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Meeting Report

New FDA Draft Guidance on Immunogenicity

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Abstract. A "Late Breaking" session was held on May 20 at the 2013 American Association of Pharmaceutical Scientists-National Biotech Conference (AAPS-NBC) to discuss the US Food and Drug Administration's (FDA) 2013 draft guidance on Immunogenicity Assessment for Therapeutic Protein Products. The session was initiated by a presentation from the FDA which highlighted several key aspects of the 2013 draft guidance pertaining to immunogenicity risk, the potential impact on patient safety and product efficacy, and risk mitigation. This was followed by an open discussion on the draft guidance which enabled delegates from biopharmaceutical companies to engage the FDA on topics that had emerged from their review of the draft guidance. The multidisciplinary audience fostered an environment that was conducive to scientific discussion on a broad range of topics such as clinical impact, immune mitigation strategies, immune prediction and the role of formulation, excipients, aggregates, and degradation products in immunogenicity. This meeting report highlights several key aspects of the 2013 draft guidance together with related dialog from the session.

INTRODUCTION

At the 2013 American Association of Pharmaceutical Scientists-National Biotech Conference (AAPS-NBC), a "Late Breaking" Session was held to discuss the FDA's 2013 draft guidance on Immunogenicity Assessment for Therapeutic Protein Products (1). Panelists included chairs of the three AAPS-Biotech Section Focus Groups: Dr. Valerie Quarmby, AAPS-Therapeutic Protein Immunogenicity Focus Group (TPIFG; Genentech), Dr. Lakshmi Amaravadi, AAPS Ligand Binding Assay Bioanalytical Focus Group (LBABFG; Biogen Idec), and Dr. Karoline Bechtold-Peters, Protein Aggregation and Biological Consequences Focus Group (PABCFG; F. Hoffmann-La Roche) along with two FDA representatives Dr. Susan Kirshner and Dr. Amy Rosenberg. The session was attended by approximately 300 delegates and was initiated by Dr. Rosenberg's presentation on the latest guidance on immunogenicity assessment of therapeutic proteins followed by an active dialogue between scientists from various biopharmaceutical companies and FDA representatives. This meeting report attempts to capture salient points from the 2013 AAPS-NBC Late Breaking News session on FDA's new draft immunogenicity guidance for therapeutic proteins. This meeting report represents the scientific discussion at the panel and may not reflect final FDA policy or the opinions of all the authors.

The following section captures the conference discussion between AAPS focus group leaders, FDA representatives, and conference participants and, as such, may not reflect final FDA policy or the opinions of all the authors.

DR. AMY ROSENBERG'S PRESENTATION

Dr. Rosenberg initiated the session by introducing the 2013 draft immunogenicity guidance for therapeutic protein products (1). The FDA intends to put forth the best guidance document based on prior experience and case studies. Hence, it encourages biopharmaceutical companies to:

- Deliver the best science to reduce risks,
- Act on what is known to help patient care, and
- Take a risk-based approach to reduce and mitigate unwanted immunogenicity.

The draft guidance outlines a risk-based approach to evaluate and mitigate unwanted immunogenicity which could otherwise adversely affect the safety and/or efficacy of a

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therapeutic protein product. Dr. Rosenberg stated that the draft guidance is a clinically focused document which provides recommendations for best practices but, as stated in the draft guidance, "does not operate to bind FDA or the public." The final version of the document will represent the FDA's current thinking on the topic and should be viewed as recommendations by the FDA. The word "should" implies that the FDA "recommends" a particular approach, but a biopharmaceutical company may approach the agency with alternative approaches to the ones mentioned in the guidance that may prove acceptable.

