytical Platform Transition, many considerations

- Transitions between LBA and LC/MS:
  - Use of incurred samples for cross platform comparison
  - Correlation between methods is expected
  - Risks: results may not agree
- Method Change (e.g. reagent change)
  - Standard method re-qualification / re-validation
  - May require analysis of incurred samples
  - Must pass pre-defined acceptance criteria



MSD platform yields higher sensitivity, dynamic range

QCs	ELISA ROQ in 5% Matrix (ng/ml)	ELISA Accuracy (% RE)	MSD ROQ in 5% Matrix (ng/ml)	MSD Accuracy (% RE)
ULOQ	34	-18	64	9.0
HQC	25	-20	50	-2.0
MQC	10	-11	25	-15
LQC	3.5	-2.7	1.3	-18
LLOQ	**1.3**	**-21**	**0.5**	**-14**

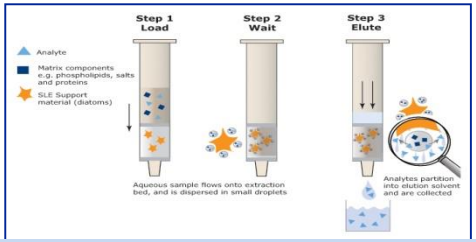
LC/MS/MS of Unconjugated and Conjugated Drug (Payload)

Plasma/serum

Tissue (bone marrow)
Homogenize (w/ PI)

Dilution/conditioning

SLE



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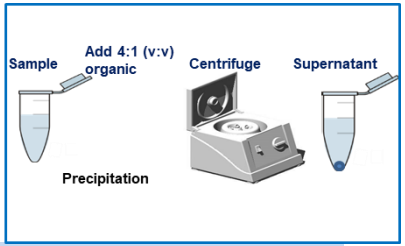


Unconjugated payload:

- ***Payload – linker metabolite assessment - early***
- ***Stability of Payload and ADC ex vivo / in vitro - Minimize unintended payload de-conjugation during sample processing and preparation***

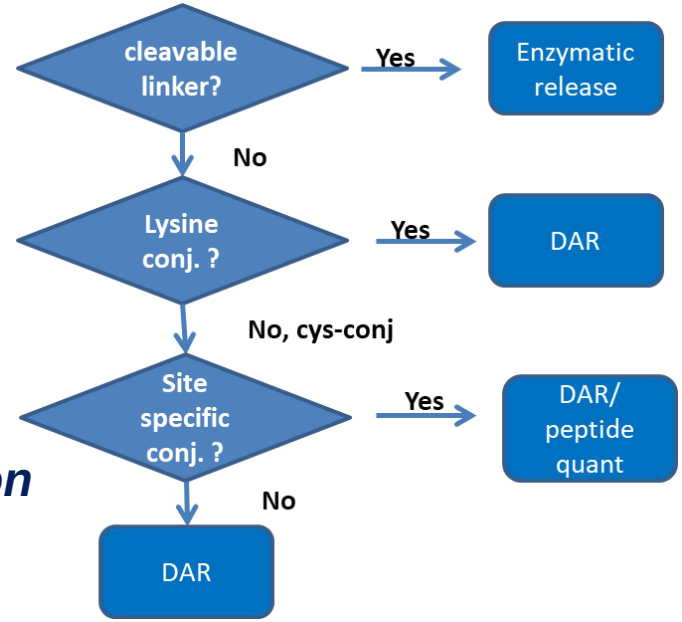
Conjugated payload

- ***Mechanism to cleave Payload from ADC***

Sample preparation

Collection/evaporation

Re-constitution



Brentuximab vedotin (SGN-35): ODAC Briefing Document - HL 06-June-2011, Seattle Genetics, Inc.

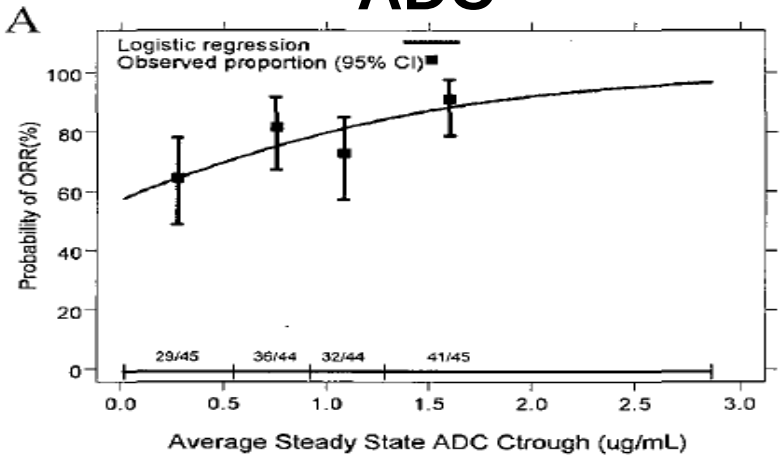
- PK measurements of:
  - ADC – brentuximab vedotin antibody-drug conjugate
  - MMAE – monomethyl auristatin E (released unconjugated small molecule)
  - TAb – total antibody (ADC plus unconjugated cAC10 antibody)
  - Anti-ADC antibody response
- Analysis was performed to determine the relationship between ADC and MMAE exposure and response

