U.S. FOOD & DRUG ADMINISTRATION

FDA Regulatory Perspectives on Neutralizing Antibody Assays

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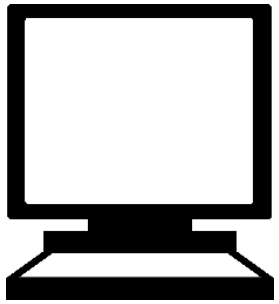
Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.


Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

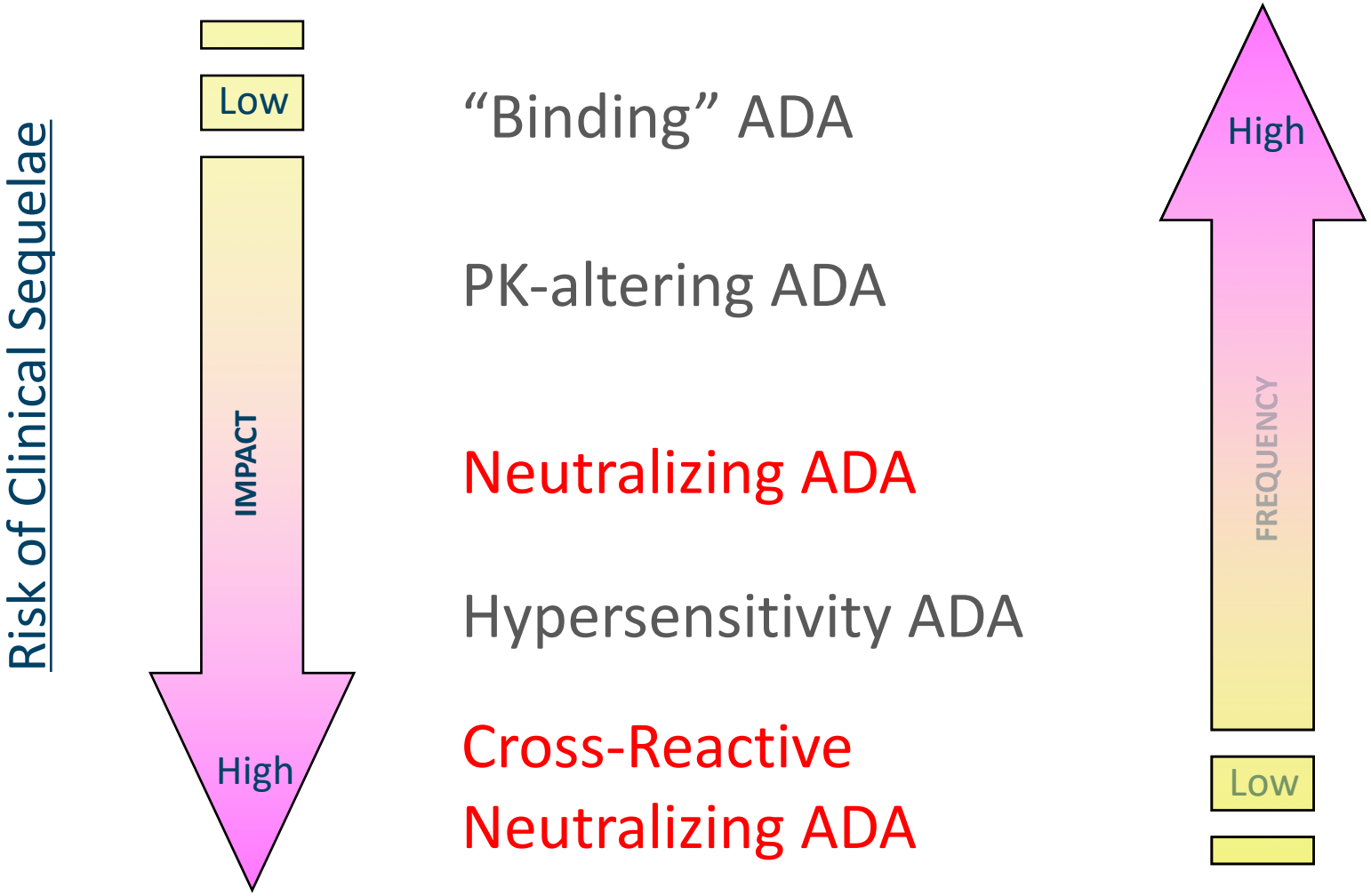
assuring *every* dose is safe and effective, free of contamination and defects.

