EMEA Guidance on Immunogenicity

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Guideline on Immunogenicity Assessment of biotechnology-derived therapeutic proteins

EMEA/CHMP/BMWP/14327/2006

• GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS – Drafted 2006
• DRAFT AGREED BY BMWP - July 2006
• ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION - 25 January 2007
• END OF CONSULTATION (DEADLINE FOR COMMENTS) - 31 July 2007
• REVISION OF DRAFT – AGREED BY BMWP - Oct 2007
Guideline On Immunogenicity Assessment Of Biotechnology-Derived Therapeutic Proteins

- Executive Summary
- Introduction
- Scope
- Legal Basis
- Main Guideline Text
- Factors that may influence the development of an immune response against a therapeutic protein
  - Patient and disease related factors,
  - Product related risk factors of immunogenicity
- Non-clinical assessment of immunogenicity and its consequences
- Development of assays for detecting and measuring immune responses in humans.
  - Assay strategy
  - Antibody assays
  - Assay validation
  - Characterization of antibodies to a therapeutic protein
- Potential clinical consequences of immunogenicity
  - Consequences on Efficacy
  - Consequences on Safety
- Immunogenicity and Clinical Development
  - Rationale for sampling schedule and kinetics of the antibody response
  - Consequences on pharmacokinetics of the product
  - Methodology aspects to assess comparability of immunogenicity potential as part of a comparability exercise
  - Immunogenicity in paediatric indications
- Risk Management Plan
- References
- ANNEX 1 - Further details on methods for assessment and characterisation of immunogenicity
- ANNEX 2 - An example of a strategy for antibody detection and characterisation.
Factors that influence the development of an immune response against a therapeutic protein

• **Patient and disease-related factors**
  - Genetic factors modulating the immune response
  - Genetic factors relating to a gene defect
  - Age
  - Disease-related factors
    - autoimmune, immunosuppressive diseases, indication-specific differences
  - Concomitant treatment
    - immunosuppressives, monotherapy or combination therapy
  - Duration, route of administration, treatment modalities
  - Previous exposure to similar or related proteins

• **Product related risk factors**
  - Protein structure
    - variations in primary structure,
    - post-translational modifications,
    - degradation
  - Formulation
  - Aggregation and adduct formation
  - Impurities
    - host cell proteins
Development of assays

• Unwanted immunogenicity - humoral and cellular immune responses.
• It is very important to select and/or develop assays & assay strategies for assessment of such immune responses.
• Most effort is usually focused on antibody detection and characterisation - technically feasible and often related to clinical safety and efficacy.
• However, cell-mediated responses are important and their assessment should be considered on a case-by-case basis.
Assay strategy

• Adopting an appropriate strategy for assessment of unwanted immunogenicity of biological products is essential.

• This should usually include
  – a screening assay for identification of antibody positive samples/patients
  – procedures for confirming the presence of antibodies
  – procedures for determining antibody specificity
  – functional bioassay(s) for the assessment of the neutralizing capacity of antibodies

• In addition, assays will be required which assess and characterize the clinical impact of antibodies (and possibly other components of immune responses) if these are detected/induced. It is important to include baseline data from all patients where appropriate.

• Further details on proposed strategy - Annex
Patient samples taken at appropriate time-points

-ve samples rejected → Screening Assay → +ve samples

Confirmatory Assay

Neutralization Assay ← Confirmed +ve samples → Characterization

Assess correlation of characterized antibodies with clinical responses to biological therapeutic

Assays for clinical markers and assessment of clinical response in patients
Screening assays

- A screening assay should be capable of detecting antibodies induced against the biological product in all antibody positive samples/patients.
- This implies that detection of some false positive results is inevitable as absolute screening-assay specificity is normally unattainable and false negative results must be avoided.
- Desirable characteristics: sensitivity, specificity, precision, reproducibility and robustness.
- The need to accommodate screening of relatively large numbers of samples necessitates use of an assay with high throughput and appropriate automation.
Assays for confirming the presence of antibodies

• These assays are necessary for elimination of false positive samples/patients following the initial screen.

