Domain Specificity of Immunogenicity Assessment for Multi-Domain Biotherapeutics

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EBF
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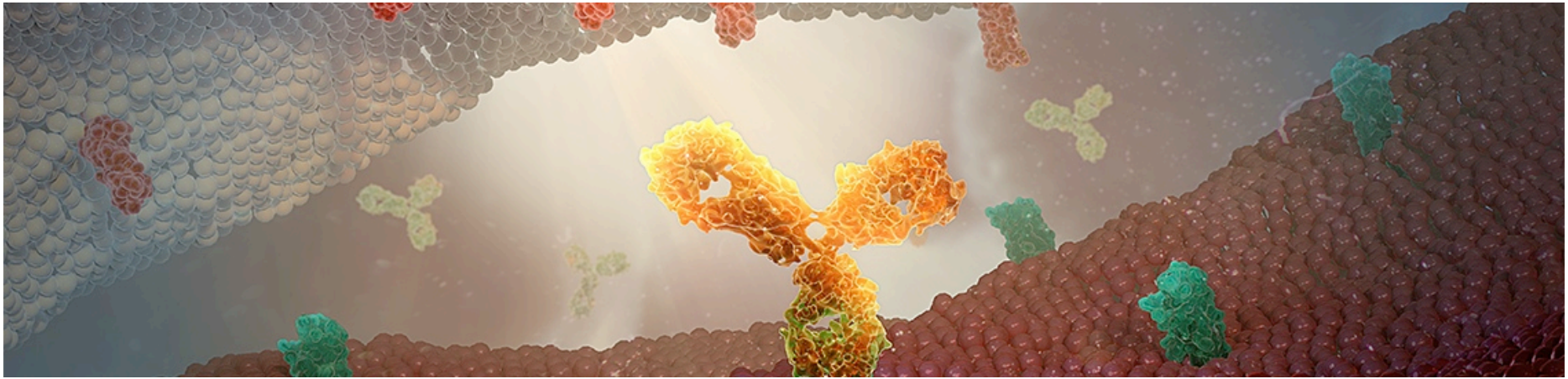
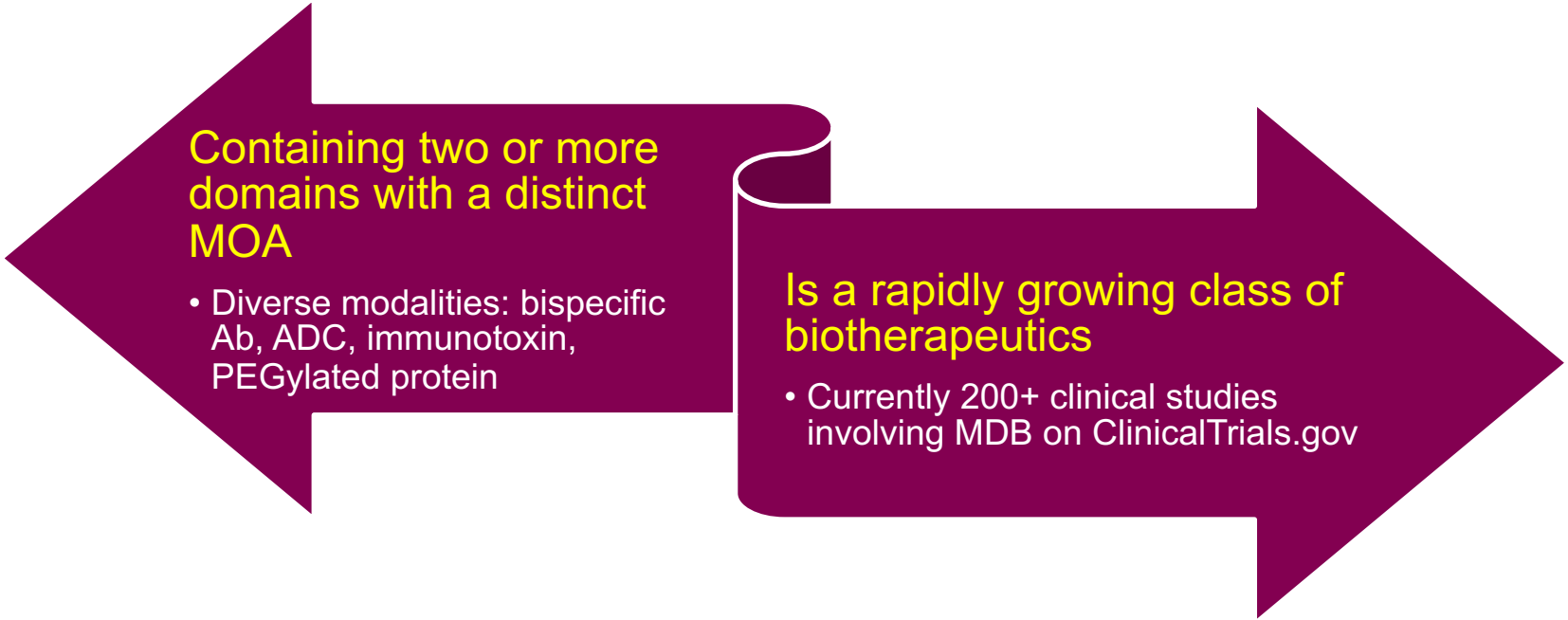


