About Pipelines and Shifting paradigms: biopharmaceuticals versus low molecular weight drugs

- Prof. dr. Daan J.A. Crommelin,
- Scientific Director TI Pharma, a Public Private Partnership, Leiden
- and Dept. Pharmaceutics, UIPS, UU

- Alicante, October 2008
‘Every protein has a life of its own’

(anonymous Ph.D. student)
With the highest growth rates within the entire pharma market, biopharmaceuticals will reach > US$ 92 billion revenues in 2011

Most biopharmaceutical proteins have small markets, but high value < 10 kg/yr, > US$10,000/g

Figure 1: Global biopharmaceuticals market revenue forecast, 2001–2011.
The number of Biotech approvals surpassed the small molecule approvals in 2002 (US)

Source: BioGeneriX
Biopharma in Perspective

- The first biotech therapy to earn FDA approval was recombinant human insulin (Genentech & Eli Lilly) in 1982.

- Since then, as of Oct 2006, more than 250 drugs & vaccines for nearly 400 indications developed by biotech companies have been approved by FDA (inc. small-molecules and tissue-engineered products). http://bio.org/speeches/pubs/er/approveddrugs.asp

- More than 400 biotech drugs & vaccines are currently in clinical trials targeting more than 200 diseases

 Courtesy ÖZCEADA
Interferons

Drugs desperately looking for a disease….

5 billion dollars!

Weimar W, Lameijer LD, Edy VG, Schellekens H.

Prophylactic use of interferon in renal allograft recipients.
PMID: 377705 [PubMed - indexed for MEDLINE]
Biopharmaceuticals = pharmaceutical biotech products = biologicals

- **Medical aspects:**
  - indications for serious diseases; meeting unmet medical needs
- **Economical aspects**
  - relatively small, but fast growing
- **Pharmaceutical aspects:**
  - delicate complex molecules
  - potent molecules (?)
  - delivery issues
### (Liver) storage diseases: orphan diseases

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>number of patients</th>
<th>treatment per year/keuros</th>
<th>total/y/product (x 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerezyme® (Imiglucerase)</td>
<td>27000</td>
<td>476</td>
<td>12852000</td>
</tr>
<tr>
<td>Fabrazyme® (Agalsidase beta)</td>
<td>1200</td>
<td>182</td>
<td>218400</td>
</tr>
<tr>
<td>Replagal® (Agalsidase alfa)</td>
<td>1200</td>
<td>189</td>
<td>226800</td>
</tr>
<tr>
<td>Aldurazyme® (Laronidase)</td>
<td>1100</td>
<td>473</td>
<td>520300</td>
</tr>
<tr>
<td>Myozyme® (alglucosidase)</td>
<td>4500</td>
<td>351</td>
<td>1579500</td>
</tr>
<tr>
<td>Elaprase® (Idurosulfatase)</td>
<td>1000</td>
<td>234</td>
<td>234000</td>
</tr>
<tr>
<td>Naglazyme® (Galsulfase)</td>
<td>400</td>
<td>100</td>
<td>40000</td>
</tr>
<tr>
<td>Elaprase® (idurosulfatase)</td>
<td>1000</td>
<td>2340</td>
<td>2340000</td>
</tr>
<tr>
<td>Grand total</td>
<td>37400</td>
<td></td>
<td>18011000</td>
</tr>
</tbody>
</table>

Adapted from Theo Dingermann
Present Arsenal

Examples of the types of product on the market:

- Hormones, growth factors, enzymes
  - Fertility hormones
  - Human insulin
  - Enzymes
  - Human growth factors (G-CSF, haematopoietic growth factors)
- Cytokines
  - Interleukins
  - Interferons
- Vaccines & antigens
  - Hepatitis B antigen
  - Cholera vaccine
- Antisense
  - Fomivirsen
- Cell therapy
  - Carticel, Epicel
Technology Evolution in Pharma Industry

- **Immature**
  - Non validated technologies
  - First products entering development phases
  - No products in market

- **Emerging**
  - Validated technologies
  - More products in development
  - Few products in the market

- **Growing**
  - Mastering technologies
  - Ongoing optimization
  - Many products in development and in the market

- **Mature**
  - Fully mastered and optimized technologies
  - Many products in development and in the market