• Various approaches can be adopted but it is necessary to select assays taking account of the limitations and characteristics of the screening assay(s).
Assays for dissecting the specificity of antibodies

• It is often possible to use an assay which provides information concerning the specificity of the antibodies detected and this contributes to confirmation of the specificity of the immune response.

• Analytical immunoassays such as immunoblotting and radioimmunoprecipitation analysis offer the advantage that they can be used to dissect the specificity of the detected antibodies as well as confirming antibody positivity.
**Neutralization assays**

- Assessment of the neutralizing capacity of antibodies usually requires the use of bioassays.
- An assay must be selected or developed which responds well to the biological product.
- Bioassays used for measuring the potency of biological products e.g. for lot release purposes can often be adapted to assess neutralising antibodies.
- However, they frequently require refining if they are to perform optimally for measuring the neutralizing capacity of antibodies.
Assay characteristics

• Adoption of the simplest assay suitable for all requirements is normally a valid approach to assay selection (particularly when high throughput is important e.g. for screening assays).

• However care with this is necessary to ensure that it does not compromise other stages of immunogenicity assessment.

• For example - direct binding ELISAs, with antigen directly immobilized on plate well surfaces are often the simplest assay approach, but may be associated with a very high incidence of false positives. In such cases, it is often necessary to adopt a more complex assay eg ‘bridging’ assays, ECL or SPR methods to avoid this.

• False negative results in screening assays due to epitope masking can be encountered and a strategy to avoid these may be necessary e.g. by using assays that avoid specific masking of particular epitope(s).
Interpretation of Results

• It is essential to establish clear criteria for deciding how samples will be considered positive or negative, and also how positive results will be confirmed.

• Approaches to these can differ according to assay etc. and need to be decided accordingly.

• A common procedure for establishing positive cut-off for immunoassays is to establish assay background and decide on a statistical (e.g. 3 SD above background value) or real data (e.g. double background value) basis of what will be considered the lowest positive result.

• Confirming positivity normally requires repeating assays, often using a different assay method(s).
Assay validation

• Assays need to be validated for their intended purpose.

• Validation studies must be conducted to establish that the assays show appropriately linear responses to relevant analytes as well as appropriate accuracy, precision, sensitivity, specificity and robustness.

• Problems encountered include matrix effects, selection of appropriate antibody controls and other reagents, residual product in samples, presence of pre-existing antibodies.
Assay Calibration

- Calibration of immunoassays is problematical - immunoglobulin present in standards and samples is heterogeneous in structure, specificity and avidity. Direct valid comparison between samples and reference materials, especially on a mass basis are therefore difficult, if not impossible.

- This implies that calibration of such assays should be carried out using an acceptable, valid approach, which is clearly described. Often the best option is to report immunoassay data as a titre based on a standard procedure for calculating this value.
Antibody Characteristics

- If antibodies are induced in patients, serum or plasma samples need to be characterised in terms of antibody content (concentration/titre) and other criteria, which need to be considered on a case-by-case basis.
- These may include antibody class and subclass (isotype), affinity, specificity,
- Antibodies present in confirmed positive samples need to be examined for specificity for the active protein and distinguished from antibodies, which bind to product-related and process-related components.
- It is also useful to screen for cross reactivity with other products based on the particular protein as well as (if possible and relevant) its endogenous counterpart.
- The neutralising capacity of antibodies present in positive samples needs to be established as this often correlates with diminished clinical responses to biological product.
Immunogenicity Assessment strategy – design and interpretation

- Immunogenicity studies need to be carefully and prospectively designed to ensure all essential procedures are in place before commencement. This includes –
  - the selection, assessment, characterisation and validation of all assays,
  - identification of appropriate sampling points,
  - sample volumes and sample processing/storage and
  - selection of statistical methods for analysis of data.