Presentation Outline

1. Multi-domain biotherapeutics
2. Immunogenicity: domain specificity
3. A case: the challenge and the solution
4. Summary

Multi-domain Biotherapeutics (MDBs)



Immunogenicity of MDBs

Regulatory Guidance

FDA

Multiple domain proteins function in different ways to mediate clinical efficacy

ADA to one domain may inhibit a specific function while leaving others intact

Screen and confirmatory test for the whole protein; May need to investigate whether the ADA binds to specific clinically relevant domains

EMA

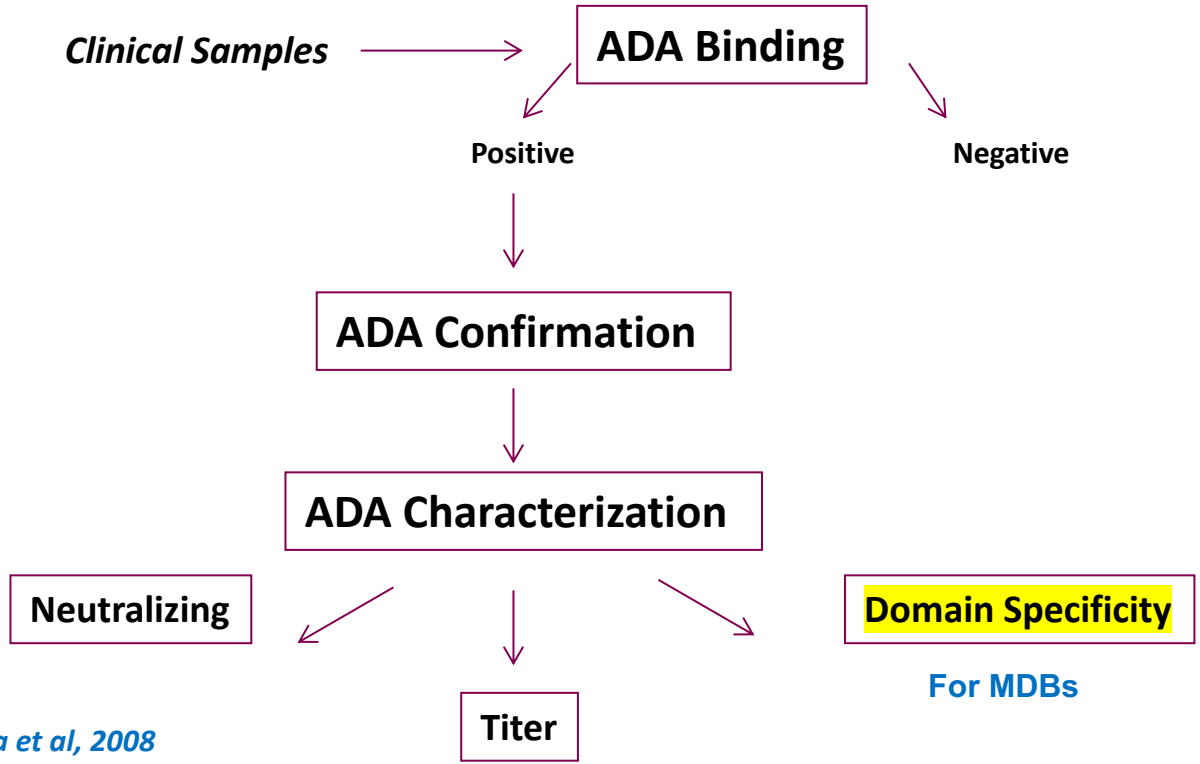
Typically, a multi-tiered approach should be employed. This includes a screening assay for identification of antibody positive samples/patients, a procedure for confirming the presence of antibodies and determining antibody specificity followed by functional assays for the assessment of the neutralizing capacity of antibodies.

Tests for determining antibody isotype and epitope specificity may also be considered on confirmed antibody positive samples.

The degree of characterization required will differ depending on the study purpose and stage of development of the product.