The document outlines risk assessment taking into consideration the severity and frequency of possible consequences pertaining to immunogenicity (2). Thus, the FDA thinks that this knowledge allows for specific and directed action rather than broad precautionary actions. Dr. Rosenberg discussed a range of potential consequences of unwanted immunogenicity from severe to no negative impact:

- Anaphylaxis (*e.g.*, factor IX): Proteins of nonhuman origin in immune competent patients or human proteins that are seen as foreign by patients who have deleterious mutations or are genetically null may be higher risk for inducing anaphylaxis.
- Neutralizing antibodies (NAbs) to non-redundant endogenous proteins can result in loss of function. Three examples are erythropoietin (Epo) where NAbs may result in pure red cell aplasia, thrombopoietin (Tpo) where NAbs can cause low platelet counts, and GM-CSF where naturally occurring NAbs have been linked to pulmonary alveolar proteinosis.
- Loss of efficacy of life-saving drugs, for example enzyme replacement therapies (ERT), where induction of immune tolerance may be warranted.
- Loss of efficacy of quality of life-improving drugs, for example TNF-α antagonists.
- Cross-reactivity of antibodies to other therapeutics in the same class or superfamily (*i.e.*, TNF-α antagonists, interferon betas) that may affect the efficacy of structurally similar drugs.
- Non-acute immune responses: immune complex formation mediating serum sickness or nephropathy.
- Altered PK: circulating immune complexes (CIC) can be formed between drug and antibodies that either increase or decrease the rate of clearance of the therapeutic protein.
- Sustained immune responses to chronically administered drugs can lead to epitope spreading and neutralization of therapeutic protein products.

Factors Which May Influence the Likelihood of an Unwanted Immune Response

Patient-specific factors: immunologic status (*i.e.*, immune competent *versus* immune suppressed), prior sensitization, allergy, route of administration, human leukocyte antigen (HLA) haplotypes, genetic polymorphisms in cytokine genes, quantity or quality of endogenous protein (*e.g.*, for enzyme replacement therapy), and pre-existing antibodies. It is not known *a priori* how pre-existing antibodies affect subsequent development of antibodies, although it has been observed that they do not always lead to boosted responses (3). Careful monitoring is recommended (1).

Product-specific factors: origin (human or foreign), structure (*e.g.*, aggregates), degradation products (*e.g.*, deamidation and isomerization), post-translational modification (*e.g.*, glycosylation and pegylation), immunomodulatory properties (*i.e.*, immune suppressive or immune stimulating activity of therapeutic protein), impurities, formulation (*e.g.*, stability), and container closure (*e.g.*, tungsten).

Mitigation of an Unwanted Immune Response to a Therapeutic Protein

Dr. Rosenberg stated that any action taken to mitigate immunogenicity should be governed by the potential consequences of unwanted immune responses (1). It was suggested that tolerance induction may be indicated when an immune response to a therapeutic protein is life-threatening and may be considered when an anti-drug antibody (ADA) response abolishes the efficacy of a highly effective therapeutic protein even if it is not lifesaving (*e.g.*, TNF antagonists). The risks associated with implementing a tolerance induction regime should be carefully considered as well as potential benefits in the context of the underlying disease (1).

A relevant risk mitigation strategy should include the following:

- Development of specific and sensitive (including drug tolerant) assays for evaluating antibodies to therapeutic proteins.
- Development of a product-specific antibody sampling plan based on stage of development: first in human (no knowledge of human immunogenicity) *vs.* later stages of product development (increasing knowledge of spectrum of human immunogenicity with increasing clinical experience).
- Development of cautious dosing and dose escalation studies. Adequate time intervals (based on product PK/PD) to assess for adverse events among patients within a dosing cohort (especially for first in human studies) and adequate evaluation of each dosing cohort prior to dose escalation
- Careful evaluation of all adverse events potentially mediated by an immune response (1)

For ERT products, where loss of efficacy can have severe consequences, immune tolerance induction may be indicated. There may be instances where de-immunization of the product to remove any identified dominant immunogenic epitopes may be considered. Protein engineering could be used to improve stability of a therapeutic protein product, thus reducing degradation without adversely affecting its activity.

