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Evaluation of immunogenicity of biosimilars

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Unwanted Immunogenicity

Proteins → Patients → Non immunogenic (G-CSF, IFN-γ)

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)

No effect (growth hormone, insulin)
Unwanted Immunogenicity

- Biological products can induce antibodies with different characteristics:
  - Non-neutralizing (binding) antibodies against active (and/or inactive) product-related substance(s).
  - Binding antibodies against contaminants.
  - Neutralising antibodies.
  - Mixtures of the above.

- Different assays are needed for detection and measurement of these antibody types.
Examples of therapeutics and their immunogenicity profile

<table>
<thead>
<tr>
<th>Product name</th>
<th>Protein</th>
<th>Indication</th>
<th>% Patients with immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td>ReFacto</td>
<td>Factor VIII</td>
<td>Hemophilia A</td>
<td>~ 30%</td>
</tr>
<tr>
<td>Intron A</td>
<td></td>
<td></td>
<td>7%</td>
</tr>
<tr>
<td>Roferon A</td>
<td>Interferon α</td>
<td>Hepatitis C</td>
<td>25%</td>
</tr>
<tr>
<td>Pegasys</td>
<td></td>
<td></td>
<td>9%</td>
</tr>
<tr>
<td>Pegintron</td>
<td></td>
<td></td>
<td>1%</td>
</tr>
<tr>
<td>Betaseron</td>
<td></td>
<td>Multiple Sclerosis</td>
<td>10-45%</td>
</tr>
<tr>
<td>Avonex</td>
<td>Interferon β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rebif</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eprex</td>
<td></td>
<td></td>
<td>Non immunogenic</td>
</tr>
<tr>
<td>Aranesp</td>
<td>Erythropoietin</td>
<td>Anemia</td>
<td>Some cases of pure red cell aplasia with Eprex</td>
</tr>
<tr>
<td>Epogen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procrit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukine</td>
<td>Granulocyte macrophage colony stimulating factor</td>
<td>Oncology</td>
<td>2.3% (neutralizing antibodies)</td>
</tr>
<tr>
<td>Neupogen</td>
<td>Granulocyte colony stimulating factor</td>
<td>Oncology</td>
<td>Non immunogenic</td>
</tr>
<tr>
<td>Neulasta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enbrel</td>
<td>TNF receptor II human Ig Fc fusion</td>
<td>Rheumatoid arthritis</td>
<td>16%</td>
</tr>
<tr>
<td>Proleukin</td>
<td>Interleukin-2</td>
<td>Oncology</td>
<td>74%</td>
</tr>
</tbody>
</table>
Antibodies and Adverse Effects

Eprex: Formulation change (1999)
Cause: Leachates from uncoated stoppers (adjuvant).
Formulation/Containers: risk factors

- MAb against EGFR – colorectal cancer, squamous cell carcinoma of head and neck
- 25/76 patients experienced hypersensitivity
- 17 had pre-existing IgE antibodies against gal-α-1, 3 gal present on Mab (expressed in murine myeloma cells)
- Cases clustered in different US states; IgE antibodies potentially due to tick bites etc

Product with same antigen as natural immunogen

PRCA cases in Thailand, Korea - many marketed products

Cross-reactivity with endogenous protein

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

Christine H. Chung, M.D., Beloo Mirakhur, M.D., Ph.D., Emily Chan, M.D., Ph.D., Quynh-Thu
Clinical Impact

- Efficacy – impaired clinical response
- Safety – Infusion reactions, hypersensitivity reactions, serum sickness
  – Cross-reactivity with an endogenous counterpart

"significant neurological abnormalities ... after... six infusions of natalizumab, .... extremely high titers of antibodies against the drug." 
"death..from 'rebound neuroinflammation as a result of the development of natalizumab anti-drug antibodies."
Biosimilar recombinant human erythropoietin induces the production of neutralizing antibodies

Kearkiet Praditpornsilpa, Khajohn Tiranathanagul, Pawinee Kupatawintu, Saengsueee Jobtia, Tanin Intragumtornchaisri, Kiang Tungsanga, Tanyarat Teeapornlertratt, Dusti Lumliertkul, Natavudh Townnamchaisri, Paveena Susantipaphat, Pisut Katavetin, Talemsak Kanjanabuch, Yingyos Avihingsanon, and Somchai Eiam-Ong