How to Test Immunogenicity for MDB

Tiered approach



Adapted from Gupta et al, 2008

When to Test Domain Specificity

Risk-based approach: determine the necessity for testing

- **So far, domain specificity was not consistently assessed**
- **~68% of MDB evaluated for domain specificity** (Gorovits et al, BioDrugs. 2020 Feb;34(1):39-54)

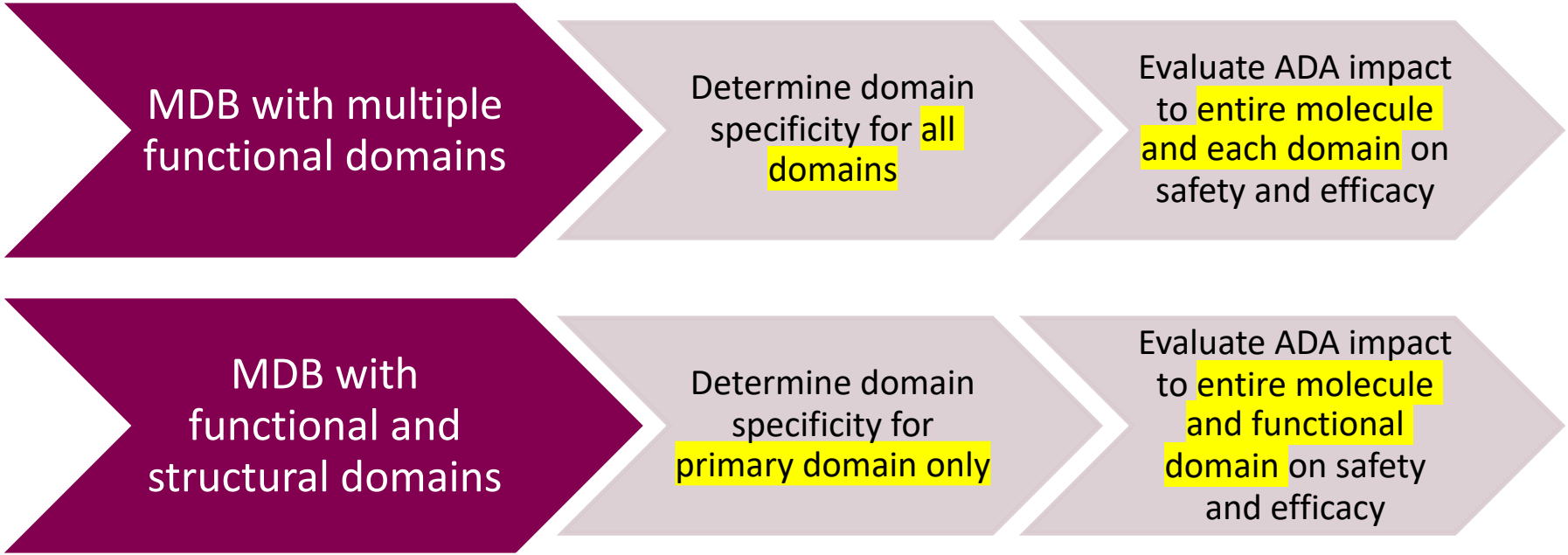
Major risk: An epitope having significant homology to an endogenous protein with a critical function

- **Require a careful evaluation of ADA domain specificity early in clinical studies**

Minor risk: An epitope spread, large immune complexes, anti-hapten-like response

- **Additional assessment of domain specificity may not be required**
- **Although it may inform development of next generation molecules**

What to Test Domain Specificity



How to Test Domain Specificity

Competition

Format:
Domains as competitors

Advantage:
Single assay, less resources

Disadvantage:
Ambiguous results for low abundant domain-specific ADA

Multiple screens

Format:
Domains as captures

Advantage:
Ability to detect a low abundant domain-specific ADA

Disadvantage:
Multiple assays, resource intensive and difficulty to compare results

Domain Nab

Format:
Nab activity for each domain

Advantage:
Ability to detect a low abundant domain-specific Nab

Disadvantage:
Limited domain specificity data for binding ADA, Nab assay, sensitivity & DT

Case Example

Novel Competition Approach Allowing Detection of Low Abundant Domain-specific ADA

