The immunogenicity of therapeutic proteins- what you don't know can hurt YOU and the patient

João A. Pedras-Vasconcelos, PhD
CMC and Immunogenicity Reviewer
Division of Therapeutic Proteins
OBP/CDER/FDA

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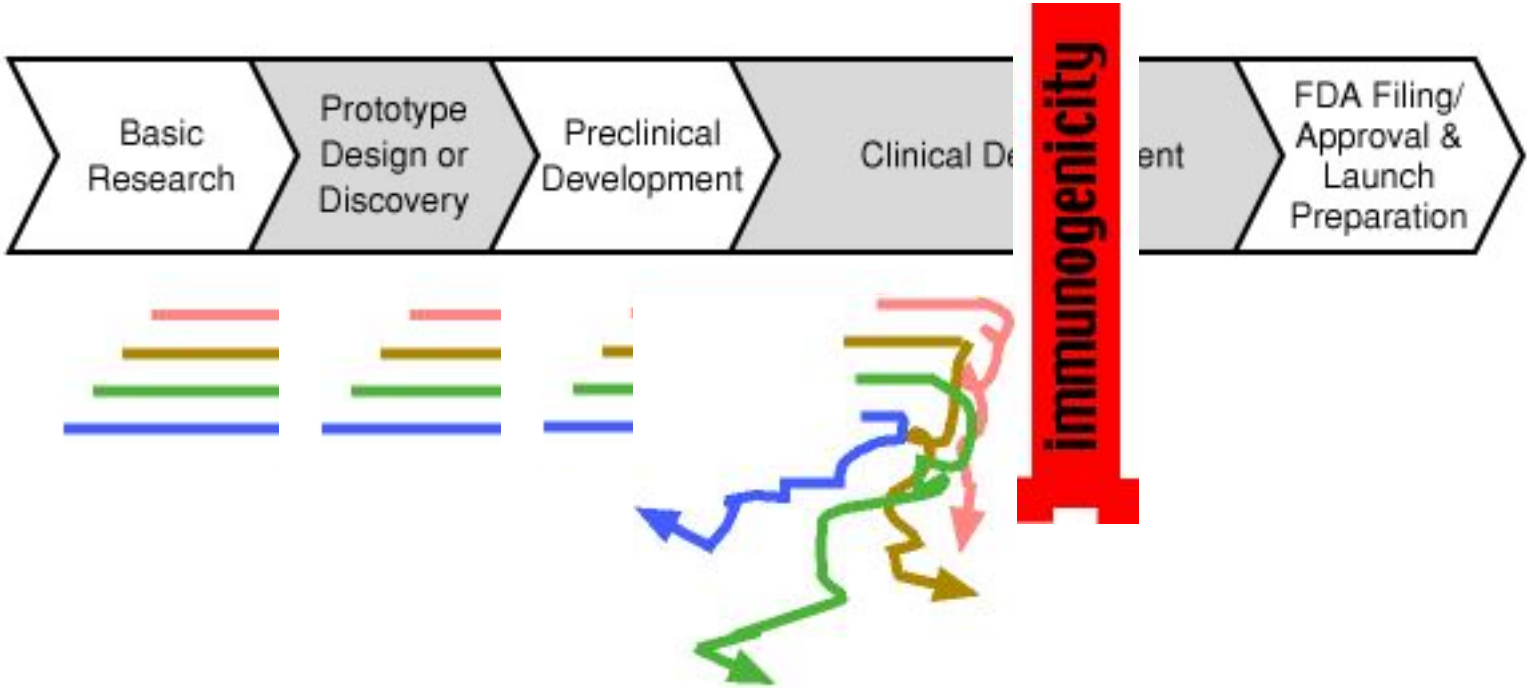
Outline

- General Introduction to Immunogenicity
- What are the consequences of immune responses to therapeutic proteins
- Evaluating Immunogenicity
  - Draft Guidance (2013): Immunogenicity Assessment for Therapeutic Protein Product
  - Draft Guidance (2009): Assay Development for Immunogenicity Testing of Therapeutic Proteins
- Summary

