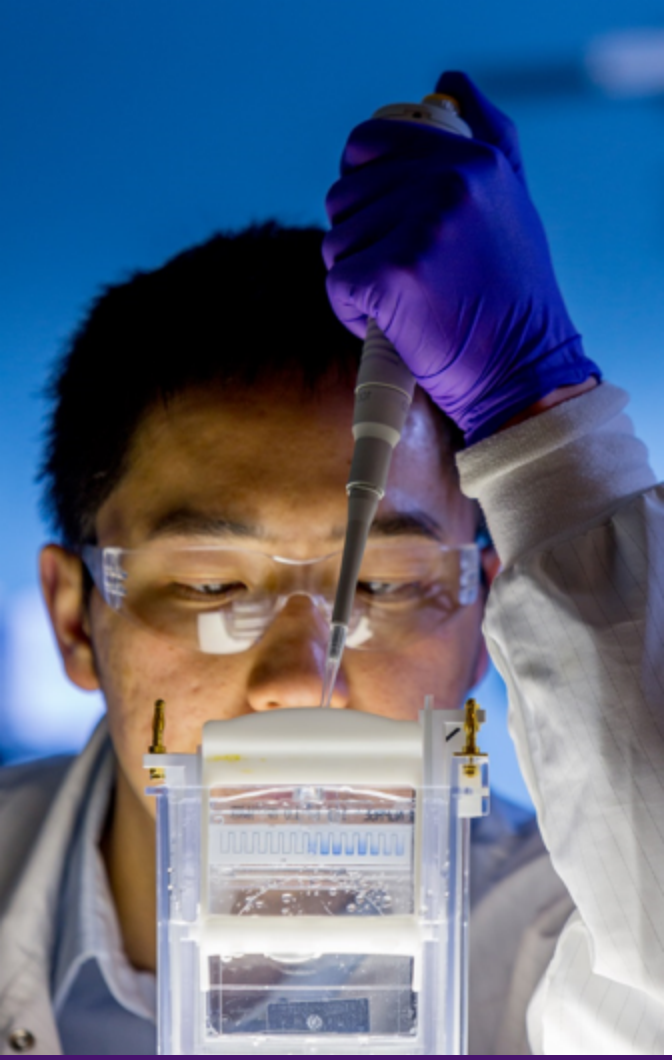

Considerations for the Development of ADA Assays for the Analysis of Novel Modalities

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Overview

1. *Standard* ADA assays approach
2. New modalities—*what to worry about?*
3. Multidomain and hybrid therapeutics
4. Gene delivery vectors
5. LC-MS technology applied to ADAs?
6. Conclusions

Standard ADA assays approach

+ Assays are required to understand immune response to a biotherapeutic along with any potential impact of ADA on safety and efficacy

+ Important for any protein-based biotherapeutic to identify potential ADA response

+ Standard recommendations: Multi-tiered approach

Screening

Confirmation

Titering

NAb

Novel Modalities in ADA Testing

- Peptides
- Multidomain and hybrid therapeutics:
  - ADCs
  - fusion proteins
  - bi-specific protein products
- AAV gene delivery vectors in NAb assays


What’s the difference?

Multidomain and hybrid therapeutics

What are they?

Biotherapeutics designed for improved half life, cellular uptake, and specificity and targeted toxicity.

More complex than standard MAb used in traditional ADA analysis

Includes: ADCs and pro-drug conjugates, bi-specific antibodies and Fc-fusion proteins