J Immunol Methods. 2016 Aug;435:68-76

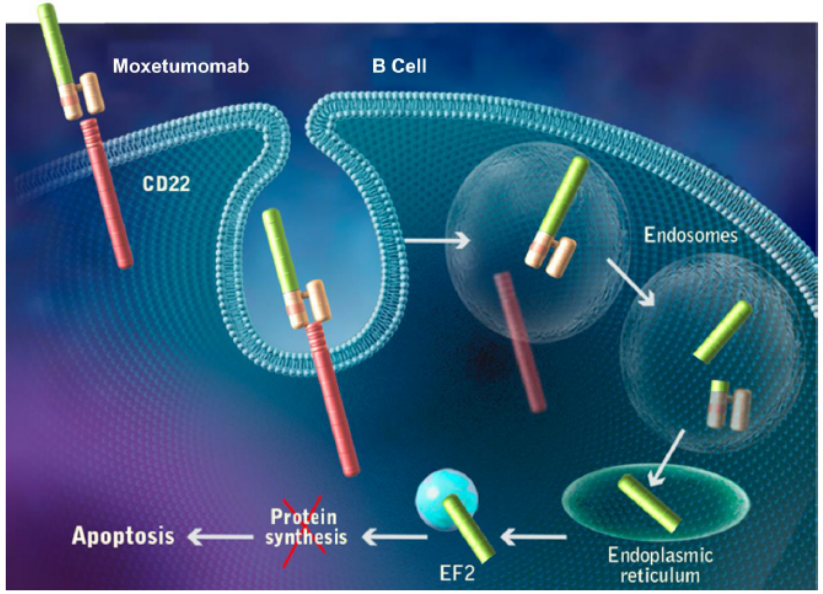
J Immunol Methods. 2020 Feb;477:112688

Lumoxiti, Moxetumomab Pasudotox

An immunotoxin composed of the binding domain of an anti-CD22 antibody fused to a truncated form of *Pseudomonas* exotoxin (PE38)

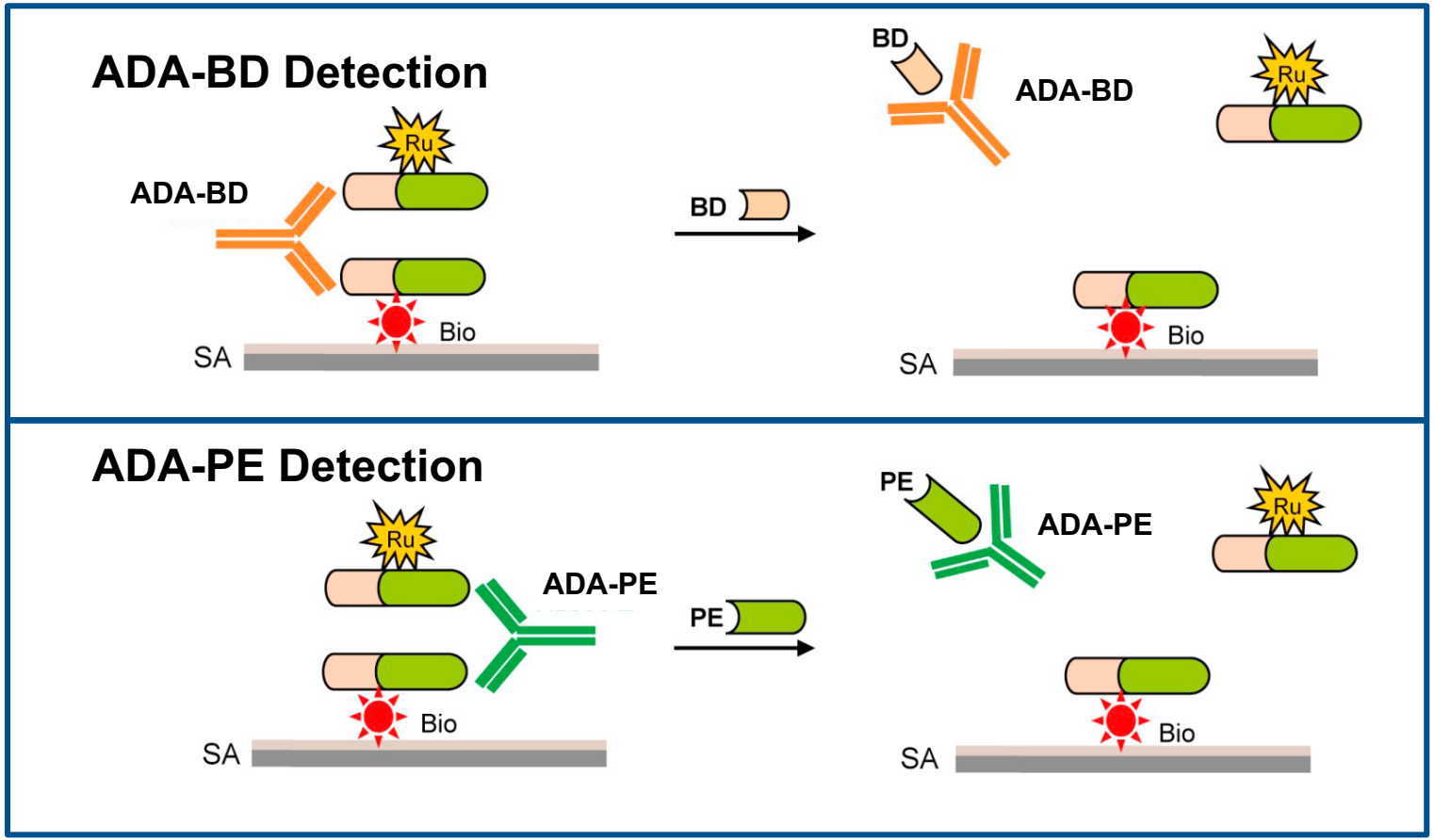
Target: CD22 is a receptor expressed on B-cells in a majority of B-cell malignancies