Therapeutic Proteins

- Therapeutic proteins (Biologics/Biotherapeutics) are >40 aa polypeptides whose active components are derived from a biological source by being produced in microorganisms and cells (humans and animals) using biotechnology
  - Not chemically synthesized
  - E.g. Hormones, cytokines, enzymes, antibodies, fusion proteins

Product Development

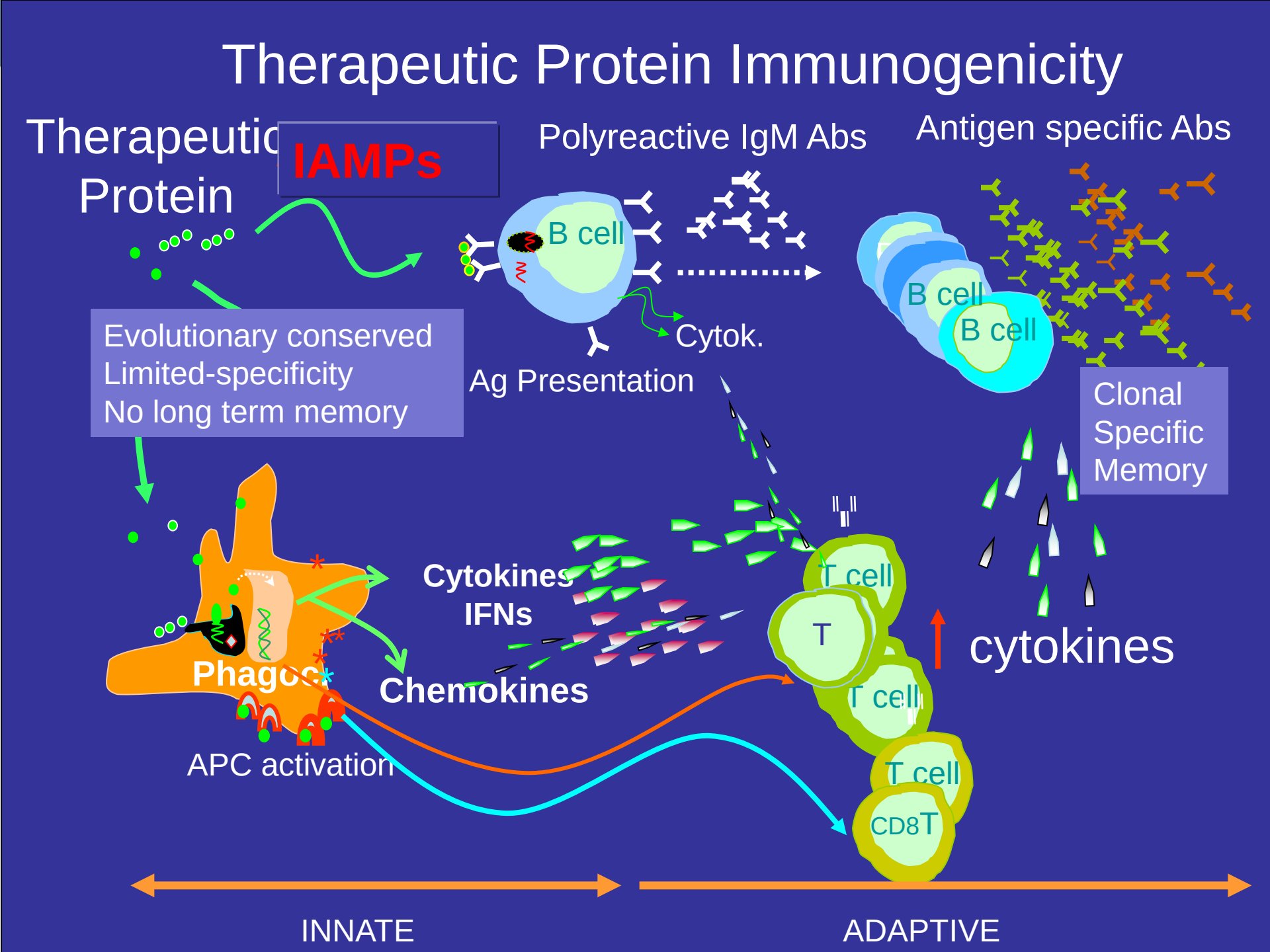


The Immunogenicity Barrier

Dr. Ed Max, DTP/OBP

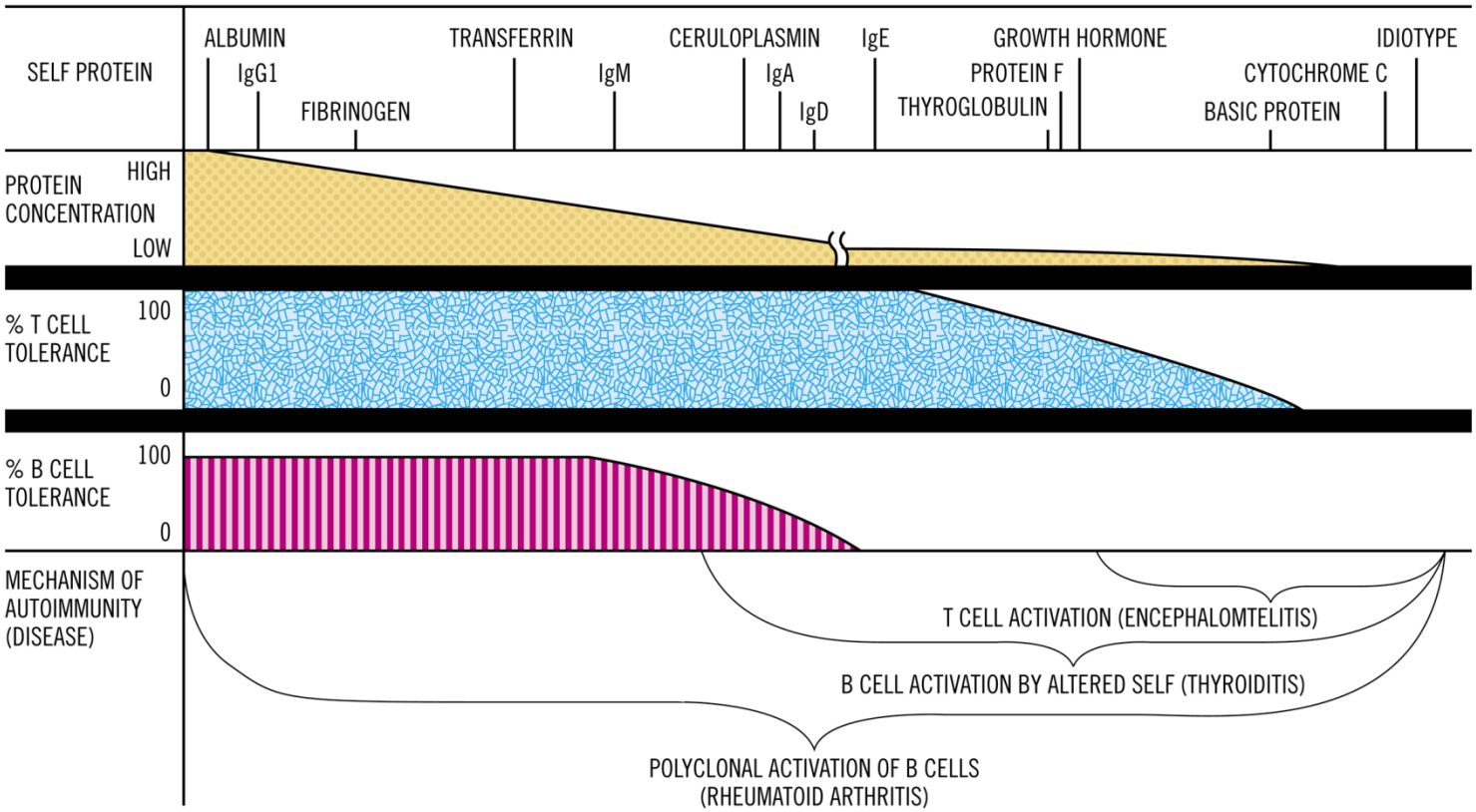
What is Therapeutic Protein Immunogenicity?