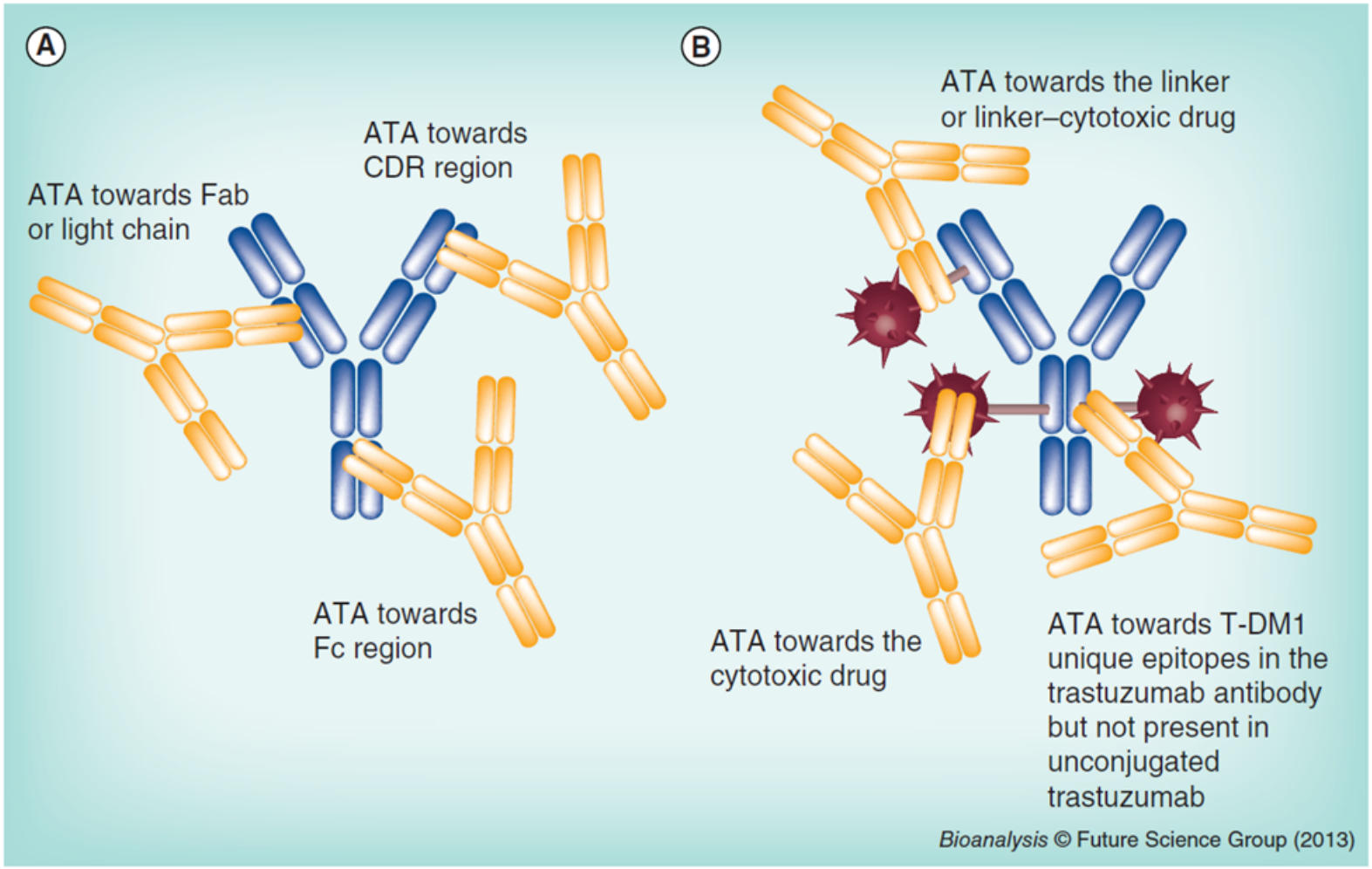
Challenge: need to characterize the ADA binding specificity and its clinical consequences

Recommendation: assess ADA to the total protein, each domain, any novel regions

Considerations

+ A multi-tiered approach for specificity/characterization cut point determination
+ Identifying the appropriate positive controls for these assessments is a common challenge