- **Decreasing**
  - Common technologies
  - Manufacturing alternatives required
  - Decreasing number of products in development and, eventually, in the market

Source: Paulo Barbanti
Gene Therapy: viruses as delivery system....
In 2002 and 2003, it was reported that three of nine children in France who had been cured of severe combined immunodeficiency disease (SCID) with gene therapy had developed cancer two to three years later. Children born with this disorder will die in the first year of life unless they can find a matching blood marrow donor, which is hard to do.
Where biopharmaceuticals differ from low molecular weight drugs

- Molecular weight
- Complexity of structure
- Characterization
  - Structure and physico-chemical properties
  - Protein purity
  - Biological activity
- Stability (shelf life/life on the shelf)
- Immunogenicity
- Needle focused

<table>
<thead>
<tr>
<th>Product</th>
<th>Molecular weight (kDa)</th>
<th>Number of amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>0.151</td>
<td>N/A</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>4.5</td>
<td>32</td>
</tr>
<tr>
<td>Epoetin-α</td>
<td>30.4</td>
<td>165</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>264.0</td>
<td>2,332</td>
</tr>
</tbody>
</table>
Protein Conformations

- Lactate Dehydrogenase: Mixed α/β
- Immunoglobulin Fold: β
- Hemoglobin B Chain: α
The conformational state of a protein

Figure 5 | The many conformational choices for a polypeptide chain. Adapted, with permission, from REF. 138 © (2003) Macmillan Magazines Ltd.
Interactions in Proteins

Wang [ref. 5]
Isoform distribution

Isoform patterns: deviations displayed by 9 of the 11 samples (including additional basic and acidic isoforms, and increased bar intensity) compared with the EPREX® standard (E)
Stability issues

**Chemical instability**
- Disulfide Exchange
- Deamidation
- Oxidation
- Proteolysis

**Physical instability**
- Denaturation
- Aggregation
- Precipitation
- Adsorption
Bottom line: complete characterization: mission impossible

The quality is in the process

---

### Table 3
(Analytical) techniques for monitoring protein structure

<table>
<thead>
<tr>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV absorption</td>
</tr>
<tr>
<td>Circular dichroism spectroscopy</td>
</tr>
<tr>
<td>Fourier transform IR</td>
</tr>
<tr>
<td>Fluorescence spectroscopy</td>
</tr>
<tr>
<td>NMR spectroscopy</td>
</tr>
<tr>
<td>Calorimetric approaches</td>
</tr>
<tr>
<td>Bio-essays</td>
</tr>
<tr>
<td>Enzyme and chemical assays</td>
</tr>
<tr>
<td>ELISA</td>
</tr>
<tr>
<td>Immune precipitation</td>
</tr>
<tr>
<td>Biosensor (SFC, CEM)</td>
</tr>
<tr>
<td>Potency testing</td>
</tr>
<tr>
<td>In cell lines</td>
</tr>
<tr>
<td>In animals</td>
</tr>
<tr>
<td>Chromatographic techniques</td>
</tr>
<tr>
<td>RP-HPLC</td>
</tr>
<tr>
<td>SEC-HPLC</td>
</tr>
<tr>
<td>Hydrophobic interaction HPLC</td>
</tr>
<tr>
<td>Ion-exchange HPLC</td>
</tr>
<tr>
<td>Peptide mapping</td>
</tr>
<tr>
<td>Electrophoretic techniques</td>
</tr>
<tr>
<td>SDS-PAGE</td>
</tr>
<tr>
<td>IEF</td>
</tr>
<tr>
<td>CZE</td>
</tr>
<tr>
<td>Field flow fractionation</td>
</tr>
<tr>
<td>Ultracentrifugation</td>
</tr>
<tr>
<td>Static and dynamic light scattering</td>
</tr>
<tr>
<td>Electron microscopy</td>
</tr>
<tr>
<td>X-ray techniques</td>
</tr>
<tr>
<td>Mass spectrometry</td>
</tr>
</tbody>
</table>
Biopharmaceutical purification means:

- The product should only contain the desired protein
  - Biopharmaceuticals are produced by living host cells that are also naturally producing many other proteins, as well as sugars, fatty acids, etc.
- All contaminants need to be excluded
  - Any trace of viruses, prions, and endotoxins needs to be removed

Purification is a complex process critical to the performance of the biopharmaceutical

Characterization: biological activity

- The biological activity (i.e. efficacy and safety) of a biopharmaceutical depends on:
  - 3D structure
  - degree of glycosylation and location of glycosylation sites
  - isoform profile
- These characteristics are unlikely (for the larger proteins) to be the same for a biosimilar and the original biopharmaceutical

It is highly unlikely that a biosimilar is identical to the original biopharmaceutical

Potency tests…….