Recombinant human erythropoietin (r-HuEpo) has been used for the treatment of renal anemia. With the loss of its patent protection, there has been an upsurge of more affordable biosimilar agents, increasing patient access to treatment for these conditions. The complexity of the manufacturing process for these recombinant proteins, however, can result in altered properties that may significantly affect patient safety. As it is not known whether various r-HuEpOs products can be safely interchanged, we studied 30 patients with chronic kidney disease treated by subcutaneous injection with biosimilar r-HuEpo and who developed a sudden loss of efficacy. Sera from 23 of these patients were positive for r-HuEpo-neutralizing antibodies, and their bone marrow biopsies indicated pure red cell aplasia, indicating the loss of erythroblasts. Sera and bone marrow biopsies from the remaining seven patients were negative for anti-r-HuEpo antibodies and red cell aplasia, respectively. The cause for r-HuEpo hypersensitivity was occult gastrointestinal bleeding. Thus, subcutaneous injection of biosimilar r-HuEpo can cause adverse immunological effects. A large, long-term, pharmacovigilance study is necessary to monitor and ensure patient safety for these agents.

EDITOR’S NOTE: Biosimilar is a term applied to subsequent versions of biopharmaceutical products that have been approved by the regulatory authorities of a given country. The pathway for approval is thus specific for that country, and because of regulatory differences, the biosimilar classification may not apply in other countries.

Worldwide consensus - A biosimilar is a biotherapeutic accepted by a regulatory pathway which requires biological and clinical comparison with the original licensed product. The ‘biosimilars’ described in this paper are NOT real biosimilars.

Under the generic drug paradigm of the Thai Food and Drug Administration, 14 biosimilar r-HuEpos were licensed by 1 January 2009. These products came from various countries such as Argentina, China, South Korea, and India. The number of cases using ‘biosimilar’ r-HuEpos have increased enormously because of their more affordable prices. With their usage, adverse effects of the less than identical therapeutic agents have started to increase. Many clinicians in Thailand were starting to see an increase in PRCA cases which raised an important issue whether the immunogenicity of biosimilar therapeutic agents were indeed equivalent to the innovative r-HuEpo.
Unwanted Immunogenicity-The Most Challenging Issues

• It is impossible to predict
  - the incidence of unwanted immunogenicity
  - the characteristics of the immune response
  - the clinical consequences & significance of such immunogenicity

• THE ABOVE NEED TO BE ASSESSED IN APPROPRIATE STUDIES
Factors Influencing Unwanted Immunogenicity

Product and Patient related

• Molecular structure, novel epitopes, glycosylation, degradation, oxidation, deamidation
• Product impurities
• Formulation
• Aggregation
• Protein – biological properties e.g., immunostimulant
• Dose, route, frequency of administration and duration of therapy
• Immune status, age, genetic profile, disease, treatment
• Previous exposure
Unwanted Immunogenicity

Current Position

Testing for unwanted immunogenicity is integral to product development (clinical & post-marketing phase) for ensuring the clinical safety of a biotherapeutic and of a biosimilar.

Animal data not predictive of immunogenicity in humans.

*In silico* and T cell methods - clinical utility in prospective studies is lacking.

Human clinical data needed

Every product needs to be evaluated for immunogenicity individually and an appropriate strategy adopted based on intended clinical use.

Guidance – EMA, FDA, WHO
# Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins

**Draft Agreed by BMWP** | July 2006
---|---
**Adoption by CHMP for Release for Consultation** | January 2007
**End of Consultation (Deadline for Comments)** | July 2007
**Agreed by BMWP** | October 2007
**Adoption by CHMP** | December 2007
**Date for Coming into Effect** | April 2008

**Keywords**
- Immunogenicity
- Unwanted immune response
- Biotechnology derived proteins
- Immunogenicity risk factors
- Assays
- Clinical efficacy and safety
- Risk management
Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins

Draft

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tr>
<td>Draft agreed by Biosimilar Medicinal Products Working Party (BMWP)</td>
<td>August 2015</td>
</tr>
<tr>
<td>Adopted by CHMP for release for consultation</td>
<td>24 September 2015</td>
</tr>
<tr>
<td>Start of public consultation</td>
<td>01 October 2015</td>
</tr>
<tr>
<td>End of consultation (deadline for comments)</td>
<td>31 January 2016</td>
</tr>
</tbody>
</table>


Comments should be provided using this template. The completed comments form should be sent to BMWP.secretariat@ema.europa.eu.