- In the context of Therapeutic Proteins product, immunogenicity refers to the immune response of the host against the Therapeutic Protein
- The immunogenic response generally includes both cellular (T cell) and humoral (antibody) arms of the immune response, however we usually measure antibodies. Antibodies directed against TP (anti-drug antibodies, ADA) may consist of IgM, IgG, IgE, and/or IgA isotypes.
  - interactions between antigen presenting cells, T-helper cells, B-cells, and their associated cytokines.


Therapeutic Protein Immunogenicity
Therapeutic Protein
IAMPs
Polyreactive IgM Abs
Antigen specific Abs
B cell
Cytok.
Ag Presentation
B cell
B cell
Evolutionary conserved
Limited-specificity
No long term memory
Clonal Specific Memory
Cytokines
IFNs
Chemokines
Phagocyte
APC activation
T cell
T
T cell
T cell
CD8T
cytokines
INNATE
ADAPTIVE

Immune Tolerance to Endogenous Proteins Present at Low Levels is Incomplete

(Weigle, 1980)

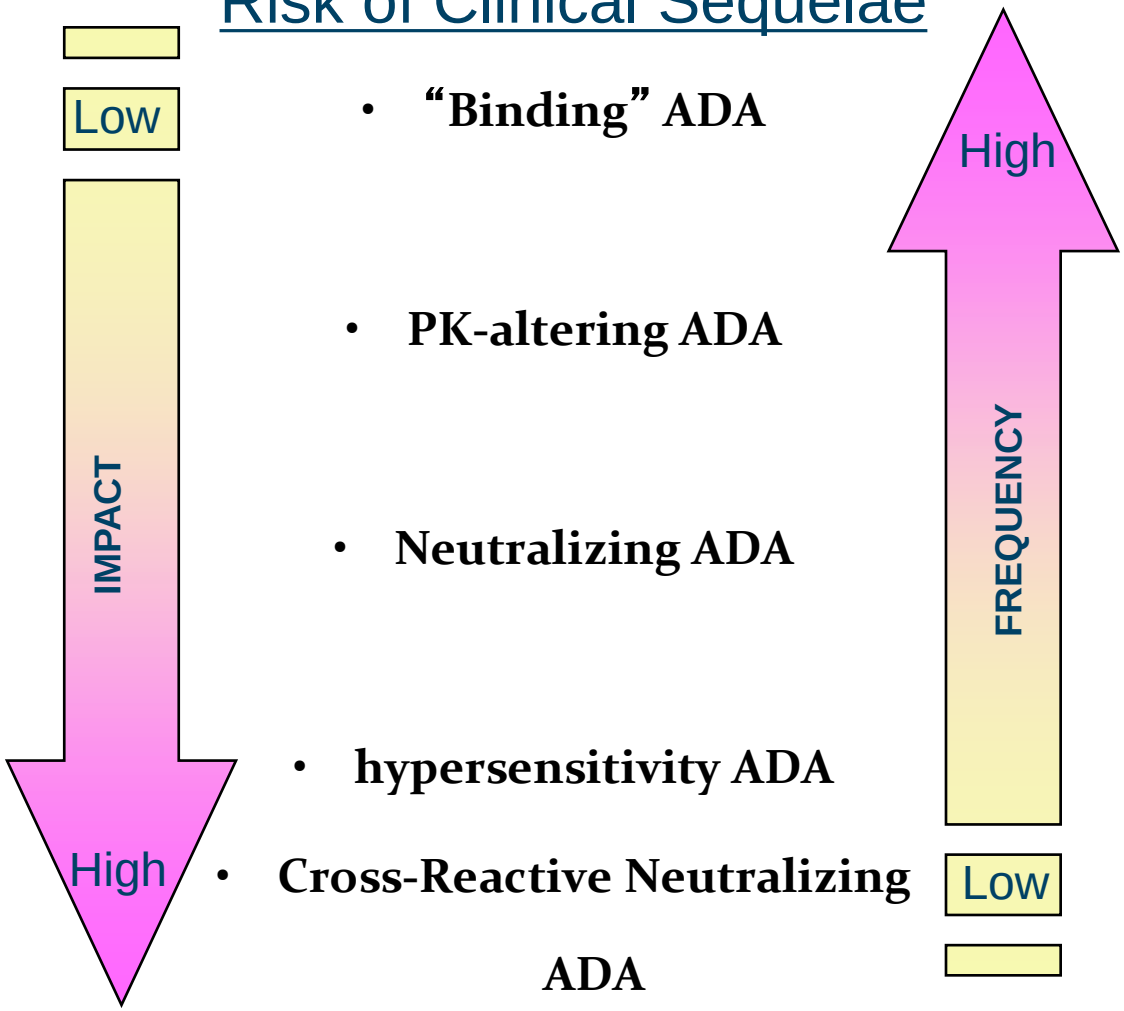


Types of ADA

- In practice, immunogenicity in the clinic is assessed by the detection of ADA.
- Binding Antibodies (BAbs)
  - All isotypes capable of binding the TP
  - Detected in an Immunoassay; common formats used:
    - Enzyme-linked immunosobent assay (ELISA),
    - Radioimmunoprecipitation assay (RIP)
    - Electrochemiluminescence immunoassay (ECLIA)
    - Surface plasmon resonance immunoassay (SPRIA)
- Neutralizing Antibodies (NAbs)
  - A subpopulation of the total BAbs
  - Inhibit functional activity of the TP; generally directed against biologically active site
  - Generally detected in a cell-based in vitro bioassay or competitive ligand binding assay

Clinical Immunogenicity

Risk of Clinical Sequelae



In general, the severity of ADA impact and its frequency of occurrence have been inversely correlated