Indication: Approved for HCL

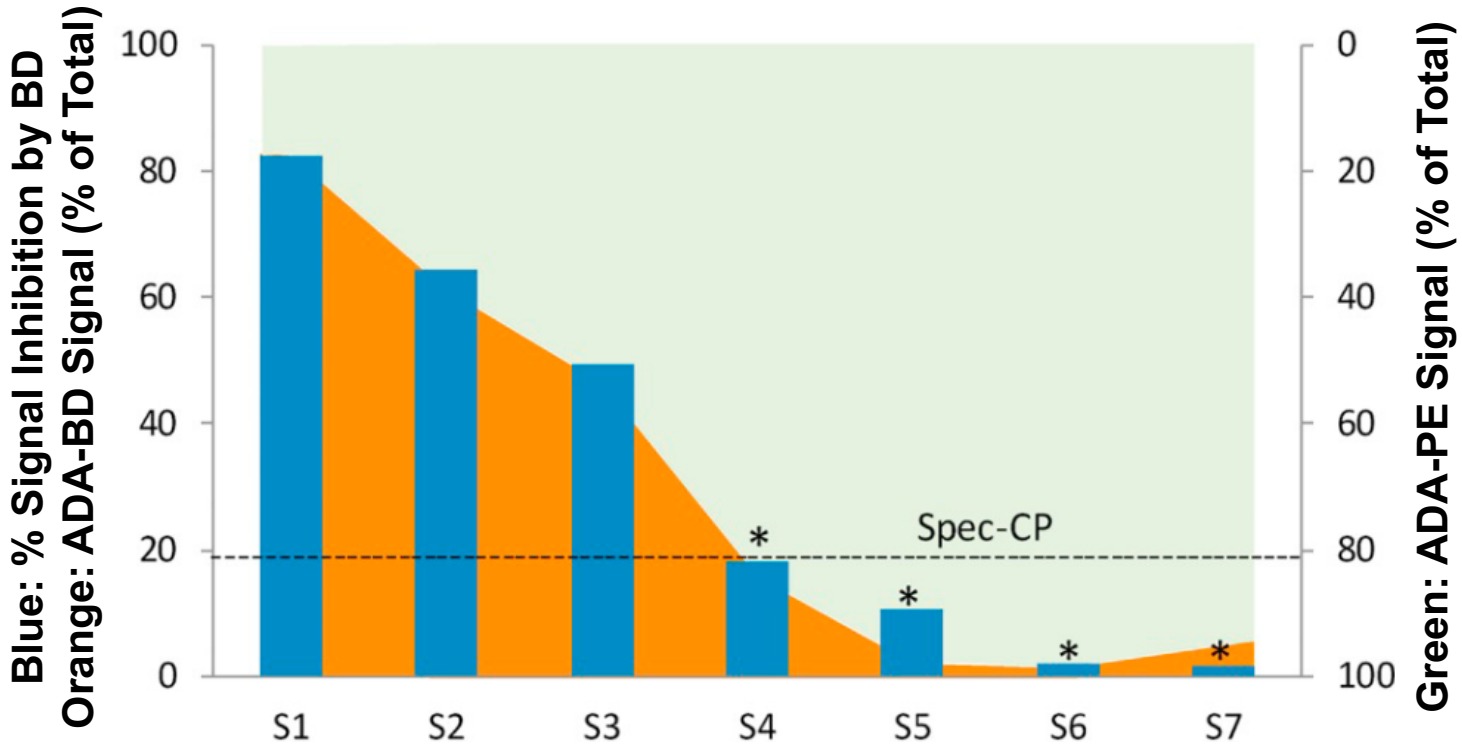


High prevalence of pre-existing anti-PE in human.