Dr. Rosenberg concluded the presentation portion of the session stating that the FDA is open to suggestions and is actively seeking feedback on improving the current draft guideline, especially focusing on areas that can have implications or concerns for patient safety and product efficacy. Suggested changes from the audience will be taken into consideration for review by the FDA.

The second and major portion of this Late Breaking Session included open discussion via a question/answer/discussion format with FDA representatives with the three focus group chairs moderating the session.

OPEN DISCUSSION

Focus on *In Vivo* Impact

A question was raised regarding how best to connect data on the *in vivo* impact of a therapeutic protein (such as local injection site responses) to data from *in vitro* assessments. There were several recommendations by Drs. Rosenberg and Kirshner on topics such as *in vivo* stability, infusion reactions, immune response monitoring, neutralizing antibodies, and classification of transient/persistent immune response. The following points highlight some recommendations made by the panelists:

- The underlying mechanisms of infusion-related reactions are not well understood but may be important for mitigating adverse clinical consequences. Thus, the FDA recommends that mechanisms underlying any observed infusion-related reaction for a therapeutic protein product be investigated and defined by the biopharmaceutical companies (1). It was suggested that drug development scientists consider adopting a universal definition or classification system for infusion-related reactions. There are current classification systems that could be further evaluated for such purpose. As an example, Oliver Hausmann and his group have designed a useful classification system for infusion-related reactions based upon the timing of the reaction relative to the infusion as well as on whether a reaction occurs at the first or a subsequent injection (4).
- Drs. Rosenberg and Kirshner suggested that drug development scientists monitor immune responses to the drug over time as the response would not remain static. Moreover, chronic administration might lead to the development of neutralizing antibodies (1). This may occur due to immune response maturation, where the immune response spreads from one or more immunodominant epitopes to other epitopes.
- It is important to understand whether the ADAs are neutralizing. Consider the clinical context where significant immune responses might impact the clinical outcome. Non-neutralizing antibodies can also have an adverse impact on a therapeutic protein product. For example, non-neutralizing antibodies at high titer can divert therapeutic proteins from the intended target tissue to FcR bearing cells or may form immune complexes which may be deposited in critical organs such as the kidney.
- It is always important to bank ADA samples as these may be helpful in enabling further characterization of any observed immune response. The type of ADA characterization (*e.g.*, epitope specificity, pro-inflammatory cytokine panels, correlation to immune-mediated adverse events, infusion-related reactions, injection site reaction, and hypersensitivity) will depend on the clinical events. Specific sampling strategies for clinical studies should be discussed with the agency to obtain agreement on moving forward.
- Definitions of "transient" *vs.* "persistent" immune responses have been addressed in an AAPS "White Paper" which has been written by Dr. Gopi Shankar and colleagues and has been submitted for publication. It was also highlighted at the session that both persistent and transient immune responses may have clinical impact and that clinical impact should be assessed for both transient and persistent ADA responses.
- Contact the FDA for a case-by-case assessment in the event that there are alternative approaches.

A suggestion was made to clarify the term "clinically relevant response" within the draft guidance. Dr. Rosenberg and Dr. Kirshner took note of this suggestion for further review by the FDA.

Immune Mitigation and Prediction

Discussions in this area touched upon topics such as desensitization of patients to therapeutic proteins and tolerance induction in patients to mitigate immune responses. It was pointed out that the current guidance does not differentiate between situations where administration of a therapeutic protein product to a particular patient population has been deemed high *vs.* low immunogenicity risk. Another suggestion brought up extension of post-marketing data to study long-term efficacy. The following points highlight recommendations made by the panelists during the session.