There have been **real life** examples in which immune responses to therapeutic proteins have had devastating consequences for healthy volunteers and patients.

- **rhEPO**
- **rhMGDF/TPO**
- **Glucocerebrosidase (Gaucher's)**
- **α-glucosidase (Pompe's)**
- **α-galactosidase A (Fabry's)**

Case Example: EPO

- 1999-2003 increased occurrence of pure red cell aplasia (PRCA) associated with the induction of neutralizing antibodies (IgG4) to native erythropoietin following subcutaneous administration for 3-67 months.
- Multiple product related-factors involved
  - Change in formulation
  - Change in container closure system
  - Improper storage conditions

Case Example: PEG-MGDF/TPO

- Biologically Unique Function of MGDF/TPO: megakaryocyte/ platelet growth and developmentfactor/thrombopietin
- Neutralizing antibody caused thrombocytopenia in healthy platelet donors (4%) and oncology patients (0.5%).
  - *Illustrates effect of immune status of host*
- In healthy donors, tolerance was easily broken (2-3 doses) in some cases
- Thrombocytopenia developed in all animal models tested, including non-human primate using species specific product
- Antibody was present in some patients prior to treatment
- Weekly (“real-time”) monitoring for patient antibodies was required during study using multiple ADA assays

Lysosomal Storage Disoders Enzyme Replacement Therapy

- Over 40 different lysosomal storage disorders that collectively occur in ~1/7000 live births

- Gaucher's: ~13% of patients develop Ab to glucocerebrosidase and 90% of patients tolerize over time.
  - A few patients develop neutralizing Ab that is associated with either a plateau in improvement or disease progression.
  - Non-neutralizing Ab are associated with infusion reactions. These disappear over time as well but have been known to return years later.
  - The development of Ab is associated with the severity of the genetic lesion.