It is what gives patients confidence in their *next* dose of medicine.

Disclaimer

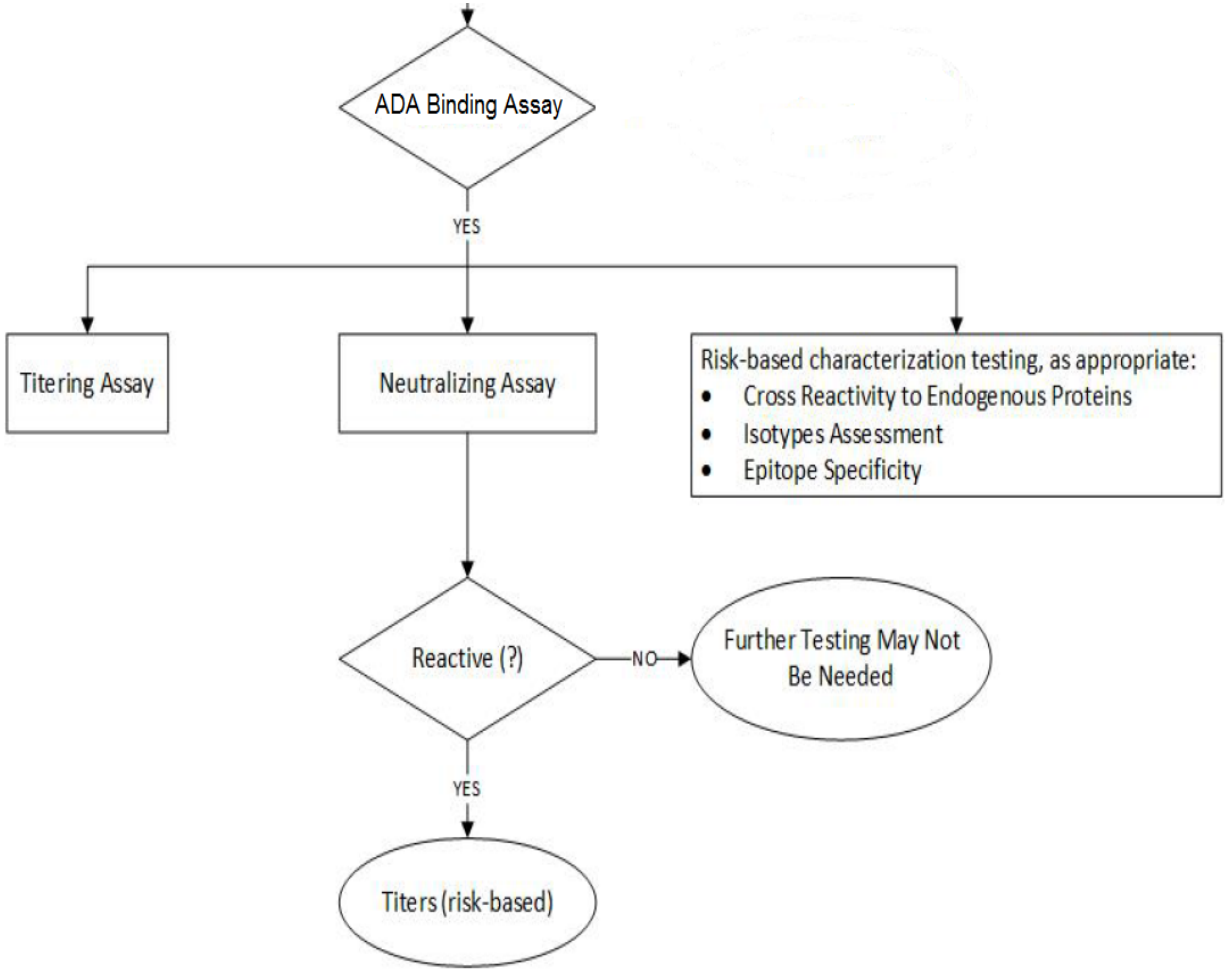
- The views and opinions expressed herein should not be used in place of regulations, published FDA guidances, or discussions with Agency
- Limited to therapeutic biologics regulated by CDER

