Immunogenicity assay strategies for antibody-drug conjugates

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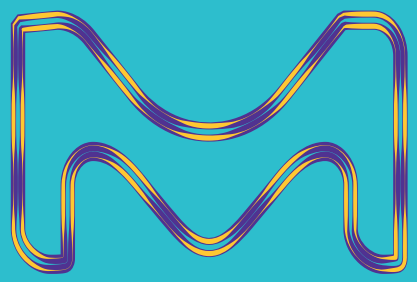

[1]A business of Merck KGaA, Darmstadt, Germany

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Disclosure statement

- Dr. Kumar is an employee of EMD Serono Research & Development Institute, Inc., a business of Merck KGaA, Darmstadt, Germany
- This presentation is based on the scientific literature, the presenter's professional experience and presentations, and work of colleagues as cited. No internal, unpublished data is presented
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IMMUNOGENICITY RISK ASSESSMENT

Risk = Probability x Severity

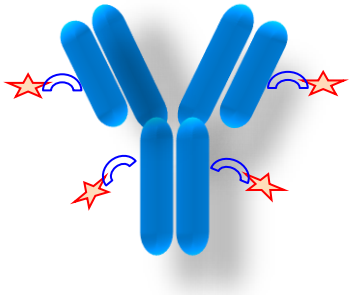
- Probability - Incidence of ADA response
  - –Risk factors (product-, patient-, treatment-related)
  - –Immunogenicity prediction tools (in silico, *in vitro, in vivo*)
- Severity – Consequences of ADA response
  - –Impact on PK, safety, efficacy
- Risk category
  - – Low, Medium, High

Immunogenicity risk level drives bioanalytical strategy!

ADCs are classified under medium risk category!

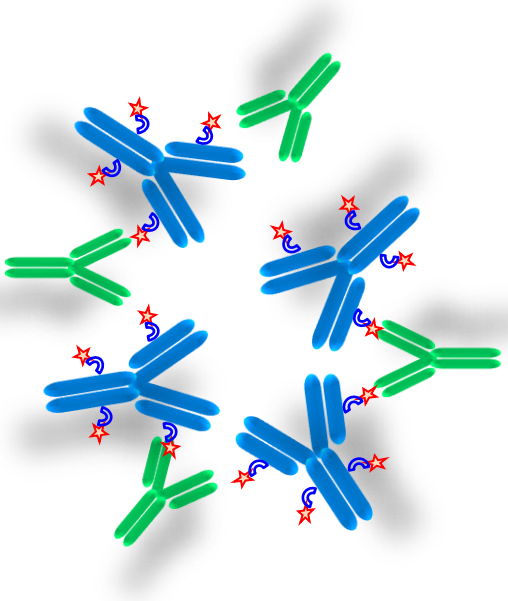
ADC IMMUNOGENICITY RISK FACTORS

- Immunogenicity may be directed against any functional domain of ADC (antibody, linker, payload)
- Intrinsic: Product-related
  - Antibody (mAb):
    - ✓Presence of non-human AA sequence
    - ✓Post-translational modifications
    - ✓Mutations in the IgG framework
  - Linker/payload:
    - ✓Payload hydrophobicity could cause ADC aggregation
    - ✓Hapten-like structure
  - ADC:
    - ✓Aggregates, impurities, degradants
    - ✓neo-epitopes formed as a result of conjugation
    - ✓Epitope spreading
    - ✓Repetitive antigenic structure due to multiple linker/payload
- Extrinsic
  - Patient-related: Immune status, Genetic factors, prior exposure to similar molecules
  - Treatment-related: Dose, frequency and route of administration, concomitant medications



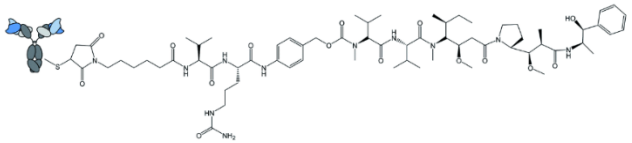
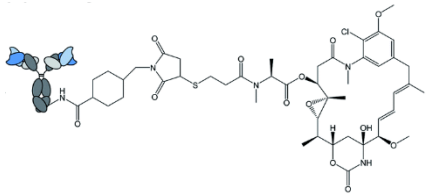
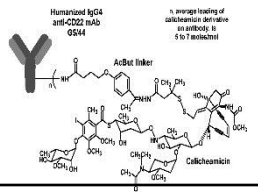
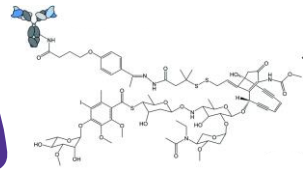
Potential consequences of ADC immunogenicity

- PK/PD
  - exposure, half-life, enhanced immune-complex mediated clearance
- Efficacy
  - activity neutralization
    - Impairment of target binding by neutralizing Ab (Nab)
    - Anti-payload ADA may form large immune complexes that prevents ADC cellular internalization
- Safety:
  - hypersensitivity reactions (prior sensitization)
  - Repetitive nature of epitope on ADC may lead to formation of large immune complexes
  - Uptake of payload by non-target immune cells during immune complex clearance may contribute to off-target ADC toxicity



Robust ADC immunogenicity monitoring strategy!