European Pharmacopoeia

- Insulin: no in vivo potency
- Human growth hormone: no in vivo potency

USP

- Insulin: in vivo potency
- Human growth hormone: in vivo potency
### EPREX-induced PRCA cases (2001-2003)

<table>
<thead>
<tr>
<th>HSA</th>
<th>Coated stoppers</th>
<th>Ab(+) PRCA cases</th>
<th>SC Exposure (pt-ys)</th>
<th>Incidence rate (per 100,000 pt-ys)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>2</td>
<td>42,305</td>
<td>4.7 (0.57 – 17.1)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>116</td>
<td>308,232</td>
<td>46.1 (38.8 – 54.3)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>1</td>
<td>36,608</td>
<td>2.6 (0.07 – 14.4)</td>
</tr>
</tbody>
</table>

Adapted from Boven et al, Kidney Int 2005; 67: 2346

PRCA = pure red cell aplasia…… by immunogenic epo
Biopharmaceutical development

Species specificity.....

- Activity non-species specific….human insulin, human growth hormone, erythropoietins, G-CSF, number of enzymes

- Activity species specific… interferons, monoclonal antibodies, GM-CSF
### TABLE 7.4 Preclinical support to phase 1

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Drug</th>
<th>Biologic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (Mo)</td>
<td>Material (g)</td>
</tr>
<tr>
<td>Assay validation</td>
<td>PK (LC/MS/MS)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>PD (LBI; FACS)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-reactivity</td>
<td>Receptor screening</td>
<td>2</td>
</tr>
<tr>
<td>Genetic toxicology</td>
<td>Mutagenicity (Ames)</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Chromosomal aberration in vitro (CHO)</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Chromosomal aberration in vivo (RMN)</td>
<td>2.5</td>
</tr>
<tr>
<td>Safety pharmacology</td>
<td>Cardiovascular, monkey</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Respiratory, rat</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>CNS, rat</td>
<td>2.5</td>
</tr>
<tr>
<td>General toxicology</td>
<td>1 mo (+2 wk rec) rat</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1 mo (+2 wk rec) monkey</td>
<td>4</td>
</tr>
<tr>
<td>Studies</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Material</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

*Note: CHO = Chinese hamster ovary cells, ELISA = enzyme-linked immnosorbent assay, FACS = fluorescent-activated cell sorting or flow cytometry; IHC = immunohistochemistry; LBI = lens binding inhibition, rec = repeat, RMN = rat microsome.*
## TABLE 7.5 Preclinical support to phase 2