Keywords

Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay strategy, binding antibodies, neutralising antibodies, risk factors, safety, efficacy, pharmacokinetics, risk management, integrated summary of immunogenicity.

Draft agreed by Similar Biological Medicinal Products Working Party | October 2010
---|---
Adoption by CHMP for release for consultation | November 2010
End of consultation (deadline for comments) | May 2011
Final agreed by BMWP | March 2012
Adoption by CHMP | 24 May 2012
Date for coming into effect | 1 December 2012

Disclaimer: This guideline is intended as an addendum to Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins EMEA/CHMP/BMWP/14327/2006 and should be read in conjunction.

Keywords | Immunogenicity, monoclonal antibodies, similar biological medicinal products, clinical use, assay strategy.
Immunogenicity Testing: A Tiered Approach

Screening assays - for ‘identification’ of all antitherapeutic antibodies
- ELISAs - direct, bridging, other formats
- Radioimmunoprecipitation assays (RIPA)
- Surface Plasmon Resonance (SPR)
- Other technologies e.g., ECL, DELFIA, Gyrolabs

Confirmatory assays - for confirming antibodies
Other assays - for specificity of the antibodies

Neutralization assays - for discriminating neutralizing & non-neutralizing antibodies.
- Cell- based assay or
- Non-cell-based ligand binding assay
Test samples

Tier 1 - screening

Test samples

Screening Assay

Confirmed positive samples

Confirmatory Assay

Confirmed positive samples

Tier 2 - confirmation

Negative samples

Neutralisation Assay

Characterisation e.g. titer, affinity, isotype

Tier 3 - characterisation

Correlation of produced antibodies with clinical responses

Assays for clinical markers & assessment of clinical response in patients

positive samples

negative samples

negative samples
Testing is challenging

- So far, there is no perfect assay for antibody screening.
- Each assay has its own relative merits and weaknesses.
- May need to evaluate more than one assay platform, assay/assay conditions dependent on therapeutic

- Assays qualitative (no reference standard); controls needed
  - Positive: for development, define sensitivity, tolerance. Hyperimmunised animal sera - affinity purified, mAbs, anti-idiotypic antibodies
  - Negative: for setting threshold/cut-off for ‘discrimination’
    Pre-therapy sera, irrelevant antibody, healthy sera (single/pooled).

- Regulatory obligation to validate assays

  Target: Measure Polyclonal response (antibodies of varying avidities, isotypes)
Immunogenicity Assessment Strategy Design and Interpretation

• Studies need to be carefully and prospectively designed to ensure all procedures are in place prior to initiation
  – Selection, assessment, characterization and validation of assays
  – Identification of appropriate sampling points, duration of testing
  – Sample volumes and sample processing/storage
  – Selection of statistical methods for analysis of data

• This applies to all assays

• Strategy needs to be established on a case-by-case basis — product, patients, expected clinical parameters
  – In chronic use — sequential sampling for a year
  – In view of variability of antibody responses, adequate numbers of patients needed

• However, unwanted immunogenicity may occur at a level, which is not detected in studies pre-approval so assessment post-approval, as part of pharmacovigilance surveillance is needed
Biosimilars as Biologicals

• As is clear from the EMA definition, Biosimilars are Biologicals. They differ from innovator Biologicals in the regulatory process used for their approval.

• As Biosimilars are ‘scientifically’ Biologicals they should be regarded as such when immunogenicity is being considered.

