Immunogenicity evaluation and testing approach to correlate immunological adverse events

Dr. Shaifali Gupta, Nilesh Patel, Hiral Surti, Sweety Shah, Arnab Sinha Roy

INTRODUCTION

Immune responses to therapeutic protein products may pose problems for both patient Safety & Product efficacy. Immunologically adverse events, such as Anaphylaxis, Cytokine Release Syndrome, and Cross-reactive Neutralization of Endogenous Proteins mediating critical functions have caused termination of the product. As most of the adverse effects resulting from elicitation of an immune response to a therapeutic protein product appear to be mediated by humoral mechanisms, circulating antibody to the therapeutic protein product has been the chief criterion for defining an immune response to this class of products.

Correlation with clinical response is typically required to determine the clinical relevance of these Anti-drug Antibodies. There are lot of factors may affect immunogenicity of therapeutic protein products. These factors are Critical Elements in the Immunogenicity Risk Assessment. Ideally, these factors should be taken into consideration in the Early stages of Therapeutic Protein Product Development. Multi-tiered Testing Approach should be properly designed to evaluate the Immunogenicity of any product.

SAFETY CONSEQUENCES OF IMMUNOGENICITY

Anaphylaxis

- Serious, acute allergic reaction
- Invoked the involvement of specific IgE antibodies

Cytokine Release Syndrome

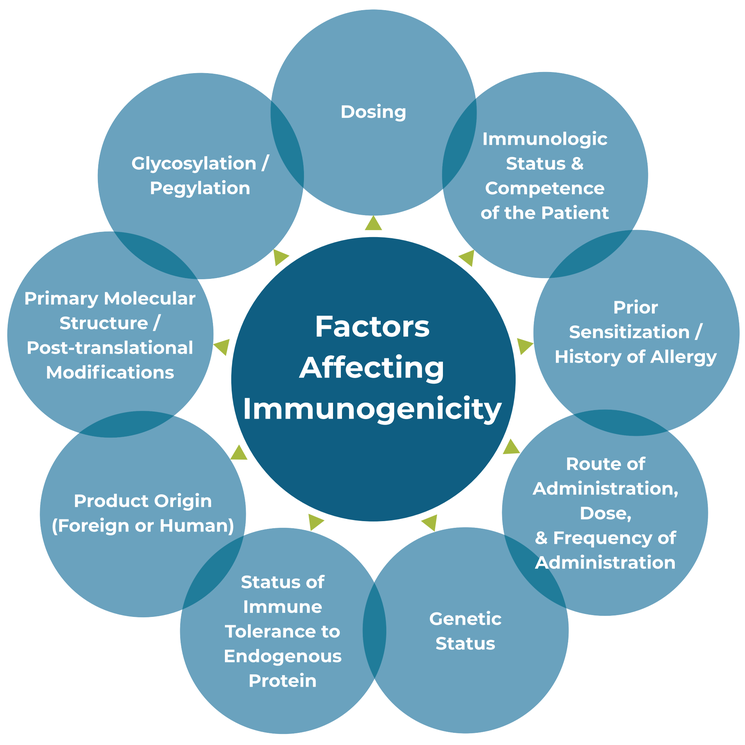
- Rapid release of proinflammatory cytokines from target immune cells
- Although cytokine release syndrome is not directly related to immunogenicity, the clinical presentation of cytokine release syndrome overlaps with anaphylaxis and other immunologically related adverse reactions

Delayed Hypersensitivity

- Immune responses secondary to immune complex formation typically have a sub acute presentation
- Clinical signs of non acute reactions often associated with immunogenicity

Cross Reactivity with endogenous products

- Cross reactivity of antibodies with cell surface receptors or proteins, causing cytokine release or other manifestations of cellular activation.
- Cross reactivity with active sites of endogenous proteins could lead severe adverse events.



CLINICAL CONSEQUENCES OF IMMUNOGENICITY

Efficacy

Antibodies to limit product efficacy in patients treated with Therapeutic Protein Products.

- Antibodies binding to either the uptake/catalytic domain of a therapeutic enzyme may lead to loss of product efficacy.
- Loss of Efficacy is problematic for all products, but is of utmost concern if the product is a lifesaving therapeutic.
- Neutralizing Antibodies to block the efficacy of therapeutic protein products by specifically targeting domains critical for efficacy.

Safety

- Anaphylaxis
- Cytokine Release Syndrome
- Infusion Reactions
- Delayed Hypersensitivity
- Cross Reactivity with Endogenous Proteins

Pharmacokinetics

Presence of Anti-drug Antibodies in clinical samples can impact the Pharmacokinetics profile of the Therapeutic Products.