<table>
<thead>
<tr>
<th>Phase 2</th>
<th>Drug</th>
<th>Biologic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (Mo)</td>
<td>Material (g)</td>
</tr>
<tr>
<td>General toxicology</td>
<td>3 mo (+1 more) rat monkey</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>3 mo (+1 more) rabbit monkey</td>
<td>7</td>
</tr>
<tr>
<td>Reproductive and developmental</td>
<td>Segment 2 RF, rat</td>
<td>2.5</td>
</tr>
<tr>
<td>toxicology</td>
<td>Segment 2 RF, rabbit</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Segment 2, rat</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Segment 2, rabbit</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Segment 1, rat, male</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Segment 1, rat, female</td>
<td>6</td>
</tr>
<tr>
<td>Studies</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Material</td>
<td>2</td>
<td>4940</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note: Rec = recovery, RF = range-finder.*
<table>
<thead>
<tr>
<th>Phase 3</th>
<th>Drug</th>
<th>Biologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive and developmental</td>
<td>Segment 3, rat</td>
<td>Segment 3, monkey</td>
</tr>
<tr>
<td>toxicology</td>
<td>9 Mo</td>
<td>18 Mo</td>
</tr>
<tr>
<td></td>
<td>250 g</td>
<td>120 g</td>
</tr>
<tr>
<td></td>
<td>350 $K$</td>
<td>1500 $K$</td>
</tr>
<tr>
<td>Chronic toxicology</td>
<td>6 Mo (+2 mo rec) rat</td>
<td>9 mo (+3 mo rec) monkey</td>
</tr>
<tr>
<td></td>
<td>11 Mo</td>
<td>16 Mo</td>
</tr>
<tr>
<td></td>
<td>1250 g</td>
<td>400 g</td>
</tr>
<tr>
<td></td>
<td>355 $K$</td>
<td>750 $K$</td>
</tr>
<tr>
<td></td>
<td>20 Mo</td>
<td>18 Mo</td>
</tr>
<tr>
<td></td>
<td>3600 g</td>
<td>1475 $K$</td>
</tr>
<tr>
<td></td>
<td>770 $K$</td>
<td>2250 $K$</td>
</tr>
<tr>
<td></td>
<td>20 Mo</td>
<td>5 Mo</td>
</tr>
<tr>
<td></td>
<td>2.5 Mo</td>
<td>520 g</td>
</tr>
<tr>
<td></td>
<td>35100 g</td>
<td>125 g</td>
</tr>
<tr>
<td></td>
<td>125 $K$</td>
<td>1800 $K$</td>
</tr>
<tr>
<td></td>
<td>22.5 Mo</td>
<td>23 Mo</td>
</tr>
<tr>
<td></td>
<td>1600 g</td>
<td>4050 $K$</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>2 yr rat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3000 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1700 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>225 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>900 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2825 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>520 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1800 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3025 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75 $K$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38 Mo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2900 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4050 $K$</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 9

Selection of Relevant Species

MEENA SUBRAMANYAM, PhD, NICOLA RINALDI, PhD,
ELISABETH MERTSCHING, PhD, and DAVID HUTTO, PhD, DVM

Contents

9.1 Introduction
9.1.1 Species Selection: Biologies versus Small Molecule Therapies 181
9.1.2 General Considerations for Relevant Species Selection 182
9.2 In vivo Pharmacodynamic Effects 183
9.3 Ex vivo Biological Effect 185
9.3.1 Fixed Endpoint Assays 188
9.3.2 Signaling Assays 189
9.4 Presence and Distribution of Receptor in Predicted Tissues 190
9.5 Ex vivo / In vitro Interaction of the Biopharmaceutical with Known Target 191
9.5.1 Binding Assays: Flow Cytometry Based Methods 194
9.5.2 In vitro Binding Affinity Determination 196
9.6 Transcript Profiling 198
9.7 In silico Analysis 199
9.7.1 Sequence Homology of Target Protein in Various Species 200
9.7.2 Utilization of Microarray Data 201
9.8 Conclusions 204
References 205

9.1 INTRODUCTION

The goal of biopharmaceutical development is to maximize therapeutic benefit while minimizing the risk of treatment-related toxicity. To mimic putative interpatient treatment differences in test article responsiveness, it is important...
### Table 9.2 Cross-species tissue cross-reactivity study of a monoclonal antibody

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type</th>
<th>Human</th>
<th>Cynomolgus Monkey</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary bladder</td>
<td>Urothelium</td>
<td>2 (3-4)</td>
<td>3 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Ureter</td>
<td>Urothelium</td>
<td>2 (3-4)</td>
<td>3 (2-4)</td>
<td>NA</td>
</tr>
<tr>
<td>Tonsil</td>
<td>Mucosal epithelium</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>NA</td>
</tr>
<tr>
<td>Uterus-cervix</td>
<td>Mucosa</td>
<td>3 (1-3)</td>
<td>1 (2)</td>
<td>NA</td>
</tr>
<tr>
<td>Eye</td>
<td>Corneal epithelium</td>
<td>1 (1)</td>
<td>NS</td>
<td>1 (1)*</td>
</tr>
<tr>
<td>Breast</td>
<td>Glandular epithelium</td>
<td>3 (1-3)</td>
<td>3 (1-3)</td>
<td>NS</td>
</tr>
<tr>
<td>Fallopian tube</td>
<td>Tubular epithelium</td>
<td>2 (1-2)</td>
<td>1 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney</td>
<td>Tubular epithelium-cortex</td>
<td>3 (3)</td>
<td>1 (3)*</td>
<td>3 (1-2)</td>
</tr>
<tr>
<td>Lung</td>
<td>Alveolar epithelium</td>
<td>3 (2-3)</td>
<td>2 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Ductular epithelium</td>
<td>3 (2)</td>
<td>2 (1-2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Prostate</td>
<td>Glandular epithelium</td>
<td>2 (1-2)</td>
<td>1 (2)</td>
<td>3 (2)*</td>
</tr>
<tr>
<td>Thyroid</td>
<td>Follicular epithelium</td>
<td>3 (2-3)</td>
<td>2 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>Uterus-endometrium</td>
<td>Endometrial mucosa</td>
<td>2 (2)</td>
<td>NS</td>
<td>3 (1-3)*</td>
</tr>
<tr>
<td>Adrenal</td>
<td>Cortical epithelium</td>
<td>3 (1-2)</td>
<td>2 (1)</td>
<td>NS</td>
</tr>
<tr>
<td>Pituitary</td>
<td>Adenohypophysis epithelium</td>
<td>3 (2)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>Sinusoidal mesenchymal cells</td>
<td>3 (2-4)</td>
<td>3 (2-3)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Immunogenicity of Biopharmaceuticals