Lysosomal Storage Disoders Enzyme Replacement Therapy

- Pompe's disease:
  - All patients developed Ab on acid β-glucosidase replacement therapy. CRIM (Cross Reactive Immunological Material) negative status was associated with high titer Nab generation.

- Fabry's disease:
  - Patients with α-galactosidase A activity $\leq$ 0.5 nmol/mg protein/hr developed Nab (10/12) than patients with > 1.1 nmol/mg protein/hr (1/4)

Evaluating Immunogenicity

- Guidance (2014): Immunogenicity Assessment for Therapeutic Protein Product
- Daft Guidance (2009): Assay Development for Immunogenicity Testing of Therapeutic Proteins

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.
  - May reveal immunogenicity differences between biosimilar and reference product.

Disclaimer

- "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

- Product derivation
  - Foreign, self, or fusion
- Product-specific attributes*
- Patient specific factors
- Trial design attributes

Predicting the Likelihood of Immunogenicity

Product Specific Attributes

- Molecular Structure
  - Aggregates
  - Changes to primary sequence
  - Fusion proteins
  - Exposure of cryptic epitopes e.g. due to glycosylation changes
  - Modified amino acids
  - Glycosylation*
    - Non-human glycoforms
    - Glycosylation patterns not native to endogenous protein

Glycosylation

- Glycans can modify epitope access
- Antibodies against non-human sugars are found with varying incidence in humans – do they impact safety and efficacy?
  - NGNA – perhaps up to 85% incidence in healthy population (Zhu A and Hurst R. 2002. Xenotransplantation 9(6)376-381)
  - Plant sugars – varies depending on the linkage and sugar
  - Galα1,3Gal – most humans
- Non-native glycans such as yeast high mannose glycans may appear foreign

Predicting the Likelihood of Immunogenicity

Product Specific Attributes

- Purity
- Process related variants
  - Host Cell Proteins
  - Host Cell DNA
- Product related variants at release and on stability
  - Clipped forms
  - Oxidized/deamidated isoAsp residue formation*
  - Aggregates*
  - Denatured product

Biotechnology Process

Impurities can come from all steps

Natural source

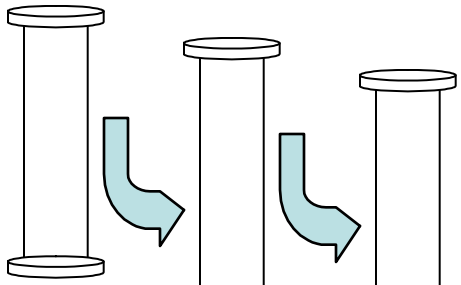
Adventitious agents

Harvest Protein mixture

Host cells

Mammalian
Bacteria
Yeast
Insect
Plant

Fermentor/ Bioreactor

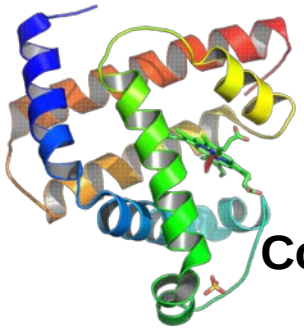


Down stream purification Chromatography Columns

Bioburden

Formulation Filling

Container/closure



Complex Drug Product

Impurities
"IIAMPs"

Immunogenicity

* Narrow definition of impurity

Protein Deamidation – Immunogenicity of isoaspartic acid

- Protein deamidation can generate isoaspartic acid, which is a ‘non-natural’ amino acid
- The enzyme PIMT repairs isoaspartylated proteins by converting isoasapartate into aspartic acid
- Antibodies to isoaspartylated histone 2b have been found in SLE patients (Doyle HA. 2013. Autoimmunity 46(1):6 -13)