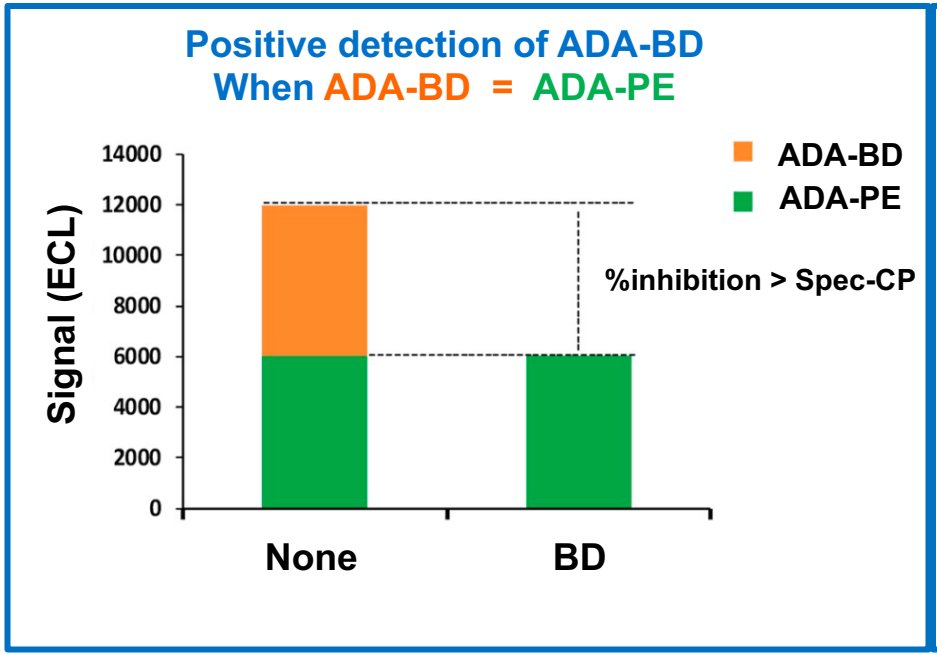
Competition Method for Domain Specificity

