NAb me well- FDA Regulatory Perspectives on Neutralizing Antibody Assays.

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Therapeutic Proteins

- Therapeutic proteins (Biologics/Biotherapeutics) are >40 aa polypeptides whose active components are derived from a biological source by being produced in a living production system using biotechnology.
  - Mammalian, Bacterial, Yeast, Insect, Plant
  - No primary chemical synthesis, but could have chemical modifications
  - Characterized by critical quality attributes (CQA) which impact safety and efficacy
Therapeutic Protein Development

The Immunogenicity Barrier
FOREIGN

• Low abundance self-protein
• Aggregates of self proteins
• PTMs or chemical degradation of self proteins
• Adjuvants

SELF

Expect Immunogenicity
No tolerance
Neutralize Product
Hypersensitivity

Potential Immunogenicity
Incomplete tolerance
Altered structure/
Antigen Present
Epitope spreading

Rare Immunogenicity
Robust tolerance
Novel Route of Administration
Adjuvants
HLA Haplotype Specific
What is Therapeutic Protein Immunogenicity?

• the immune response of the host against the therapeutic protein

• The immune responses generally include both innate and adaptive responses.

• Adaptive responses include both T cell and B Cell responses
  – Immunogenicity assessment primarily measures antibodies directed against TP (anti-drug antibodies, ADA) are
    • Analytically defined as “Binding” and/or “Neutralizing antibodies” depending on assay used to detect them
    • may consist of IgM, IgG, IgE, and/or IgA isotypes.
## Concerns for Antibodies in the Clinic

<table>
<thead>
<tr>
<th>Clinical Concern</th>
<th>Clinical Outcome</th>
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| **Safety**           | • Neutralize activity of endogenous counterpart with unique function causing deficiency syndrome  
                         • Hypersensitivity reactions |
| **Efficacy**         | Enhancing or decreasing efficacy by:  
                          • changing half life.  
                          • changing biodistribution. |
| **Pharmacokinetics** | • Antibody production may dictate changes in dosing level due to PK changes. |
| **None**             | • Despite generation of antibodies, no discernable impact |
Therapeutic Protein Immunogenicity at the FDA

• Who reviews it?
  – CBER- allergenics, blood and blood components, cellular and gene therapies, vaccines

  – CDER - Hormones, cytokines, enzymes, monoclonal antibodies, fusion proteins, growth factors, thrombolytics, therapeutic toxins
Office of Biotechnology Products (OBP)

- CMC for therapeutic proteins under CDER purview
- Responsible for reviewing immunogenicity risk assessments and clinical immunogenicity assays for biologics and drugs at CDER
  - Involved in writing FDA Immunogenicity guidances:
    - Draft Guidance (2012): Scientific Considerations In Demonstrating Biosimilarity To A Reference Product
    - Guidance (2014): Immunogenicity Assessment for Therapeutic Protein Product
OBP

• Previously (2002-2014) •
two divisions divided along product lines
  – Division of Therapeutic Proteins
    • Cytokines, growth factors, therapeutic toxins, non-Fc fusion proteins
  – Division of Monoclonal Antibodies
    • MAbs and therapeutic Fc-Fusion proteins

January 2015- Four divisions with mixed Biologics portfolios
  – Divisions of Biotechnology Review and Research I-IV
  – OBP Immunogenicity Working Group
Neutralizing Antibodies (NAbs)

- NAbs are a subset if BAbs
- Binding inhibits receptor/ligand interactions
- Detected using a cell based or non-cell based assay
  - Functional definition
Clinical Significance of NAbs

• In a patient both BAbs and NAbs can lead to loss of efficacy and/or negatively impact safety, therefore both may be clinically important

• NAbs may be more effective in directly impacting efficacy

• Importance of well performed immunogenicity risk assessment
Evaluating TP Immunogenicity

• Guidance (2014): Immunogenicity Assessment for Therapeutic Protein Product


• Guidance (2012): Scientific Considerations In Demonstrating Biosimilarity To A Reference Product
I. Introduction

“FDA guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.”
Guidance for Industry
Immunogenicity Assessment for Therapeutic Protein Products

GUIDANCE

This guidance document is being distributed for comment purposes only.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
August 2014
Clinical/Medical
Predicting the Likelihood of Product Immunogenicity

• Patient related factors

• Treatment-related factors

• Product/CMC related factors
Patient Related Factors

- Patient population
  - Healthy Subjects
  - Immune-competency of patient population
  - Proinflammatory environment
    - innate or adaptive
- Genetics
- Age
- Gender
- Pre-existing antibodies
  - Prior exposure to antigen
  - Cross-reactive antibodies
Trial Design Specific Factors

- Route of Delivery (Oral, IV, IM, SC)
- Dose and Frequency of Administration
- Immunomodulatory Properties of Product
- Stage of product development
Product Specific Attributes