Predicting the Likelihood of Immunogenicity

Product Specific Attributes

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

- **Product mechanism of action**
  - Immunosuppresive vs. pro-inflammatory

Hypersensitivity Responses Induced by Denatured Aggregated Proteins

- Early preparations of IVIG and HSA had substantial aggregate content causing severe “anaphylactoid” responses (Barandun 1962; Ellis et al 1969)
  - Product aggregates directly fixed complement
  - Generation of immune response to aggregate specific determinants
  - Generation of immune response to native determinants in Ig deficient populations

Consequences of Immune Responses to Aggregates

- Neutralizing antibody that blocks efficacy/potential for cross reaction on endogenous protein
  - IFN-α
  - IL-2
  - Epo
  - mAb/fusion proteins

Patient Specific 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

Timing of an Antibody Response

- Single Exposure
  - Mostly IgM
  - Limited Magnitude
- Two Exposures
  - Isotype Switching, higher magnitude
- Multiple/Continuous Exposure
  - Isotype Switching
  - Higher Magnitude
  - Higher Affinity

Concerns for Antibodies in the Clinic

Clinical Concern	Clinical Outcome
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

General Discussion

- Assays are critical when neutralizing immunogenicity poses a high-risk therefore “real time” (weekly) data concerning patient responses are needed

- Preliminary validated (“qualified”) assays should be implemented early (preclinical and phase I).

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* (February 2013)

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)

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.
  - E.g. Biosimilar vs Reference product discussed in Draft Guidance *Scientific considerations in Demonstrating Biosimilarity to a Reference Product* (February 2012)

Lessons Learned

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

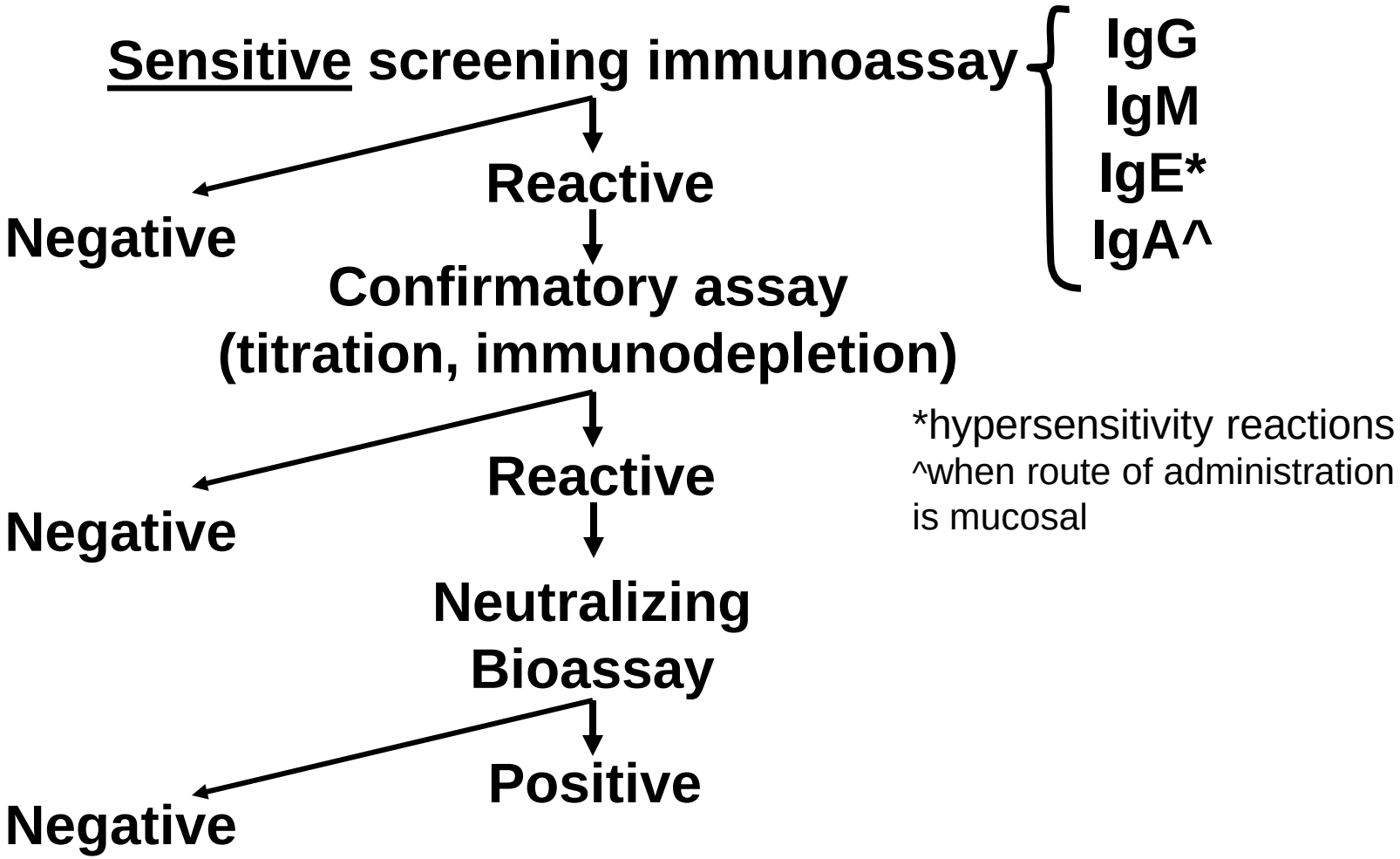
Likelihood of developing an immune response