Huub Schellekens, Daan Crommelin* and Wim Jiskoot

Dept. Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, UIPS
Scientific Director of the Dutch Top Institute Pharma*, Leiden
Co-founder of OctoPlus, Leiden*
Selected papers (2004) on the immunogenicity of recombinant human interferon beta

Antonio Bertolotto
Florian Deisenhammer
Paolo Gallo
Per Sölberg Sørensen

Immuneogenicity of interferon beta: differences among products

Neutralizing antibodies reduce the efficacy of βIFN during treatment of multiple sclerosis

S. Malucchi, MD; A. Sala, PhD; F. Gilli, PhD; R. Bottiero, MD; A. Di Sapio, MD;
M. Capobianco, MD; and A. Bertolotto, MD

Neutralizing antibodies against IFN-β in multiple sclerosis: antagonization of IFN-β mediated suppression of MMPs

Francesca Gilli,1 Antonio Bertolotto,1 Arianna Sala,1 Francine Hoffmann,2 Marco Capobianco,1 Simona Malucchi,1 Tracy Glass,2 Ludwig Kappos,3 Raija L.P. Lindberg2,4 and David Leppert2,4,*

Hans-Peter Hartung
Huub Schellekens
Frederick E. Munschauer III

Neutralizing antibodies to interferon beta in patients with multiple sclerosis: scientific background and clinical implications

In the EU ca. 100 million euro/year is spent on useless IFN-β therapy
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 Self-Antigen (complexes)

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?
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
Relation between sustained response and antibody level in IFN alpha-2a treated HCV patients

![Bar chart showing the relation between sustained response and antibody level](chart.png)
Prediction of immunogenicity
Immuno-genicity

Sequence variation
- human
- non-human

Glycosylation

Contaminants & impurities

Formulation

Application route

Dose

Length of treatment

Assay technology

Patient features

Unknown factors

Prediction of immunogenicity

- Quality of the product
- Sequence analysis
- Reactivity with antibodies
- Animal studies
  - Conventional animals
  - Non-human primates
  - Transgenic immune tolerant mice
Transgenic immune tolerant mice to test immunogenicity of non-structural factors

The Hu IFN alpha 2 transgenics

Hermeling, Crommelin, Jiskoot, Schellekens
Hu IFN alpha 2 immune tolerant mice

- Current use: to study immunogenicity of modified Hu IFN alpha 2b preparations
- Obtained from Roche
The immunogenicity of HuIFN alpha 2 stored at different pH

![Graph showing IgG titer for Wildtype and Transgenic samples at different pH levels. The graph indicates significant differences in immunogenicity between the two types, with the Transgenic samples showing higher IgG titers.](image)

Hermeling et al.
Conclusions about transgenic immune tolerant mice

- **It is the aggregates!**
  - Native epitopes
  - Very sensitive ( <1 µg)
  - Aggregates should be not too big

- Beware of the mouse strain

- Always test antibodies to final product

- The immune reaction in wild type mice is different from breaking B cell tolerance
EU: Innovative Medicines Initiative

- EU Commission and EFPIA have established a Strategic Research Agenda (SRA), the ‘roadmap’ for the Innovative Medicines Initiative (IMI) – FP7

- Recommendations to address the key ‘bottlenecks’ in four areas of the biomedical R&D process:
  - (Predicting) drug safety
  - (Predicting) drug efficacy
  - Knowledge management
  - Education and Training

- Call for project proposals came out April 2008

- Immunogenicity of biopharmaceuticals on top…..