- This applies to assays used to measure and characterise antibodies and to methods employed for assessing clinical responses to antibodies if they are induced. Much of this needs to be established on a case-by-case basis, taking account of product, patients, expected clinical parameters.
Immunogenicity Assessment strategy – design and interpretation

- Such studies can provide valuable information concerning significant immunogenicity of biological products, its characteristics and potential clinical consequences.
- They can be valuable for preliminary comparative immunogenicity studies for biosimilar products or following production/process changes introduced for established products.
- However, unwanted immunogenicity can occur at a level, which will not be detected by such studies when conducted at a pre-approval stage, due to the restricted number of patients normally available for study.
- It is usually necessary to continue assessment of unwanted immunogenicity and its clinical significance post-approval, usually as part of pharmacovigilance surveillance.
Potential Clinical Consequences of immunogenicity

• Can range from benign, non-significant to serious life-threatening
  - depends on the therapeutic
  - very much a case-by-case scenario
  - consider risk factors
• Consequences on efficacy
• Consequences on safety
Unwanted Immunogenicity

Proteins → Patients

Immunogenic (induce antibodies)

Neutralise biological effects and compromise further therapy (factor VIII, IFNα2a, GM-CSF)

Alter PK/PD

Cross-react with native protein and induce adverse symptoms (Epo, MGDF)

Non immunogenic (G-CSF, IFN-γ)

No effect (growth hormone, insulin)
Consequences of immunogenicity

Clinical Safety

- Safety issues can occur even when there is no loss of efficacy.
- Acute consequences
  - Infusion reactions, anaphylactic reactions
- Non-acute consequences
  - Delayed-type hypersensitivity/immune complexes
  - Cross-reactivity with an endogenous counterpart
Immunogenicity and Clinical Development

• Rationale for sampling schedule and kinetics of the antibody response
  – Sampling schedule very important
    - Needs to be adapted to individual treatment modalities
    - Baseline sampling, repetitive sampling etc
    - intended for chronic use
    - determined by the type of therapeutic
Considerations for evaluation of relative immunogenicity

• Variations in production process can influence a product’s immunogenicity. Studies on relative immunogenicity need to be considered.
  
  • For this, a homogeneous and clinically relevant patient population should be selected. Healthy volunteers are not suitable substitutes.
  
  • Evaluation should preferably involve head-to-head studies of pre- and post-change product. The same assays should be used.

• Immunogenicity in paediatric indications

Therapeutic proteins are used in children. Children may differ from adults in their immune response. Results should be analysed by age groups.
**Risk Management**

- Recommendations for routine monitoring of changes in clinical response and linking immunological findings to clinical events
  - Immunogenicity as part of all clinical trials
  - Evaluate all patients
  - Standardise sampling schedule as much as possible
  - Specifically analyse adverse events for linkage to an unwanted immune response (i.e. also in a symptom driven-manner)
- Provide guidance on how prescriber should handle patient in case unwanted immune response occurs
EU Guidelines-Biosimilars

- Similar biological medicinal products (Oct 05)
- Similar biological medicinal products containing biotechnology-derived proteins
  - quality issues (effective - June 06)
  - non-clinical & clinical issues (June 06)
- Biosimilar medicinal products
  - Insulin, GCSF, Somatropin (June 06)
  - EPO (July 06), alpha-IFNs (being drafted).
- Guideline on immunogenicity assessment of therapeutic proteins - currently being re-drafted
Guidance on Similar Biological Medicinal Products containing biotech-derived proteins as active substance: Non-Clinical & Clinical Issues: IMMUNOGENICITY

• In view of the unpredictability of the onset and incidence of immunogenicity, long term results of monitoring of antibodies at predetermined intervals will be required. In case of chronic administration, one-year follow up data will be required pre-licensing.

• The applicant should consider the possibility of antibodies to process related impurities.
IMMUNOGENICITY

• There is considerable interindividual variability in antibody response in terms of different antibody classes, affinities, and specificities. Thus, data should be collected from a sufficient number of patients to characterise the variability in antibody response.
EPO GUIDANCE – IMMUNOGENICITY ASPECTS

4.3 Clinical safety

Comparative safety data from the efficacy trials are sufficient to provide an adequate pre-marketing safety database.

The applicant should provide at least 12-month comparative immunogenicity data pre-authorization. Retention samples for both correction phase and maintenance phase studies are recommended. For detection of anti-epoetin antibodies, a validated, highly sensitive assay should be used.