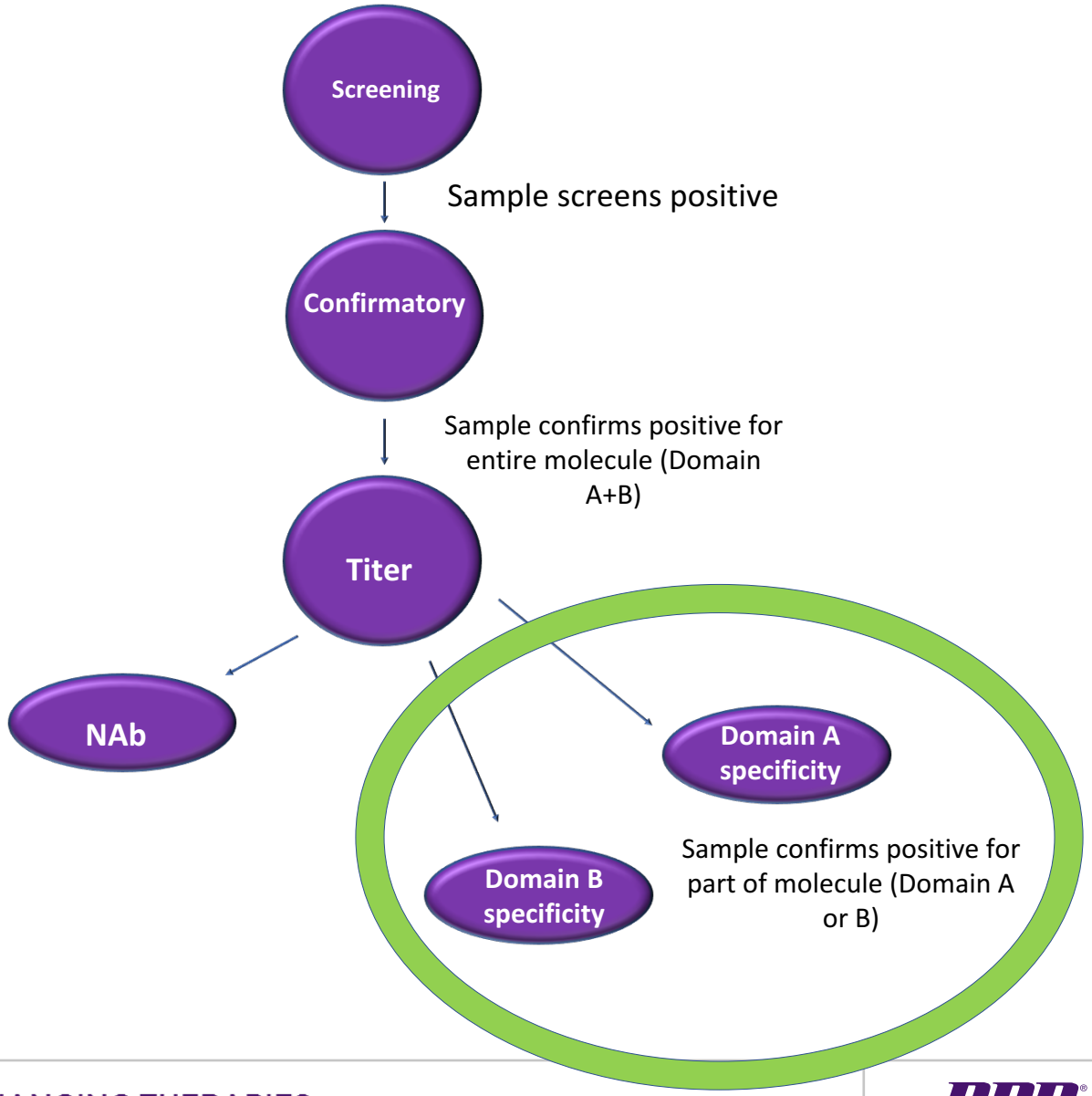
Multidomain and hybrid therapeutics



Carrasco-Triguero, *et al.* / *Bioanalysis* (2013) 5(9), 1007-1023

Unique challenges in ADA analysis

- Characterize the ADA response
  - Based on risk factors and **phase of clinical development**
  - Determine specificity of the ADA response