• Molecular Structure
  • Source of protein sequence (foreign, self, hybrid/fusion)
    • Modified amino acids,
    • Neo-epitopes

• Production system
  • Impact on glycosylation*
    ▪ Non-human glycoforms
    ▪ Glycosylation patterns not native to endogenous protein
      ▪ Exposure of cryptic epitopes
Manufacturing related issues

- Purity
  - Process related impurities
    - Host Cell Proteins
    - Host Cell DNA
  - Product related variants at release and on stability
    - Aggregates
    - Clipped forms
    - Oxidized/deamidated
    - Denatured product
Product Specific Attributes

- Formulation
  - Control of product degradation and aggregation
  - Glycation
  - PK control

- Product mechanism of action
  - Immunosuppressive vs. pro-inflammatory
Recommendations

A RISK BASED approach is required to balance the potential harm with potential good of new product

- Likelihood of developing an immune response
- Risk of immune response to patient
- Are there alternatives
- Stage of Development
- Reversibility
Guidance for Industry
Assay Development for Immunogenicity Testing of Therapeutic Proteins

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
December 2009
CMC
I. Introduction

• Recommendations to facilitate development of immune assays for assessment of the immunogenicity of therapeutic proteins during clinical trials
  – binding assays
  – confirmatory assays
  – neutralizing assays

• Does not specifically discuss the development of immune assays for preclinical studies, however the concepts discussed are relevant

• Does not discuss the product and patient risk factors that may contribute to immune response rates.
  – Discussed in detail in draft guidance titled *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014)
I. Introduction

- This guidance does not pertain to immunogenicity assays for assessment of immune response to preventative and therapeutic vaccines for infectious disease indications.
  For information on Vaccine products, see guidance titled *General Principles for the Development of Vaccines to Protect against global infectious diseases* (December 2011)

- In addition, this document does not specifically discuss how results obtained from immunoassays relate to biosimilars.
  For information on proposed biosimilar products, see draft guidance titled *Scientific considerations in Demonstrating Biosimilarity to a Reference Product* (February 2012)
General Discussion

• Assays are critical when neutralizing immunogenicity poses a high-risk therefore real time data concerning patient responses are needed

• Preliminary validated assays should be implemented early (preclinical and phase I).
General Discussion

• Therapeutic proteins are frequently immunogenic in animals.
  – Immunogenicity in animal models is not predictive of immunogenicity in humans.
  – Assessment of immunogenicity in animals may be useful to interpret nonclinical toxicology and pharmacology data.
  – Immunogenicity in animal models may reveal potential antibody related toxicities that could be monitored in clinical trials.
Immunogenicity Testing During Product Development

• Assay differences can make immunogenicity comparisons across products in the same class invalid.
• Therefore, in the product labeling, FDA does not recommend comparing the incidence of antibody formation between products when different assays are used.
• A comparison of immunogenicity across different products in the same class can best be obtained by conducting head-to-head patient trials using a standardized assay that has equivalent sensitivity and specificity for both products.
Overview of Design Elements: A Multi-Tiered Approach

Sensitive screening immunoassay

Reactive

Confirmatory assay (titration, immunodepletion)

Reactive

Neutralizing Bioassay

Positive

Negative

IgG
IgM
IgE*
IgA^*

*hypersensitivity reactions
^when route of administration is mucosal
Obtaining Patient Samples

- Pre-exposure samples should be obtained from all patients.
- Subsequent samples should be obtained with timing depending on the frequency of dosing.
- Samples should be obtained when there will be minimal interference from product present in the serum.
- If drug-free samples cannot be obtained during the treatment phase of the trial, then additional samples should be obtained after an appropriate washout period (e.g., five drug half-lives).
- If the product in is an immune suppressant samples should be obtained from patients who have undergone a washout period
Reporting Results

• Results of patient sample testing are often reported as positive vs. negative

• An assessment of antibody levels may be informative. FDA, therefore, recommends that positive antibody responses be reported as a titer (e.g., the reciprocal of the highest dilution that gives a value equivalent to the cut point of the assay).
Reporting Results

- Values may also be reported as amount of drug (in mass units) neutralized per volume serum with the caveat that these are arbitrary in vitro assay units and cannot be used to directly assess drug availability in vivo.
- Antibody levels reported in mass units based on interpolation of data from standard curves generated with a positive control standard antibody are generally less informative because interpretation is based on the specific control antibody.
Types of assays generally used: cell-based biologic assays and non cell-based competitive ligand-binding assays.

FDA considers that bioassays are more reflective of the in vivo situation and are recommended.

The bioassay should be related to product mechanism of action to be informative as to the effect of NAb on clinical results.

Competitive ligand-binding assays may be the only alternative in some situations.

Assays may use direct (inhibition of stimulation) or indirect (inhibition of inhibition) assessment.
Neutralizing Assay: Activity Curve

• Most commonly the neutralization assay employs a single concentration of product with different concentrations of antibody samples added to determine neutralizing capability.
• A product concentration whose activity readout is sensitive to inhibition should be used.