IMMUNOGENICITY ASSESSMENT STRATEGY

- **Multi-tiered Testing approach:**
  A sensitive screening assay is initially used to assess clinical samples to gain a more accurate understanding of the natural history of the ADA response where low and high affinity antibodies are detected. Samples testing positive in the screening assay are then subjected to a confirmatory assay to demonstrate that ADAs are specific for the therapeutic protein product. Samples tested positive in confirmatory assay are further characterized by Titer assay to quantitate the level of antibody present in the sample and NAB assay to determine the neutralizing capacity of the antibody.
- **Immunoglobulin Isotypes:**
  The screening assay theoretically detect antibodies of most isotypes but does not provide information on which isotypes are being detected. Assessment of ADA subtype may be informative in some situations. For example, the generation of IgG4 antibodies has been associated with immune responses generated under conditions of chronic antigen exposure, such as factor VIII treatment or presence of IgE with high risk of anaphylaxis.
- **Domain Specificity:**
  Some proteins possess multiple domains that function in different ways to mediate clinical efficacy. An immune response to one domain may inhibit a specific function while leaving others intact. Examination of immune responses to therapeutic protein products with multiple functional domains such as bispecific antibodies may require development of multiple assays to measure immune responses to different domains of the molecules .

ADVERSE EVENTS WITH THE ADMINISTRATION OF BIOPHARMACEUTICALS

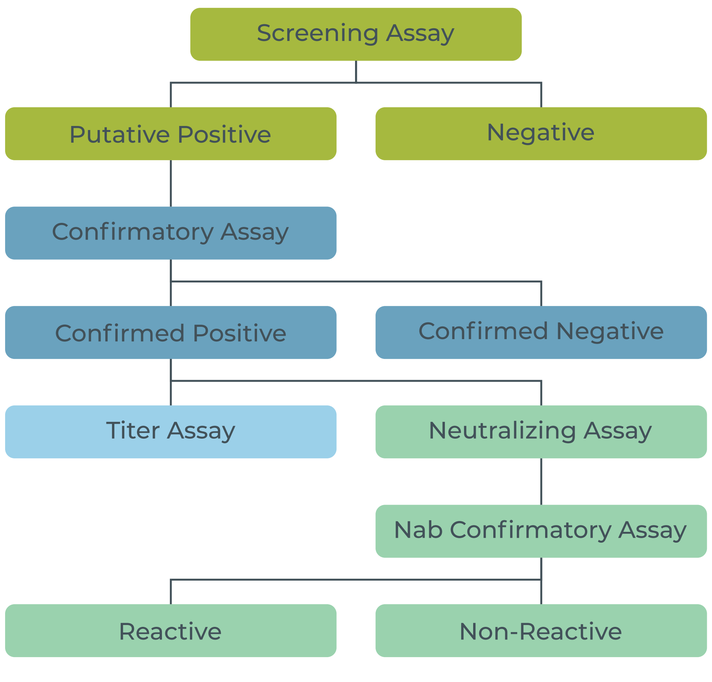
- **Neutralizing antibody that blocks efficacy / potential for cross reaction on endogenous protein**
- IFN-Alpha,
- IL-2,
- Erythropoietin,
- mAb /Fusion protein

Case Study - Erythropoietin

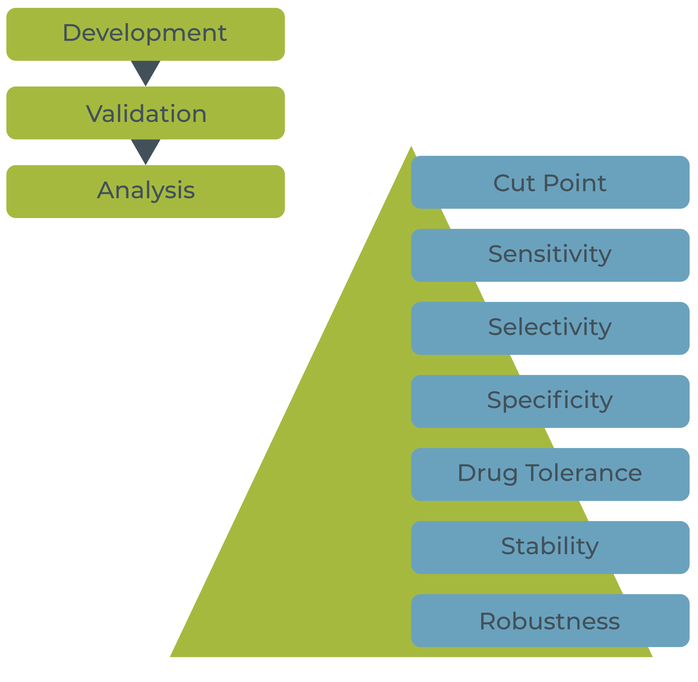
Several cases of neutralizing anti-erythropoietin antibody development and subsequent adverse events (pure red cell aplasia) have been reported following subcutaneous administration of erythropoietin biologics from different manufacturers in patients with chronic renal failure; this effect has been attributed to the route of administration as well as product-related factors, such as changes in formulation and in container closure systems

Assessment of immunogenicity is of critical importance when establishing the safety profile of all biologics, and head-to-head assessment of immunogenicity with the reference product, ideally demonstrating similarity, is considered a key component of a biosimilar's clinical development program.

MULTI-TIERED TESTING APPROACH



METHOD VALIDATION PARAMETERS



METHODS & ASSAY PLATFORMS