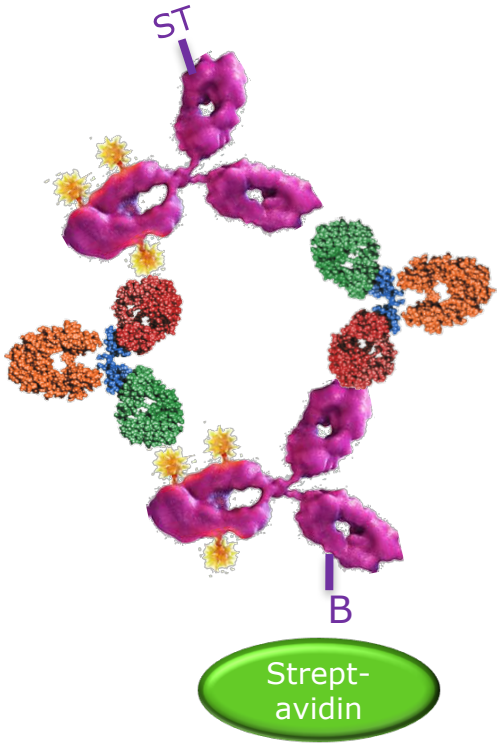
Multidomain and hybrid therapeutics

ADCs

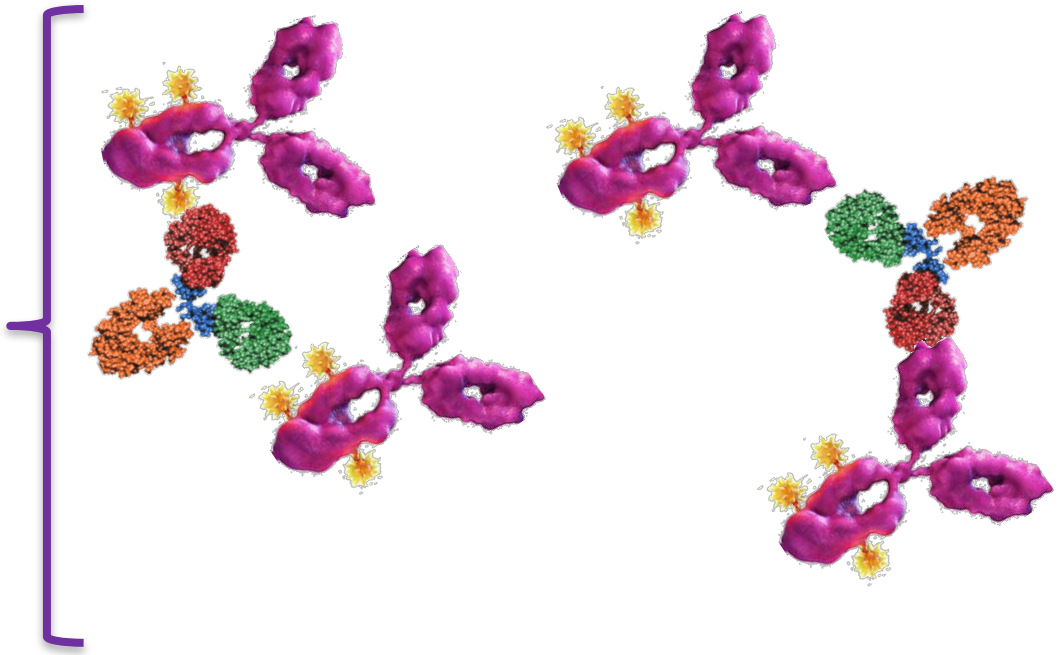
- Careful reagent conjugation of ADCs to reduce further molecular structural change
- Increased instance of hydrophobicity/aggregation = characterize stability and solubility
- Buffers containing blocker protein to prevent non-specific binding
- mAbs ideally specific to linker, small molecule drug or both helpful for assay development
- Screening and confirmatory against entire ADC molecule, characterization of domain specificity