- **Risk of immune response to patient**
- **Are there alternatives**
- **Stage of Development**
- **Reversibility**

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 Immunogenicity: A Tiered Approach



*hypersensitivity reactions
^when route of administration is mucosal

Function of Immunoglobulin Types

Class	Isotype	Timing	Conc. mg/ml	Function
IgM	NA	1º (7d)	1.5	-Binding sol Ag - C' Act
IgG	IgG1 IgG2 IgG3 IgG4	2º (25-35d)	9 3 1 0.5	-Binding Sol Ag -C' Activation -FcR binding
IgE	NA		0.05	Immediate Hypersensitivity
IgA	IgA1 IgA2		3 0.5	Mucosal Immunity
IgD	NA	Naïve Cell		Surface Receptor

Draft Guidance: Assay development for immunogenicity testing of therapeutic proteins

Established and optimized during development:

- Assay format
- Assay matrix - serum, plasma, buffer
- Minimum required dilution (MRD)
- Optimal reagent concentrations
- Assessment of plate effects
- Robustness (some aspects of robustness are also validated)

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

Assay Odds and Ends--Cutpoints

- The cutpoint is the assay decision point
  - Screening, confirmatory, titration, neutralizing assays may have different assay baselines (background) and sources of variability that suggest the need for a different cutpoint for each.
- The validation cutpoint may not be appropriate for the study population
  - Commercial samples may not be representative of the study
  - Confirm with study population samples
  - Different indications are likely to have different cutpoints and immunogenicity rates—extrapolation is risky scientifically and from a regulatory standpoint.

Binding Antibody Assessment

- Qualitative Status
  - Positive, negative or negative with onboard drug
- Confirmation with cold competition
- Titer
- Specificity
- Relevant isotype distribution,
- Time course of development,
- Persistence/disappearance,
- Association with clinical sequelae

Neutralizing Assay: Selection of Format

- 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
  - Known relevant functions (e.g., uptake and catalytic activity, neutralization for replacement enzyme therapeutics).
- Competitive ligand-binding assays may be the only alternative in some situations (e.g. some mAbs)
- Assays may use direct (inhibition of stimulation) or indirect (inhibition of inhibition) assessment

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

In Summary

- 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.
- There are many factors which influence immunogenic responses to therapeutic proteins
  - It is a safety concern, there is a need to assess/measure it.
  - Correlate with clinical data (AE, pK and pD)

Regulatory Expectations

- There are regulatory expectations from the FDA
  - Provide risk assessment and appropriate sampling plan
  - 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
  - COMMUNICATE WITH AGENCY

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