Biologics: Pharmacokinetics and Drug Development

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Presentation Overview

- Introduction on Biologics
  - Biologics landscape

- Overview of DMPK Properties of Large Molecules (LM)/Biologics
  - ADME processes
    - Approaches to increase half-life

- Overview of Large Molecules (LM) Drug Discovery and Development
  - High level DMPK support
  - Human PK prediction
    - First Human Dose prediction
  - Comparability exercise
  - Biosimilar development
  - Other DMPK studies
    - Linking to SM Experience
What is Biologics - Definition

- Generally derived from living material--human, animal, or microorganism-- are complex in structure, and thus are usually not fully characterized.

- Examples – US FDA (CDER)
  - Monoclonal antibodies for in-vivo use
  - Cytokines, growth factors, enzymes, immunomodulators; and thrombolytics
  - Proteins intended for therapeutic use that are extracted from animals or microorganisms, including recombinant versions of these products (except clotting factors)
  - Other non-vaccine therapeutic immunotherapies
  - Vaccines, blood products and gene therapy - not covered in this presentation
FDA-Approved Biologics

Numbers of FDA-Approved Biologic Products of Various Types Available for Treating or Preventing Various Conditions.
• Number of Approved Large Molecules (LM), although accounting for a smaller fraction vs Small Molecules (SM), is on the rise
• mAbs represent the fast growing sector among Biologics
Relative Size of Small Molecules and Proteins

Monoclonal antibodies (mAbs)
Monoclonal Antibodies (mAbs)

J Clin Pharmacol 2007;47:553-565

Fig. 2. A schematic representation of the structural and functional domains of IgG.
Fig. 1. A historical timeline of therapeutic monoclonal antibody approvals in the United States relative to the development of antibody generation technologies.

*Fig. 3. Plasma half-lives of approved therapeutic antibodies of different formats.*[6]

**SM and LM DMPK Analogy**

**Drug**

\[ C_p \quad \text{keo} \quad C_e \]

\[ H \quad \text{k}_{in} \quad \text{k}_{out} \]

**Biosignal**

**R**

**Pharmacokinetics**

**Pharmacodynamics**

**Response**

*Similar high level concept*

*Different ADME mechanisms*

*Different underlying ADME determinants*

**Jusko et al., JPB 23: 5, 1995**

**Mager DE, Wyska E, Jusko, WJ, DMD. 31: 510 (2003).**

Prueksaritanont, Presented at SUNY Buffalo, Aug 2011
ADME of Biologics

- Absorption
- Distribution
- Metabolism
- Elimination

References:
Jiunn H. Lin, *Current Drug Metabolism*, 2009, 10, 661-691
Absorption of Biologics

- **Molecular Weight (MW):**
  - $\uparrow MW = \uparrow T_{\text{max}}$
  - Absorption via capillaries $MW < 1,000$ Da
  - Via lymphatic $MW > 16,000$ Da

- **Route of Administration (IV, SC, IM):**
  - *Currently there are no oral biologics on the market*

- **Immunogenicity may differ based on route**
  - SC $>$ IM $>$ IV

  More on Subcutaneous absorption
SC Absorption of Macromolecules

- Proteins larger than 20kD are generally believed to be taken up mostly by the lymphatic systems following SC administration
  - Consistent with the known structure differences of lymph and blood capillaries

**SC** ➔ **Injection site**

**Interstitial transport** ➔ **Lymphatic capillary** ➔ **Lymphatic transport** ➔ **System circulation**

**MW-dependent lymph recovery in sheep**

Overview of the Lymphatic System

- The lymphatic is a **one-way transport system** for fluid and proteins by collecting them from interstitial space and returning them to blood circulation.

- Net flow rate of lymph is \(~100-500\) times **less than** that of the blood.

- The protein composition of lymph is nearly equivalent to that of interstitial fluid.

- The lymphatic system also serves as a major transport route for immune cells and macromolecules.