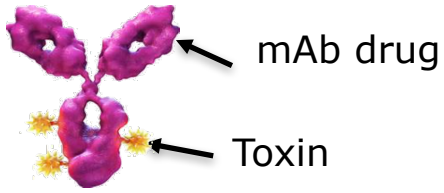
First confirmation step

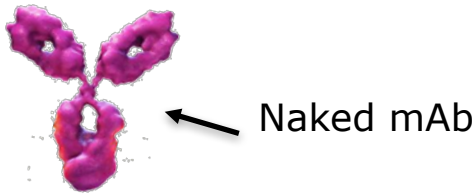
Add excess ADC to the reaction mix, which will complex with ADAs and diminish the assay response, confirming their presence. ADAs against all ADC epitopes will be inhibited from reagent binding.

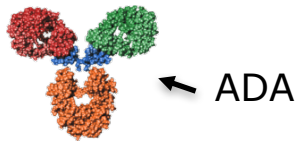


No assay signal is generated from these complexes



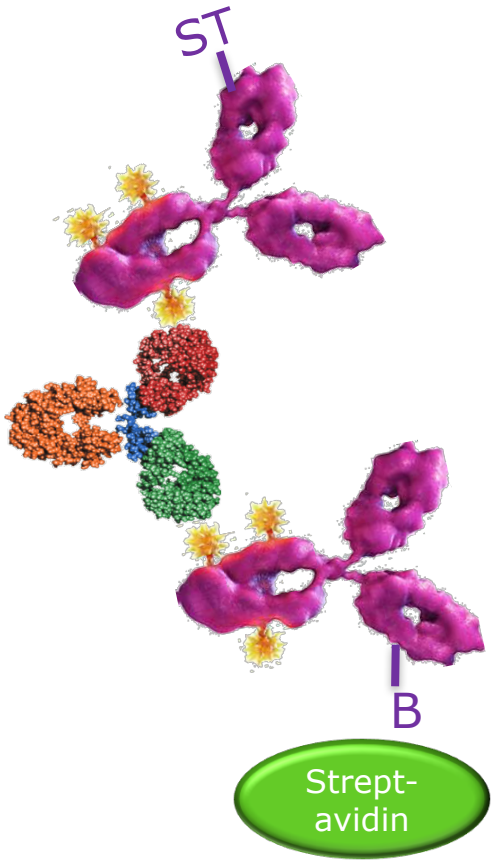




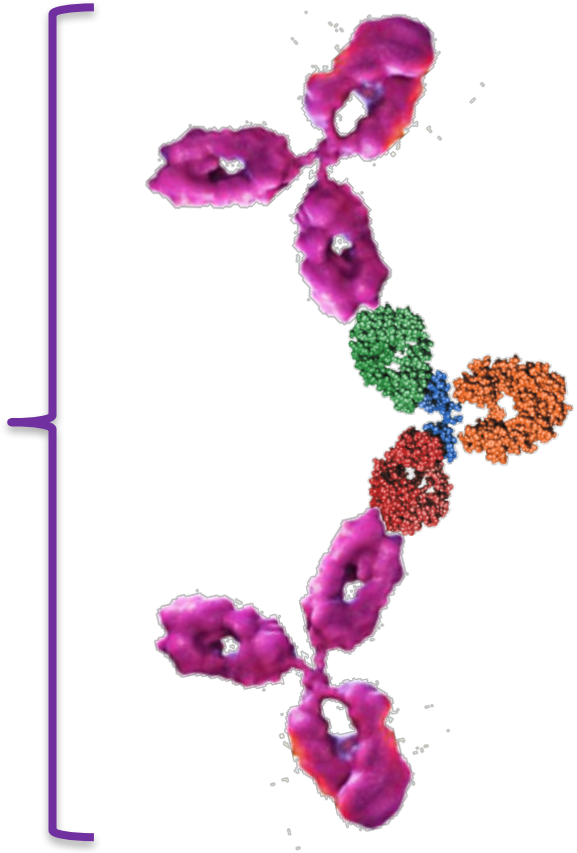


Second confirmation step

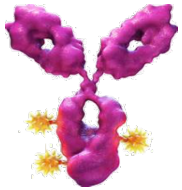
Add excess *naked* antibody (i.e., ADC mAb with no conjugated drugs) to the reaction mix, which will complex with ADAs specific to only the mAb portion of the ADC. ADAs against other epitopes (linker or conjugated drug) will be not be inhibited from reagent binding.