Clinical significance of Anti-drug Antibodies



What is Neutralizing ADA (NAb)?

- A subset of binding ADA
- Interfere with the clinical activity of a therapeutic protein product by
  - Preventing product binding to the target
  - Interfering pharmacological activity (such as receptor-ligand interaction)
  - Preventing product cellular uptake
- NAb assay results indicate the neutralizing potential of ADA



Clinical Significance of NAbs

- Binding ADA may impact PK which can lead to loss of efficacy
- NAb directly inhibits drug function (loss of efficacy) and potentially inhibits biological function of the endogenous counterpart (safety).
- NAbs may be more likely to directly impact drug efficacy
  - e.g. IFN-b and IL-2

Risk Based Assay Development Strategy

- NAb assays are critical when neutralizing immunogenicity poses a high-risk to patient safety
  - **High Risk**: qualified/validated assays should be implemented early (phase I)
  - **Medium to Low risk**: validated assay prior to testing clinical phase 3 study samples.

NAb Assay Development: Assay Format

- Types of assays generally used:
  - Cell-based biologic assays (CBA) and
  - Non-cell-based competitive ligand-binding assays (CLBA).

FDA recommends that neutralization assays use a cell-based bioassay format; alternative strategy might be acceptable.

- Factors affect selection of appropriate assay format:
  - mechanism of action (MoA) of the therapeutic protein product, the selectivity, sensitivity, precision, and robustness of the assay.

- In selected cases, where there is a highly sensitive PD marker or an appropriately designed PK assay or both that generate data that inform clinical activity, it may be possible to use these in lieu of a NAb assay. This determination should be done in consultation with the Agency.

MoA Guided Assay Format Selection

MoA	Drug Modality	Drug Target	Drug-target Interaction	Examples	Recommended Assay Format
Agonist	Recombinant protein or antibody	Cellular receptor	Drug binds and activates cellular receptor	cytokines, growth factors, EPO	Cell-based assay [1]
				Agonists with no homology to endogenous protein	Cell-based assay as primary choice, non cell-based as an alternative [1]
Antagonist	Monoclonal antibody	Humoral target	Drug binds and inhibits the target	Golimumab, Ustekinumab, Adalimumab	Non cell-based CLB assay
	Monoclonal antibody	Cellular receptor	Drug binds cellular receptor and competitively inhibits receptor-ligand interaction	Natalizumab, Trastuzumab, Tocilizumab	Cell-based assay or non cell-based CLB [2]
	Soluble receptor	Ligand	Soluble receptor binds ligand and blocks receptor-ligand interaction	Etanercept, Abatacept	Non cell-based CLB or cell-based CLB assays
Targeted intra-cellular delivery of a potent cytotoxin mediated by antibody	ADC	Cellular receptor	ADC binds to cellular receptor and mediates the internalization of payload	Brentuximab vedotin, Ado-trastuzumab emtansine	Cell-based assay(s)
Target cell lysis through antibody effector function	Monoclonal antibody	Target cell receptor, FcγR or complement	Antibody binds to target cell receptor through variable region and FcγR or complement through Fc domain	Rituximab, Cetuximab, Alemtuzumab	Cell-based effector assay recommended, cell-based binding assay or non cell-based CLB assay acceptable with justification [4]
Enzyme replacement	Enzyme	Cellular receptor for enzyme uptake	Enzyme functions in circulation or through cellular uptake	Human factor IX, Imiglucerase, Idursulfase, Galsulfase	Enzyme bioactivity assay and/or cell-based assay; two assays may be needed [5]

Wu B et al AAPS J. 2016 Nov;18(6): 1335-1350.