- More info: www.imi-europe.org
Welcome to Innovative Medicines Initiative Online

The Innovative Medicines Initiative is a proposed partnership between the European Commission and the European Federation of Pharmaceutical Industry and Associations (EFPIA).

For more, please take a look at the new IMI Flyer or at the new IMI Overview Presentation.

More...

**IMI Objectives**

The vision of IMI is to create Biomedical Research & Development leadership for Europe to benefit patients and society.

More...

**Why IMI Matters to You**

IMI will drive the creation of a vibrant and dynamic scientific environment and ensure a strong European biomedical science base.

More...
Present Arsenal

Examples of the types of product on the market:

- Homones, growth factors, enzymes
  - Fertility hormones
  - Human insulin
  - Enzymes
  - Human growth factors (G-CSF, haematopoietic growth factors)
- Cytokines
  - Interleukins
  - Interferons
- Vaccines & antigens
  - Hepatitis B antigen
  - Cholera vaccine
- Antisense
  - Fomivirsen
- Cell therapy
  - Carticel, Epicel

Endogenous products
Delivery of Proteins

Welcome to the kingdom of the needle?

- Are we stuck to the needle?
Get rid of the protein.....

**Insulin-alternatives: small is beautiful...**

- **Vaccines**
  - Diamyd, Diapep277
- **Thiazolinedione-derivatives**
  - PPAR agonists e.g. netoglitazone, balaglitazone, rosiglitazone
- **DPP4- inhibitors,**
  - e.g. sitagliptine, vildagliptin, saxagliptin, alogliptine
- **GLP-1 analogues**
  - e.g., liraglutide
- **Metaglidasen**
- **Succinobucol**
- **Managlinat dialanetil**
- **Solabegron**
- **BGP15**
Further paradigm shifts at the horizon

• New production approaches
  – Transgenic animals, transgenic plants
  • siRNA, gene therapy
  • Stem cell therapy
• Modified proteins
  – IgG fragments
  – Fusion proteins
Modified proteins

Challenging Mother nature
Five expression technologies for protein production

- Transgenic Animals: Sheep, goat, cow
- Saccharomyces: Yeast
- Mammalian Cells: CHO
- Transgenic Plants: Tobacco, moss
- Bacteria: Escherichia coli
FOR IMMEDIATE RELEASE
February 16, 2005

BIOLEX AND OCTOPLUS ANNOUNCE JOINT DEVELOPMENT OF LOCTERON™: A NOVEL, CONTROLLED RELEASE FORMULATION OF ALFA INTERFERON

Clinical Trials to Commence in 2005
Biolex Interferon alpha

- **Biolex’ LEX™ system**
  - Aquatic higher plant, Lemna
  - Secretes recombinant protein (e.g. IFNa2b)
  - Fast, inexpensive process
  - High expression levels
  - Highly scalable

www.biolex.com
A double-blind placebo-controlled clinical trial evaluated the immunogenicity of hepatitis B surface antigen (HBsAg) expressed in *potatoes* and delivered orally to previously vaccinated individuals. The potatoes accumulated HBsAg at 8.5 gg of potato tuber, and doses of 100 g of tuber were administered by ingestion. The correlate of protection for hepatitis B virus, a nonenteric pathogen, is blood serum antibody titers against HBsAg. After volunteers ate uncooked potatoes, serum anti-HBsAg titers increased in 10 of 16 volunteers (62.5%) who ate three doses of potatoes; in 9 of 17 volunteers (52.9%) who ate two doses of transgenic potatoes; and in none of the volunteers who ate nontransgenic potatoes. These results were achieved without the coadministration of a mucosal adjuvant or the need for buffering stomach pH. We conclude that a plant-derived orally delivered vaccine for prevention of hepatitis B virus should be considered as a viable component of a global immunization program.
‘Every protein has a life of its own’

(anonymous Ph.D. student)