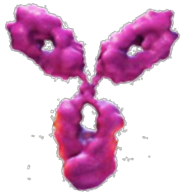


No assay signal is generated from this complex



Samples results comparing *confirmation* with ADC to *characterization* with naked antibody





	Screening Assay	Confirmatory Assay	%INH	Characterization	%INH
Sample 1	10,000	200	98	4000	60
Sample 2	10,000	100	99	100	99
Sample 3	10,000	500	95	9000	10

Assay should measure ADA responses to all potential ADC epitopes, incl. mAb, linker and conjugated small molecule drugs

Multidomain and hybrid therapeutics

Bi-Specific Antibodies

+ PCs against each arm (or whole molecule)
+ Need Cut Points for each arm/Fab and the whole molecule

Fusion Proteins

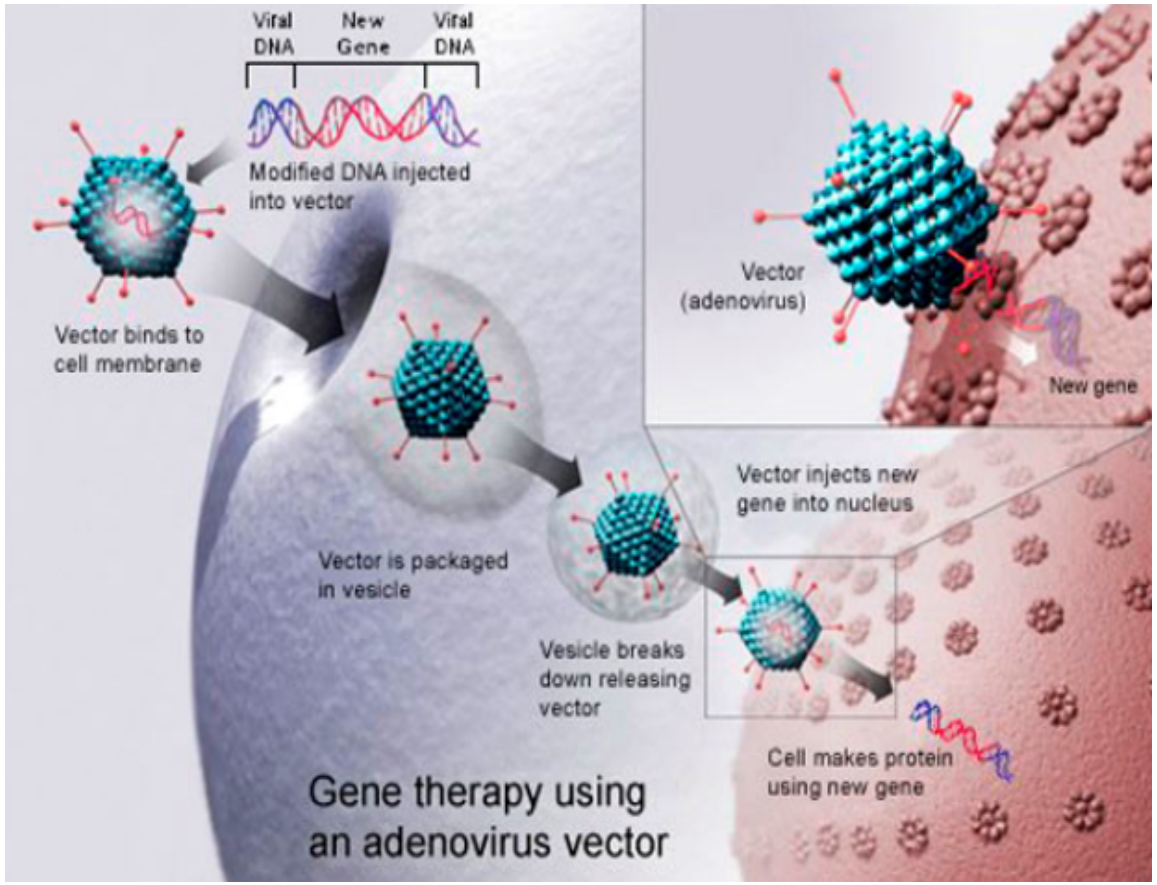
+ Epitope spreading in fusion proteins
+ RF interference – binds Fc regions in Fc-fusion proteins, resulting in false positives
+ Multiple assays to different domains and junction neoepitopes

