Safety of biopharmaceuticals immunogenicity

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The history of the use of proteins in medicine
An example of an animal derived biotech product: diphtheria antitoxin
The most used animal derived biologic: porcine/bovine insulin
A human protein from natural source: human growth hormone
The first phase of protein drugs

- Based on
  - Recombinant DNA technology
  - Hybridoma technology
- Copies of natural products
Bacteria making insulin
First generation biopharmaceuticals

- Insulin
- Growth hormone
- Interferon alfa
- Interferon beta
- Interferon gamma
- G-CSF
- GM-CSF
- EPO
- FSH
- HBV vaccine
- Monoclonal antibodies (MAb)

G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; EPO, erythropoietin; FSH, follicle stimulating hormone; HBV, hepatitis B virus
Failed biopharmaceuticals

- TNF
- IL-1,2 etc
- MDGF
- Centoxin
- TNFR-Ig

TNF, tumour necrosis factor; IL, interleukin; MDGF, macrophage-derived growth factor; TNFR-Ig, tumour necrosis factor receptor I protein.
Problems with biopharmaceuticals

- Specificity
- Immunogenicity
- Parts of complicated network
- Unknown mode of action
- Unfavourable pharmacokinetics
Second generation biopharmaceuticals

- Sequence variants
- Variants of post translational modification
- Hybrid molecules
- Unnatural modification
- New forms of administration
Immunogenicity of therapeutic proteins

A key issue
History of the medical use of proteins

- Proteins of animal origin (e.g., equine antisera, porcine/bovine insulin): foreign proteins

- Human derived proteins (e.g., growth hormone, factor VIII): no immune tolerance

- Recombinant human proteins (e.g., insulin, interferons, GM-CSF): ??
Most biopharmaceuticals induce antibodies

Two mechanisms

• Reaction to neo-antigens

• Breakdown of immune tolerance
### Types of immune reaction against biopharmaceuticals

**Reaction to foreign proteins**

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Products of microbial or animal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of antibody production</td>
<td>Fast, often after a single injection, neutralising antibodies, long duration</td>
</tr>
<tr>
<td>Cause</td>
<td>The presence of foreign antigens</td>
</tr>
</tbody>
</table>
### Types of immune reaction against biopharmaceuticals

**Breaking of self-tolerance**

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Human homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of antibody production</td>
<td>Slow, after long treatment, binding antibodies, disappear after treatment</td>
</tr>
<tr>
<td>Cause</td>
<td>Mainly impurities and aggregates</td>
</tr>
</tbody>
</table>
Fate of auto-reactive B cells after encountering conjugated VLPs

Monomeric BCR/self-Ag complexes

Oligomerization of BCR/self-Ag signaling complexes

Toleragenic signals

Survival/Proliferative signals

Q’s: Qualitative or Quantitative differences in signaling? Involve initial activation of B cells or reactivation of anergic B cells?
Factors influencing immunogenicity

Structural properties
Sequence variation
Glycosylation

Other factors
Assays
Contaminants and impurities
Formulation
Downstream processing
Route of application
Dose and length of treatment
Patient characteristics
Unknown factors
Structural properties

- Degree of “non-self”: biopharmaceuticals of bacterial and plant origin (Streptokinase, staphylokinase, asparaginase)

- Glycosylation
  - Protection of antigenic sites (GM-CSF)
  - Influence on solubility (Interferon beta)
Factors influencing immunogenicity

Assays
Neutralising antibodies standard serum in different laboratories

Neutralizing Activity

Serum Dilution

Neutralizing Activity

Serum Dilution

400 200 100 50 25 12.5 6.25 3.1
Factors influencing immunogenicity

Formulation: the interferon alpha 2 case
## Two main IFN alpha-2 preparations

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Commercial name</th>
<th>Aa position 23</th>
<th>Natural allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu IFN alpha-2a</td>
<td>Roferon</td>
<td>Lys</td>
<td>No</td>
</tr>
<tr>
<td>Hu IFN alpha-2b</td>
<td>Intron</td>
<td>Arg</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Hu IFN, human interferon
Antigenicity of different IFN alpha-2a formulations
Other factors influencing immunogenicity

- Downstream processing
  - Viral inactivation factor VIII

- Impurities and contaminants
  - Insulin
  - Growth hormone

- Duration of treatment
  - Avonex/Rebif versus Betaseron
Other factors influencing immunogenicity

• Route of administration
  – SC>IM>IV>local

• Type of disease

• Genetic background of patients
  – MHC?
  – Haemophilia

• Unknown factors
Antigenicity of identical Hu IFN beta produced at different sites