• There is no reason to treat approved Biosimilars any differently from all Biologicals (including innovator products) from the immunogenicity perspective.
Biosimilars: Comparability Concept

Comparability studies are needed to generate evidence substantiating the similar nature, in terms of quality, safety and efficacy, of the new similar biological medicinal product and the chosen reference medicinal product authorised in the Community.
Comparative Immunogenicity

- Compares immunogenicity of different products; Studies need to be designed to demonstrate whether the immunogenicity of the products is the same or significantly different.
- This is likely to affect the design of the studies & their interpretation.
- For this, a homogeneous and clinically relevant patient population should be selected. Head-to-Head studies needed. Same assays & sampling strategy should be used.
- The consequences of immunogenicity also must be compared.
- Post-approval assessment may be necessary, usually as part of pharmacovigilance surveillance.
Relative Immunogenicity

Comparative Clinical Trial using RP and NP

Patient samples

Screening Assays

Using RP

Using NP

-ab +ve samples followed

RP

NP

Confirmation & further characterization as per strategy using RP and NP

Provide information regarding immunogenicity profile of each product – antibody types, kinetics of antibody development, cross-reactivity. Assess correlation of characterized antibodies with clinical responses to biologic therapeutic.
Relative Immunogenicity

Provide information regarding immunogenicity of each product – titres etc, kinetics, cross-reactivity. Assess correlation of characterized antibodies with clinical responses to biologic therapeutic.
<table>
<thead>
<tr>
<th>Biosimilar</th>
<th>Ab frequency</th>
<th>Reference</th>
<th>Ab frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omnitrope (SC)</td>
<td>0/51 (0.0%)</td>
<td>Genotropin</td>
<td>1/44 (2.3%)</td>
</tr>
<tr>
<td>Valtropin (SC)</td>
<td>3/98 (3.4%)</td>
<td>Humatrope</td>
<td>1/49 (2.0%)</td>
</tr>
<tr>
<td>Binocrit (IV)</td>
<td>2/314 (0.6%)</td>
<td>Erypo</td>
<td>3/164 (1.8%)</td>
</tr>
<tr>
<td>Silapo (IV)</td>
<td>0/305 (0.0%)</td>
<td>Erypo</td>
<td>0/304 (0.0%)</td>
</tr>
<tr>
<td>Silapo (SC)</td>
<td>0/323 (0.0%)</td>
<td>Erypo</td>
<td>0/230 (0.0%)</td>
</tr>
<tr>
<td>Ratiograsstimal (SC)</td>
<td>7/356 (2.0%)</td>
<td>Neupogen</td>
<td>2/134 (1.5%)</td>
</tr>
<tr>
<td>Zarzio (IV / SC)</td>
<td>0%</td>
<td>Neupogen</td>
<td>0%</td>
</tr>
<tr>
<td>(Phase 1, crossover)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nivestim</td>
<td>3/183 (1.6%)</td>
<td>Neupogen</td>
<td>0/95 (0.0%)</td>
</tr>
<tr>
<td>Bemfola</td>
<td>0/249 (0%)</td>
<td>Gonal-f</td>
<td>0/123 (0%)</td>
</tr>
<tr>
<td>Insulin Marvel §</td>
<td>T1DM: 25/114 (21.9%)</td>
<td>Humulin</td>
<td>T1DM: 16/114 (14.0%)</td>
</tr>
<tr>
<td></td>
<td>T2DM: 14/131 (10.7%)</td>
<td></td>
<td>T2DM: 17/136 (12.5%)</td>
</tr>
<tr>
<td>Remsima - AS - RA</td>
<td>37.5%</td>
<td>Remicade</td>
<td>36.1%</td>
</tr>
<tr>
<td></td>
<td>55.6%</td>
<td></td>
<td>54.3%</td>
</tr>
</tbody>
</table>

Data from EPARs at [www.ema.europa.eu](http://www.ema.europa.eu)

§ Application withdrawn. Table courtesy of Martina Weise
Immunogenicity Studies: Biosimilars

The immunogenicity of the marketed product does not influence the need for comparative immunogenicity studies.

However, if the immunogenicity profiles of marketed and biosimilar products are significantly different, they can be considered DISSIMILAR.
Conclusions

- Immunogenicity issues occur all along the life cycle of a product and particularly when:
  - a new therapeutic protein is developed and used for various clinical indications
  - a change is introduced e.g. process, formulation, storage conditions etc
  - a biosimilar product is proposed

- Assessment requires:
  - an optimal antibody testing strategy
  - validated methodologies and reference standards
Biosimilars: Unwanted Immunogenicity

Quote from EMA BMWP chairmen:

‘Unwanted Immunogenicity is the biggest challenge for the approval of Biosimilars’
Acknowledgements

Meenu Wadhwa
Chris Bird
Isabelle Cludts

Colleagues of the BMWP