Clinical Immunogenicity data from approved ADCs

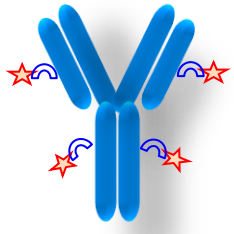
ADC	Assay	Immunogenicity Incidence	Nab	Impact
Brentuximab vedotin 	ECL	• 30% (47/156) transiently positive - against chimeric mAb domain of ADC • 7% (11/156) persistently positive	Detected in 62% of the ADA positive subjects	• Impact on efficacy or safety unknown • Higher incidence of infusion related reactions
Ado-trastuzumab emtansine 	ECL, ELISA	• 5.3% (44/836) tested positive - primarily against linker-payload and/or neoepitopes in the mAb • Circulating drug interference at the time of sampling may have interfered with ADA detection; May not accurately reflect the true incidence	Not assessed	No observed impact on PK, efficacy and safety
Inotuzumab ozogamicin 	ECL	• 3% (7/236) tested positive - majority against calicheamicin domain. Low titer pre-existing antibodies in some patients that became undetectable during the study	None detected (Cell based Nab assay)	No impact on clearance
Gemtuzumab ozogamicin 	ELISA	• 1.1% (2/182) tested positive - antibodies against the calicheamicin/linker domain	Not reported	Transient shortness of breath

https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125388Orig1s000ClinPharmR.pdf
http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/125427Orig1s000ClinPharmR.pdf
https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21174_MYLOTARG_biopharmr.pdf
http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/004119/WC500231263.pdf

Immunogenicity assay strategies for ADCs

Risk-based tiered approach:

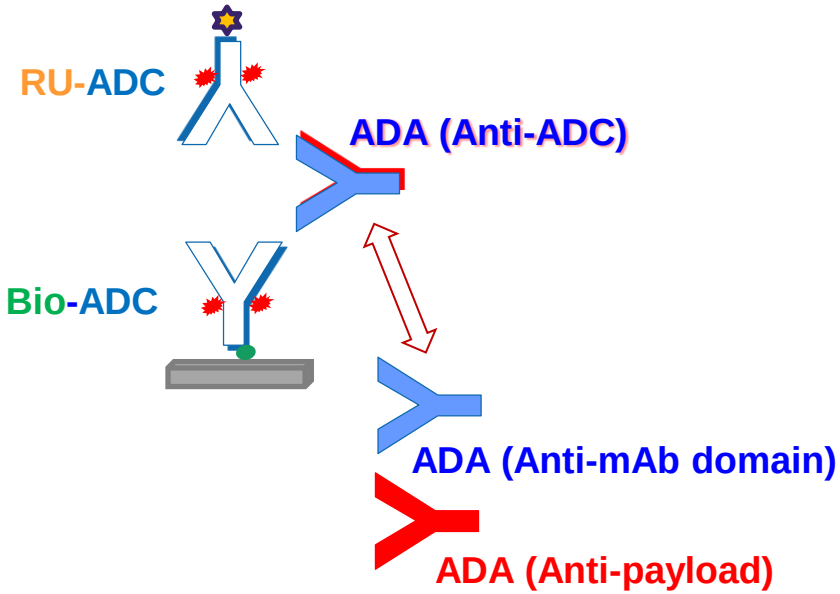
- Screening assay
- Confirmatory assay (whole drug)
- Additional characterization
  - Titer assay
  - Neutralizing Ab testing
  - Domain specificity