Multiple domains

+ Multiple assays to measure immune response to each domain
+ Functional and non-functional (PEGylated)

Gene delivery vectors

- **What are they?**
  - Small nonenveloped virus that packages a linear stranded DNA
  - Able to attach and enter target cell and successfully transfer to the nucleus



U.S. National Library of Medicine

Gene delivery vectors

Examples

Adeno–associated virus vectors – 12 human serotypes

- \+ Recombinant AAV (rAAV) vectors
- \+ Self-complementary AAV (scAAV) vectors

Considerations

- Pre-existing antibodies present in matrix correlates with decreased efficacy
- Determination of how to derive an appropriate cut point
- Pre-screening for enrollment into gene therapy programs
- After dosing, individuals should be tested for the immune response via titration.
- Sample result TAT speed is crucial due to the chance of exposure to AAV in the environment

AAV Pre-Screen and Post-Enrollment

Example:

AAV9 NAb assay

- MD/MV according to 2016 FDA guidelines for cell-based NAb assays
  - Assay sensitivity of <60 ng/mL
  - Precision <15%
- Pre-enrollment screening
  - NAb positive subjects excluded from study participation
  - 1-2 week TAT from sample receipt to provide enrollment eligibility to sponsor
  - Post-dose monitoring

LC/MS as a new technology to aid ADA analysis

Format: affinity capture methods most common

Protein Analysis

- Bottom up analysis for the quantitation of therapeutic proteins and biomarkers
  - Universal peptide for pre-clinical applications
  - CDR or proteolytic peptide for clinical applications
- Top down and middle up analysis for qualitative/semi-quantitative analysis of intact proteins

Supporting LBA

- Evaluation/optimization of drug depletion procedures
- Evaluation of labeled reagents (e.g. biotinylated and ruthenylated drug)
- Assist in the development of NAb assays – supporting role for drug tolerance, elimination, confirmation

Direct ADA analysis

- ADA isotyping

LC/MS as a new technology to aid ADA analysis

LC/MS assays are rarely applied in ADA programs mainly due to their cost-prohibitive nature, but could prove beneficial in the right programs.

- Immunoassay collaboration
  - Combine technologies to increase selectivity and sensitivity
  - Better characterize what is being captured/detected
  - Aid LBA and ADA method development

Final Thoughts

Biotherapeutic molecules continue to evolve in the direction of complex modalities

+ Complex biotherapeutics may have unknown impacts on immune response which result in unique challenges associated with ADA assay development

+ Common themes for ADA assay development:
  - Careful considerations when conjugating reagents to already modified molecules
  - Composition of buffers used to eliminate non-specific binding
  - Cut Points: characterization tier; appropriate false positive rate
  - Stability and solubility of molecule
  - Generation of appropriate positive control(s)
  - Specific domain appropriate reagents which can be difficult to obtain

Keys to Successful Complex ADA Method Development and Validation

+ Open communication
  + Free exchange of ideas and experience is particularly beneficial during early development or method troubleshooting

+ Disclosure of critical therapeutic product information at project start
  - Formulation and design of the molecule
  - Mechanism of action
  - Drug target
  - Type of drug, linker
  - Stability issues

+ Critical path with milestones for method development; consider time, scope and resources

Thank you

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+ Kelli Phillips

Questions?