NAb Assay Validation

- Where should I set the cut point?
  - 1% false-positive rate (for NAb assay performed on confirmed ADA positive samples)
- What if my NAb assay sensitivity is poor?
  - Activity curve and selection of therapeutic product concentration
  - Further optimization?
  - Change from bioassay to CLBA, if applicable.
  - Neutralizing activity of positive control
  - Clinical data interpretation
- What if my cell based NAb assay precision is poor?
  - Further optimization
  - Analysts training
  - Consider performing more replicates

NAb Assay Validation

- Matrix Interference
  - matrix components may enhance or inhibit the activity of a therapeutic protein product in bioassays.
  - Test results from baseline pre-exposure samples may be informative.
- Specificity
  - Confirmatory NAb assay is not common, but it should be considered in certain cases
- Drug Tolerance
  - Further optimization: consider introducing procedure(s) to remove drug in patient samples
  - Change from bioassay to CLBA, if applicable.
  - Clinical interpretation

How Can Industry facilitate assessment of NADA assays by regulators?

POSSIBLE SOLUTIONS

Harmonized Validation Template(s)

- Allow for triage of immunogenicity assays
  - –Help with setting workload timelines
  - –Facilitate assay assessments by OBP and inspections by OSIS
- Standardize quality of validation reports
  - Common terminology of parameters
  - Common order for data presentation
- May make immunogenicity assessments less time-consuming

OBP ADA Validation Assessments

- OBP is currently using tabular summaries adapted from Harmonized ADA Validation Template as part of its BLA Immunogenicity Assessment Memos:
  - Binding antibody assays (screening, confirmatory, titer)
  - Neutralizing antibody assays
  - Adapted for use with 351 (a) innovator products and 351 (k) biosimilars ADA assay validation requirements

Example of an OBP 351 (a) innovator product NADA Assay Validation Summary

- Intended to reduce size of OBP immunogenicity assessments

- Detailed data is discussed in "additional assessor comments" section when validation issues are uncovered

- We also include an assessment of assay performance in the pivotal clinical studie in a separate memo section

- For 351 (k) biosimilar product table we include section on antigenic equivalence and information on DP lots for both biosimilar and RP used in the validation
  - Relationship to clinical material

Table A: Validation Results and Assessor Analysis for NADA assay(s) used in Pivotal studies (Validation Reports xxx)

Validation Parameter	Clin Study XX Validation Report: XXX	Clin Study YY Validation Report: YYY	Assessor Comment
Contract Research Org			
Assay principle			
Sample Pretreatment (Acid dissociation)			
Positive control (PC)			
PC Dose Curve and Hook Effect			
LPC			
HPC			
Matrix and NC			
MRD			
NC system suitability range			
LPC system suitability range			
HPC system suitability range			
NAb assay cut- point (NACP) Normalized CP: mean S/N-3.09*SD			
Assay Drug tolerance			
Target tolerance			
Sensitivity			
Repeatability/Intra-assay variability	NC %CV LPC %CV HPC %CV	NC %CV LPC %CV HPC %CV	
Intermediate Precision (IP)/inter-assay variability	NC %CV LPC %CV HPC %CV	NC %CV LPC %CV HPC %CV	
Selectivity			
Robustness			
Stability			
Lipemia			
Hemolysis			
NADA Assay Assessment	**Suitable/Not suitable for Intended purpose**	**Suitable/Not suitable for Intended purpose**	

Summary

- NAb can affect product efficacy and safety
- Risk based assay development strategy
- Selection of NAb assay format:
  - Based on MoA,
  - Consider assay performance.
- NAb assay validation
  - Use of harmonized template could facilitate review processes

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