Hock *et al*. AAPS J. 2015 Jan; 17(1): 35–43

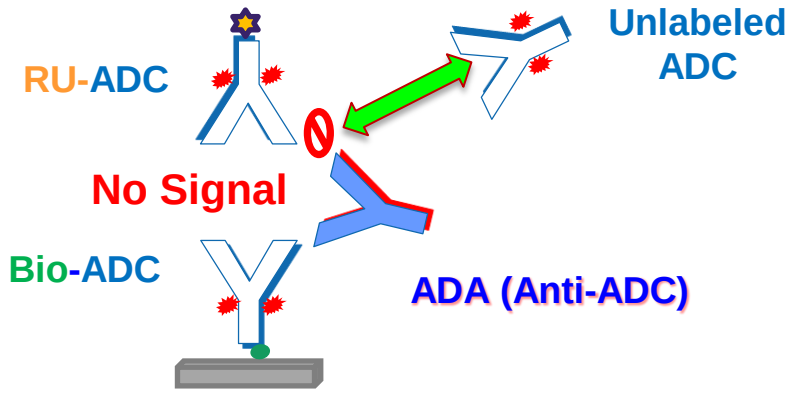
Bridge ADA Assay Formats for ADC

Screening Assay



1 anti-ADC PC
or
2 domain specific PC

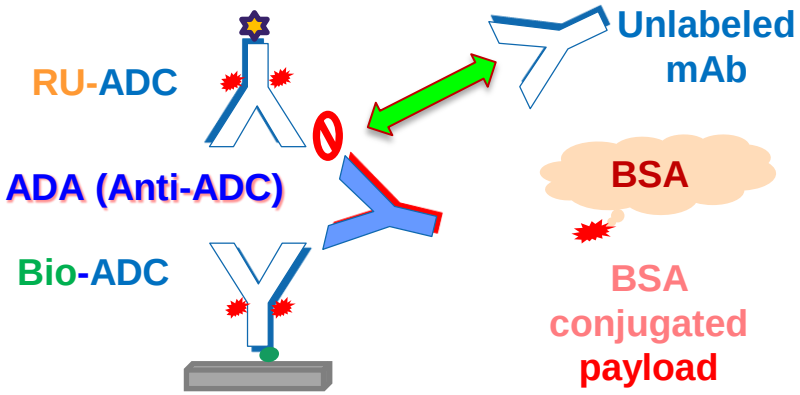
Whole Drug Confirmatory Assay



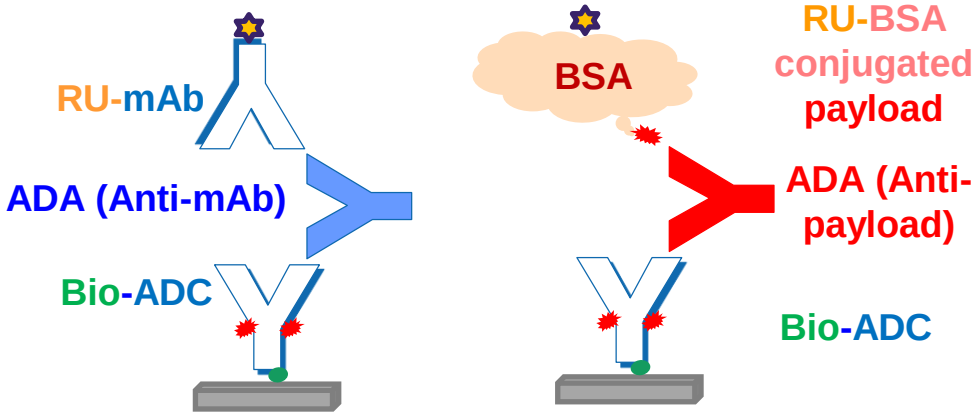
Competitive inhibition using unlabeled ADC

Domain specific characterization

Domain Specific Competition (DSC)



Domain Specific Detection (DSD)



One assay with 2 competitors vs 2 individual domain-specific assays!

Adapted from Hoofring *et al.* 2013. *Bioanalysis*, 5(9), 1041-55

KEY CONSIDERATIONS FOR ADC IMMUNOGENICITY ASSAYS

- ✓ More complex or more engineered structure = more # of ADA assays
  - more challenges in assay development (soluble target interference, drug tolerance, pre-existing reactivity etc.)
  - specialized critical reagents (e.g. domain specific positive control, domain specific competitor molecules or domain specific labeled ADC component reagents) and multifaceted data interpretation from multiple assays
- ✓ Challenges in generating high affinity and high avidity anti-payload reagents (PC for ADA and assay reagent for Nab assay)
- ✓ Challenges in labeling ADC- insufficient labeling because labeling reagent (biotin/ruthenylated/digoxigenin) may compete for the same conjugation site on mAb as payload particularly for high DAR ADCs
- ✓ High label to ADC coupling ratio could result in epitope masking on ADC for ADA detection and may contribute to aggregation, poor solubility and instability of the labeled ADC reagents
- ✓ Antibodies to linkers? Or develop linker-specific reagents? Or use inferential approach?
- ✓ Complex neutralizing antibody (Nab) testing strategy against multiple functional domains

SUMMARY

- ✓ Limited information on immunogenicity of ADCs from few approved ADCs
  - Information from numerous ongoing clinical trials and post-marketing data will provide a broader understanding of ADC immunogenicity risk and its impact on clinical consequences
- ✓ Similar to other large molecule biotherapeutics, ADCs should follow tier-based testing approach for immune response evaluation
- ✓ Screening and Confirmatory assays should include ADA positive control against whole ADC or individual positive controls against Ab framework and linker-payload
- ✓ Risk-based, fit-for-purpose approach and product development stage should drive the need for domain specific characterization assays against individual ADC domains
- ✓ Focus must remain on understanding ADA incidence, and their impact on PK, PD, efficacy and safety

ACKNOWLEDGEMENT

My colleagues present and past for the invaluable discussions!

Thank you for your attention!