- Drs. Rosenberg and Kirshner pointed out that the guidance encourages biopharmaceutical companies to explore options that may help reduce the immunogenicity of a therapeutic protein such as de-immunization of the protein by removal of the putative immunodominant epitopes. A note of caution was added regarding such engineering, which is that, sequence or other changes to the therapeutic protein should not alter other critical product quality attributes. Application of the guidance to a specific situation could be resolved through dialogue with the FDA on the scientific approach chosen by the biopharmaceutical company.
- It is important for biopharmaceutical companies to study post-marketing data as some therapeutics are administered long-term and little is being done to study the long-term efficacy of these drugs during registrational clinical trials. For example, loss in efficacy of chronically administered monoclonal antibody is not well understood. In most cases, the basis for loss of efficacy may determine subsequent alteration in clinical therapy. Investigations should be initiated if loss of efficacy is observed following successful treatment in the initial period (1).
- For a PEGylated therapeutic protein, a validated, sensitive, and specific ADA assay for the conjugate would be accepted by the FDA and could be used to detect both PEG-directed antibodies as well as therapeutic protein-directed antibodies during the

initial screening tier. Specificity can be assessed in subsequent tier analysis. Drug development scientists continue to debate the reliability of anti-PEG antibody assays.

- The existence of pre-existing antibodies to certain components of therapeutic proteins, as exemplified by pre-existing antibodies to the sugar linkage Gal alpha1, 3 Gal, also needs to be taken into consideration. Drs. Rosenberg and Kirshner acknowledged that the significance of pre-existing antibodies is not well understood, and hence, the FDA cannot comment with certainty on whether a therapeutic protein can or cannot be administered in patients with pre-existing antibodies. They suggested that when there are pre-existing antibodies that will react with a life-saving therapeutic protein, then a tolerance protocol could be followed once a boost in the antibody response is detected or an immunologically adverse event is experienced (1). The presence of pre-existing antibodies also depends on the drug, patient, and the type of therapeutic protein. The degree to which a biopharmaceutical company understands the type of pre-existing antibodies that are present in a population (*e.g.*, directed to the protein, a specific sugar moiety, IsoAsp, PEG, protein, *etc.*) could help inform prediction of their impact.
- There were several aspects of immune prediction and mitigation that still need to be explored; hence, Dr. Rosenberg and Dr. Kirshner encouraged biopharmaceutical companies to conduct more exploratory analyses in these two areas.

Excipients and Aggregates

Discussions pertaining to the use of excipients and the clinical impact of aggregates in a therapeutic product were numerous and lively, and several recommendations were made by Drs. Rosenberg and Kirshner. Some of the discussion touched upon topics such as understanding the impact of aggregates in a product and incorporating appropriate mitigation strategies. Other suggestions from Drs. Rosenberg and Kirshner included tracking the exact lots of product that each patient in a clinical trial receives as a means to evaluate whether adverse events are linked to specific quality attributes (1). For example, observations of increased levels of immunogenicity in patients could be linked to lots containing higher levels of host cell proteins. They also suggested that drug development scientists integrate the work ongoing in CMC and immunogenicity groups within a company during product development. The following are a detailed list of suggestions made by the panelists during the session:

- Drs. Rosenberg and Kirshner clearly stated that they do not recommend clinical testing of artificially created high levels of protein aggregates in humans for ethical reasons. The use of clinical product with somewhat higher aggregate levels is not unusual for early stage clinical material, and this will enable "qualification" of the material in the clinic. Animal studies may be performed to help understand the qualities and quantities of aggregates that trigger immune responses and their consequences. They recommended that each protein therapeutic product be extensively characterized with regard to potential critical quality attributes during development. It was suggested that assessing the safety and efficacy of a product that is close to the end of its shelf life is also an important consideration.
- Manufacturing control systems play a crucial role in maintaining low aggregate levels during product development, but, as noted above, to better characterize and isolate the cause of any unwanted immune response, Drs. Rosenberg and. Kirshner recommended tracking the drug product lots from which patients received the treatment (1). Indeed, this approach identified the critical factor responsible for unwanted immune responses to a biosimilar Epo product (5). This may enable drug development scientists to understand if patient(s) with ADA received a particular batch of material that was associated with immunogenicity, and, if so, what product attributes could be responsible or if the development of ADA was related to some other aspect of exposure (1). It was recommended that biopharmaceutical companies perform such studies and understand how the product performs at stages of development, *i.e.*, in clinical trials in which lots of product with defined attributes are still traceable. Drs. Rosenberg and Kirshner also pointed out that legislation to track the exact vials of drug product that each patient receives is currently being discussed. Comments from the audience were that de-convoluting the generated data is impossible since multiple factors can contribute to a patient's immune response beyond the individual drug product batch, thereby making this approach extremely challenging for drug development scientists. Drs. Rosenberg and Kirshner took note of this comment for further review by the FDA.
- Drs. Rosenberg and Kirshner suggested that the use of transgenic animals would improve correlation of immunogenicity data from animal models to humans (1). Results from transgenic animal models may not translate exactly to humans but would help in the understanding of broad principles such as immune response to dimers.
- The draft guidance suggests that animal immunogenicity studies are expected prior to use of clinical material. Drs. Rosenberg and Kirshner clarified that immunogenicity testing in animals is critical for (1) understanding the toxicology studies and (2) evaluating the risk of potential consequences of immune responses for certain types of therapeutics. The FDA understands that conventional animal models are not adequate for the prediction of immune response incidence and does not endorse unnecessary animal studies. Drs. Rosenberg and Kirshner acknowledged the lack of concrete predictive models for immunogenicity and suggested that in early clinical trials, multiple lots of a particular drug product containing a limited range of levels of process- and product-related impurities, depending on manufacturing

capabilities, be administered to patients to elaborate appropriate acceptance criteria (1).

- Drs. Rosenberg and Kirshner recommended that biopharmaceutical companies undertake studies to characterize other impurities and understand any possible high risk interactions within the product. FDA has found that some excipients can interact with drug product to form adducts. For example protein-phenol adducts were formed in a product formulated with a phenol-containing preservative (6). Drs. Rosenberg and Kirshner strongly recommended that the CMC, immunogenicity, and clinical groups within a company work together to understand the product and the potential risks that might arise. This would help identify potential sources of immunogenicity during development. Once clinical data are available, they suggested that it was important to add that information to help elucidate any product quality-related basis for clinical phenomena pertaining to unwanted immune responses to a product (1).
- Drs. Rosenberg and Kirshner stated that if a detailed list of components within the container closure system (*e.g.*, stoppers) is not available to the drug development scientist, a reference or cross-reference to a drug master file (DMF) would be acceptable by the FDA in cases where suitable data are found in those DMF submissions.

  Drs. Rosenberg and. Kirshner took note of one comment which suggested providing a risk ranking list of excipients within the guidance that may/may not be used in the product owing to their antigen- or adjuvant-like properties.

CONCLUSION

AAPS-NBC 2013 held a Late Breaking session to discuss the recently formulated FDA draft guidance document on immunogenicity of therapeutic proteins. Dr. Rosenberg highlighted several key aspects within the guidance during her presentation and discussed the scientific basis on which the guidance was drafted. A detailed discussion followed Dr. Rosenberg's presentation. The session was very well received by the audience since critical concerns relating to the draft guidance could be discussed with Drs. Rosenberg and Kirshner. They gave several recommendations during the session on a range of topics discussed within the draft guidance. The discussions helped Dr. Rosenberg and Dr. Kirshner in receiving active feedback from drug development scientists regarding the guidance document, and a few suggested changes were noted by them for further review and consideration. Scientists participating in this exciting session were able to hear the FDA's perspective on the intent of the guidance in context and in real time.

The meeting organizers would like to thank Dr. Rosenberg and Dr. Kirshner for joining their colleagues from various biopharmaceutical companies at this session and for their very active participation in this dynamic discussion. The meeting organizers would also like to thank the TPIFG, PABC, and LBABFG membership and session scribes: Dr. Heather Myler and Ashwin Parenky (TPIFG), Dr. Paolo Mangiagalli, Dr. Hanns-Christian Mahler and Dr. JanOlaf Stracke (PABCFG) for their active participation in this event.

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