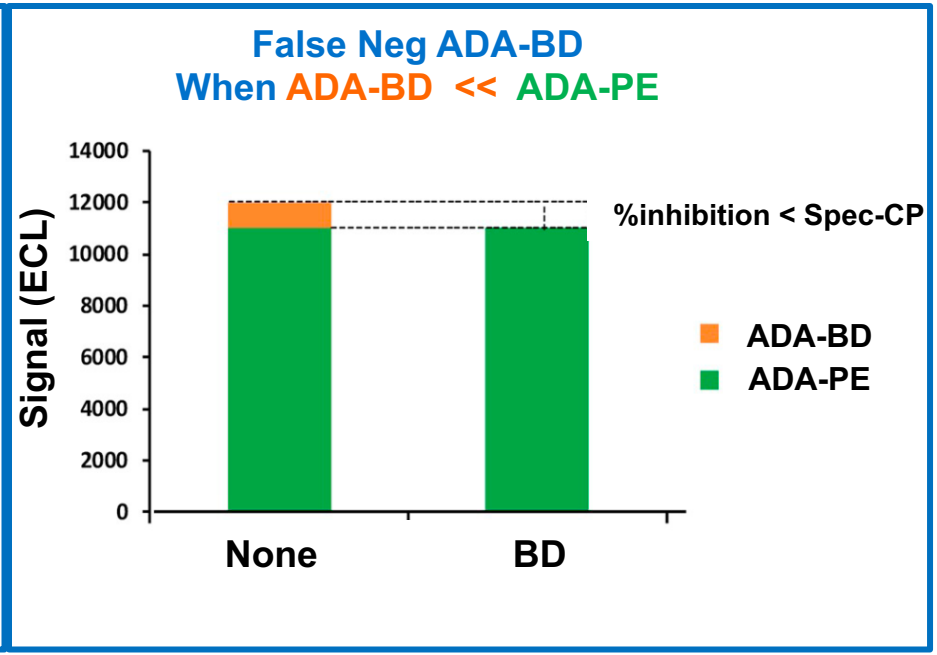


Difficulty to Detect Low Abundant ADA-BD

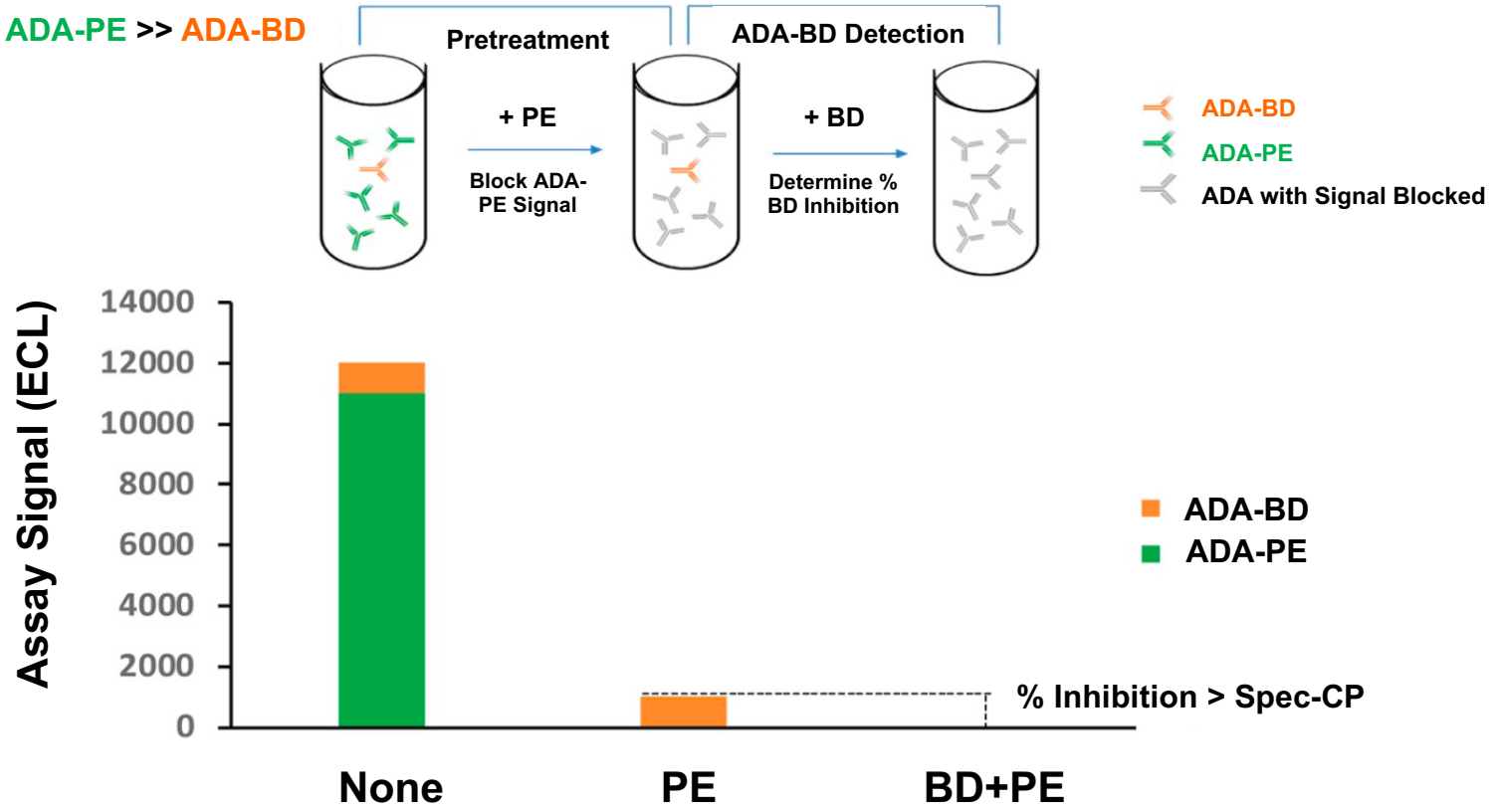


ADA Abundancy Impacts its Reliable Detection





Approach Enabling Detection of Low Abundant ADA

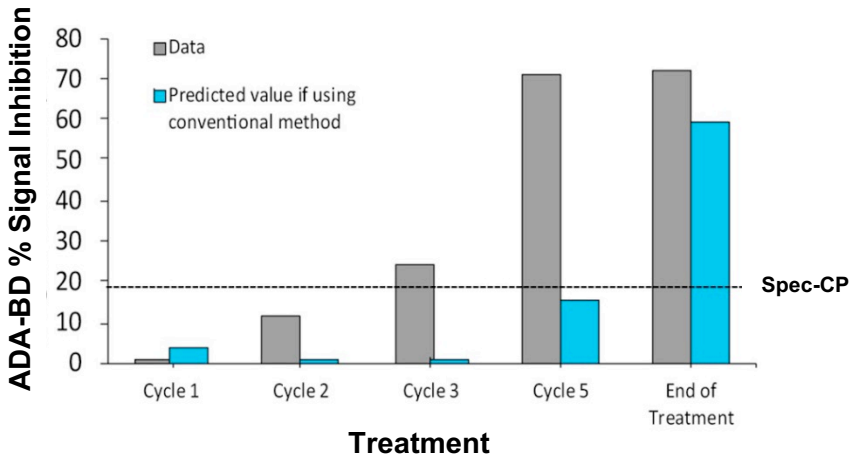


Comparison: Conventional and New Methods

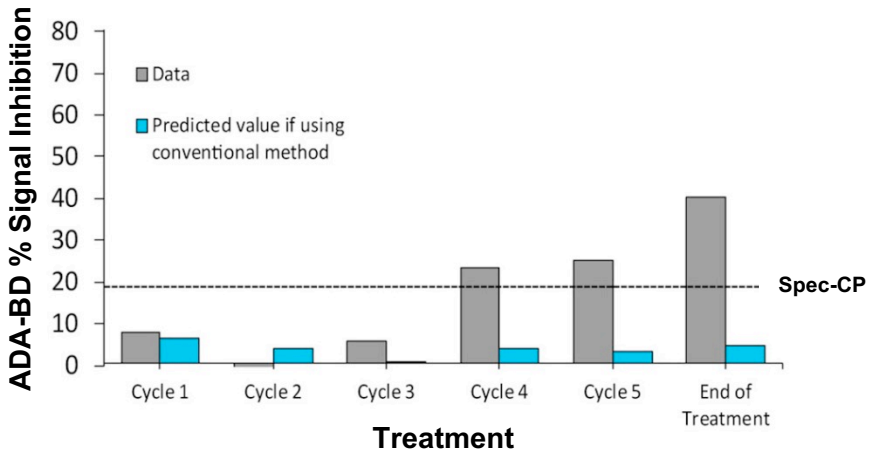
ADA Content, ECL			ADA-PE		ADA-BD	
Endogenous ADA-PE	**Spiked ADA-BD**	**ADA-PE : ADA-BD**	**Conventional**	**New**	**Conventional**	**New**
377	1760	1 : 5	False Neg	Pos	Pos	Pos
1524	1604	1 : 1	Pos	Pos	Pos	Pos
19410	1518	13 : 1	Pos	Pos	False Neg	Pos

Conventional Approach Could Result in Inaccurate ADA-BD

Patient A: conventional approach could lead to mis-interpret the onset of ADA-BD response

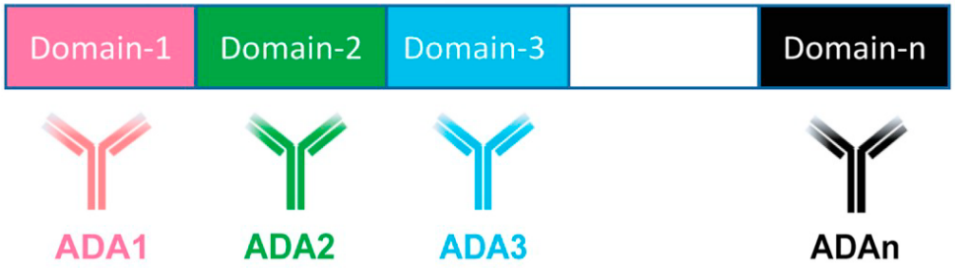


Patient B: conventional approach could lead to false negative classification of ADA-BD

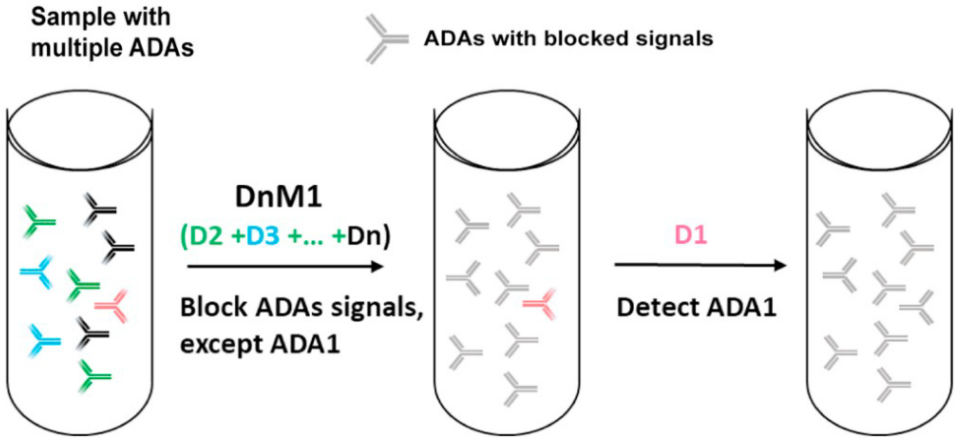


Novel Approach Applicable to All MDBs

Multi-domain molecule



Scheme of ADA1 Determination



Summary: Assessment of ADA Domain Specificity for MDBs

Regulatory Expectation

Risk-based assessment of domain specificity for MDBs

Challenge

Detection of low abundant ADA in a polyclonal sample for the commonly used competition method

Solution

A novel approach proven to effectively overcome the challenge can be broadly applied to all MDBs

If in doubt about whether domain-specificity assessment is needed, sponsors should **consult with the corresponding regulatory agencies**

Acknowledgements

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Vainshtein I, Sun B, Roskos LK, Liang M. A novel approach to assess domain specificity of anti-drug antibodies to moxetumomab pasudotox, an immunotoxin with two functional domains. J Immunol Methods. 2020 Feb;477:112688

Thank You