Figure 1. Activity Curve for a Representative Therapeutic Protein
Neutralizing Assay Validation: Cut Point

- The determination should be statistically based and derived from assays using samples from patients not exposed to the product.
- If variation makes it difficult to assess neutralizing activity, other approaches may be considered but should be discussed with FDA.
- Alternatively, exploring other assay formats that lead to less variability and provide a more accurate assignment of cut point may be necessary.
Neutralizing Assay Validation: Specificity

• Assay specificity should be demonstrated.

• For cells that may be responsive to stimuli other than the specific therapeutic protein, the ability to demonstrate that NAb only inhibit the response to product and not to other stimuli is a good indication of assay specificity.

• The applicant should also confirm the absence of alternative stimuli in patient serum/matrix.
In-study performance: Concurrent Positive and Negative Quality Controls

- Positive control or QC samples are critical and should be run concurrently with patient samples.

- QC samples should have known negative, low, medium, and high reactivity in the assay.

- More importantly, the samples should be diluted in the assay matrix

- Low positive control samples should be selected based upon statistical analysis that would lead to the rejection of an assay run 1 percent of the time.

- Should be detected by the secondary detecting reagent, to ensure that negative results that might be observed
Additional Parameters

• The following are parameters that should be established but may not need to be confirmed in a validation exercise. [However supporting data should be provided to the Agency to establish assay suitability.]
  – Amount of drug used if applicable
  – MRD
  – Robustness
    • Cell passage number
    • Incubation times
    • Media components
    • Others as applicable
  – Reagent stability
  – Positional effects
Additional Validation Parameters

• Sensitivity - Similar to the binding assay sensitivity should be reported in mass units.

• Precision
  – Intra-assay and inter-assay

• Ruggedness (if applicable)
Guidance for Industry
Scientific Considerations in Demonstrating Biosimilarity to a Reference Product

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Center for Biologics Evaluation and Research (CBER)

February 2012
Biosimilarity
Biosimilarity Guidance: the clinical program for a 351(k) application

• must include a clinical study or studies including an assessment of immunogenicity and PK or PD sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for which the reference product is licensed and intended to be used and for which licensure is sought for the biological product, as set forth in the Public Health Service Act.

• Lessening the number or narrowing the scope of any of these types of clinical studies (i.e., human PK, PD, clinical immunogenicity, or clinical safety and effectiveness) should be scientifically justified by the sponsor.
Biosimilarity and immunogenicity

- Immunogenicity (the capacity of the product to induce an immune response) is evaluated separately from pK (how the body acts on a product) and pD (how the product affects the body).

- Immunogenicity study(ies) are part of the totality of evidence required to establish “biosimilarity” between a 351 (a) reference product and 351 (k) biosimilar applicant.
  - A key element to demonstrate there are “no clinically meaningful differences”.

Clinical immunogenicity assessment

• The goal of clinical immunogenicity assessment is to evaluate potential differences between the proposed biosimilar product and the reference product in the incidence and severity of human immune responses.

• Assays must be capable of sensitively detecting immune responses, even in the presence of circulating drug product (proposed product and reference product).
Anti-Drug Antibody Assessment

• Binding antibody:
  – titer, specificity, relevant isotype distribution, time course of development, persistence, disappearance, and association with clinical sequelae.

• Neutralizing antibody:
  – all of the above, plus neutralizing capacity to all (known) relevant functions (e.g., uptake and catalytic activity, neutralization for replacement enzyme therapeutics).
Assay development

• One assay based on the biosimilar product used to test serum samples from both biosimilar and reference product treated subjects.
  – For NAb assay, if bioassay is used, should show cellular responsiveness to both biosimilar and reference product.
Performance characteristics assessed in validation exercise

• Assay cut points
• Sensitivity
• Specificity
• Selectivity/interference
• Precision
• System suitability acceptance criteria (QC)
• Robustness
• Stability*
• Ruggedness (when applicable)

*Some argue that this does not need to be part of the validation exercise
LESSONS LEARNED

• Immunogenicity *will* likely happen for most therapeutic proteins
  – Multi-disciplinary risk based analysis early in product development.
  • The higher the risk category for the product, the faster the pace of assay development should take place.
LESSONS LEARNED

• There are many product related factors which influence immunogenic responses to therapeutic proteins
  – It is a safety concern, there is a need to assess/measure it.
    • NAb me well
    • Correlate with clinical data (AE, pK and pD)
Regulatory Expectations

- There are regulatory expectations from the FDA
  - Sponsors need to provide risk assessment and appropriate sampling plan
  - Sponsors need to develop validated immunogenicity assays
    - Binding antibody assay
    - Neutralizing antibody assay
  - phase dependent assay development
    - Have assay validated prior to testing clinical phase 3 study samples
    - Crucial to have appropriately stored study samples
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