•Using trough concentrations, safety and efficacy were correlated to the exposure of the Conjugated Antibody

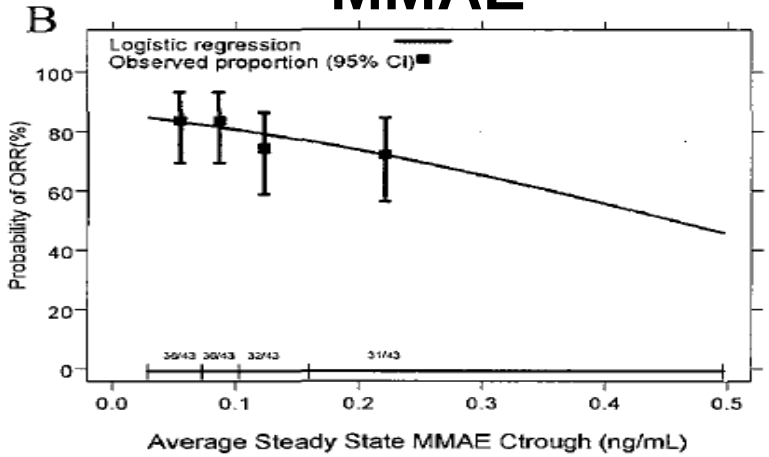
Center for Drug Eval. & Re. Clin Pharm & Biopharm Review. Application Number: 125388Orig1s000. US FDA, Washington, DC, USA (2011)
Brentuximab Vedotin (SGN-35) Briefing Document: ODAC Briefing Document HL 06-06-2011, Seattle Genetics, Inc.

Brentuximab vedotin experience. Stacy S. Shord, NBC 2013

ADC



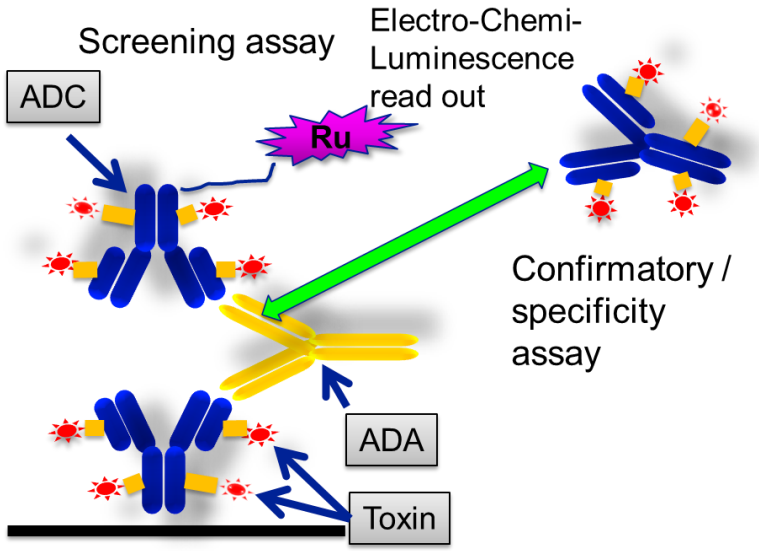
MMAE

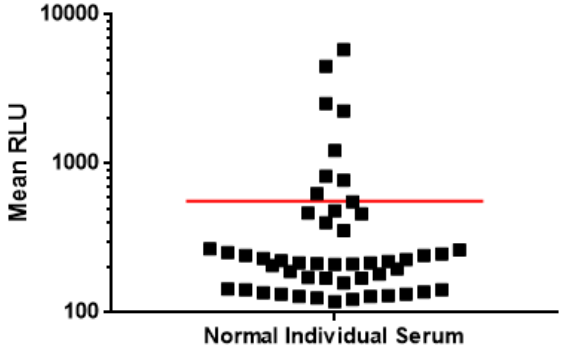


- Building an Exposure – Response relationship can be challenging but particularly challenging for unconjugated payload analyte due to low / non-existent PK profiles and scarce measurable concentrations
- Probability of overall response rate
  - increases with increasing ADC Ctrough (left)
  - decreases or flattens with increasing MMAE Ctrough (right)

Immunogenicity evaluation is important part of BA strategy

- What to consider:
  - Pre-existing reactivity to payload and to the protein component when patient had prior exposure to similar biologic
  - ADA assay Cut-point assessment
  - Expected ADA / NAB assay sensitivity
  - Interference of circulating target - although ADCs are mainly anti-cell surface targets, some targets may be shed





Summary

- Initial ADC reference material is heterogeneous mixtures of various drug-to-antibody ratio (DAR) species
- ADC heterogeneity continues to evolve in vivo
- DAR IN-sensitive LBAs are preferred for clinical studies
- There is no single bioanalytical strategy that fits all
- It is possible that none of the formats will deliver best conditions – consider using an alternative analyte (e.g. Conjugated Drug)
- A fit-for-purpose assay used in discovery may not translate to a robust and reliable assay for GLP and clinical support

Acknowledgements

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AAPS BA community

Thank you!