[Graph showing antigenicity over time for BG9015 and AVONEX™]
## Consequences of antibodies

### Loss of efficacy
- Insulin
- Streptokinase
- Staphylokinase
- ADA
- Salmon calcitonin
- Factor VIII
- Interferon alpha 2
- Interferon beta
- IL-2
- GnRH
- TNFR55/IgG1
- Denileukin diftitox
- HCG
- GM-CSF/IL3

### Enhancement of efficacy
- Growth hormone

### Neutralization of native protein
- MDGF
- EPO

### General immune effects
- Allergy
- Anaphylaxis
- Serum sickness, etc

HCG, Human chorionic gonadotropin; ADA, adenoside deaminase; GnRH, gonadotropin-releasing hormone
Relation between sustained response and antibody level in IFN alpha-2a treated HCV patients

HCV, hepatitis C virus
Consequences of antibodies

**Loss of efficacy**
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- Salmon calcitonin
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**Neutralization of native protein**
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- Allergy
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AMGEN DISCONTINUES DEVELOPMENT OF MGDF

FOR IMMEDIATE RELEASE

THOUSAND OAKS, Calif., September 11, 1998 -- Amgen (NASDAQ:AMGN) today reported that it has discontinued development of its megakaryocyte growth and development factor (PEG-rHuMGDF) due to evidence of neutralizing antibodies in a few patients participating in cancer clinical trials and in additional people in platelet donor clinical trials.

Amgen is a global biotechnology company that discovers, develops, manufactures and markets cost-effective human therapeutics based on advances in cellular and molecular biology.

CONTACT: Amgen, Thousand Oaks
David Kaye, 805/447-6692 (media)
Denise Powell, 805/447-4346 (investors)

EDITOR’S NOTE: An electronic version of this news release may be accessed via our web site at www.Amgen.com. Visit the Corporate Center and click on Amgen News. Journalists and media representatives may sign up to receive all news releases electronically at time of announcement by filling out a short form in the Amgen News section of the web site.
Prediction of immunogenicity

• Quality of the product
• Sequence analysis
• Reactivity with antibodies
• Animal studies
  – Conventional animals
  – Non-human primates
  – Transgenic immune tolerant mice
What caused Eprex associated PRCA?
Normal bone marrow

PRCA bone marrow

PRCA, pure red cell aplasia
Pure red cell aplasia associated with anti-EPO antibodies

Nicole Casadevall

- 1996 PRCA case with natural antibodies
- 2002 13 cases with antibodies associated with epoetin treatment
Why was Eprex implicated?

- High association between Eprex and PRCA
- Geographic distribution
- Association with formulation change
PRCA cases reported by the FDA and Johnson & Johnson

- **Epoetin alfa assoc. cases (ex USA)**
- **Epoetin alfa assoc. cases (in USA)**

**Cumulative number, Medwatch**

- **1997**: 0
- **1998**: 15
- **1999**: 30
- **2000**: 45
- **2001**: 60
- **Up to 30.04.02**: 120
- **Up to 31.07.02**: 180
- **Up to 31.10.02**: 210
- **Up to 31.12.02**: 225
- **Up to 31.07.03**: 240

**Modification of Eprex® formulation outside USA**
Recent concern over use of HSA in Europe because of potential transmission of infectious viruses or BSE prions

In 1998, HSA was replaced with polysorbate 80 in prefilled syringes of Eprex® distributed ex-US
## Main stabilizers used in the epoetin formulations

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<tbody>
<tr>
<td>HSA</td>
<td>HSA</td>
<td>Polysorbate 80</td>
<td>Polysorbate 20</td>
<td>Glycine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycine</td>
<td>Glycine</td>
<td>Complex of 5 other amino acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium chloride</td>
<td>Urea</td>
</tr>
</tbody>
</table>
Factors potentially contributing to the immunogenicity of Eprex®

- Formation of micelles associated with Epo (Hermeling et al. 2003)
- Silicon droplets in the prefilled syringes
- Leachates from rubber stoppers
- Mishandling
Mishandling

- Mishandling with a slightly less stable product may explain all features of PRCA
  - Biological rationale
  - Fits with data concerning other product
  - Fits the pathogenesis
  - Fits with the epidemiological data
Conclusion

• The mystery of Eprex® associated PRCA has not been solved, but aggregates are the most likely explanation
• Immunogenicity is an issue with all therapeutic proteins