Swartz MA, Adv Drug Delivery Reviews 50:3-20, 2001
Distribution

- Limited and slow tissue distribution
  - Generally approximates plasma volume (3-5% of TBW)

- Involves 2 steps
  - extravasations from the blood circulation into interstitial space
  - diffusion through the extracellular matrix to the cell surface target

- Key mechanisms - MW and charge dependent:
  - Diffusion
    - For *most* small molecules, drug entry/loss from tissue is via diffusion, and 
      \[ Cu_{tissue,ss} = Cu_{blood,ss} \]
  - Convection
    - For *most* large molecules, drug entry/loss from tissue is via convection (solvent drag by pressure gradient, and 
      \[ Cu_{tissue,ss} \ll Cu_{blood,ss} \]
  - Pinocytosis/transcytosis
    - Active/facilitated receptor-mediated endocytosis
    - Non-specific endocytosis

*Important to know if the biologic reaches its target*
Estimation of Volume of Distribution

Most standard PK analyses estimate Vss via non-compartmental techniques (i.e., \( V_{ss} = D \times AUMC/AUC^2 \)) or via mammillary compartment modes (i.e., \( V_{ss} = \sum V_i \)). These methods ARE NOT APPROPRIATE unless all CL is from the central compartment!!

A. Linear PK (diffusion/convection), no tissue metabolism
   - Standard compartmental & non-compartmental approaches

B. Non-linear distribution and/or tissue metabolism (e.g., receptor-mediated uptake, saturable tissue binding) – applicable to a number of Therapeutic Proteins/mAbs
   - Direct assessment of plasma + tissue concentrations
   - \( V = \text{Mass} / \text{Cp} \)
   - PBPK

Balthasar, Merck Presentation, 2007
Elimination of Biologics

- Elimination kinetics may be nonlinear
  - Immune-mediated (Immunogenicity)
    - Unique to Biologics
  - Catabolism/Proteolysis
    - Main reason for peptides’ short t1/2
    - Degraded to small peptides / amino acids
  - Receptor-mediated
    - Responsible for non-linear
  - FcRn salvage pathway
    - Specific to mAbs
  - Renal elimination
  - Hepatic elimination
Renal Elimination

- Filtration
  - Glomerular filter is negatively charged - Anionic molecules repelled/not filtered as effectively
  - MW <69 kDa
  - Glomerular sieving coefficient (GSC) – hydrodynamic volume

- Brush-border catabolism

- Reuptake/secretion (+ catabolism)

Fig. 4 Pathways of renal elimination of proteins, including glomerular filtration, catabolism at the luminal membrane, tubular absorption followed by intracellular degradation, and postglomerular peritubular uptake followed by intracellular degradation. [From Rabkin et al. (12)].
Renal Elimination of mAbs and Fragments

• Renal CL of protein is underestimated by GSC

• Current data suggests that renal CL accounts for ca. 65 –100% of the total CL of single chain Fv (MW ~ 25 kDa) and Fab (MW ~50 kDa)

• However, for healthy individuals, true IgG renal CL is only ~0.5 ml/h, representing <10% of total CL
  • Nephrotic syndrome: IgG renal CL may increase by > 5-fold

Hepatic Uptake and Metabolism

Primary receptors mediating hepatic uptake of proteins

• Asialoglycoprotein receptors – ASGPR
  • Recognizes galactose and N-acetylglactosamine
    • Asialoglycoprotein bearing terminal N-linked Gal or GalNAc cleared fast
    • Highly sialated O-glycan exhibited lowerest clearance and the least distribution to liver NPC
  • Primarily expression on hepatocytes (used for targeting)

• Mannose receptor
  • Recognizes terminal mannose, glucose, N-acetylglucosamine.
  • Expressed on Kupfer cells and macrophages, endothelial/dendritic cells.

• Both internalize ligands through endocytic pathway for lysosomal degradation.
FcRn Plays An Essential Role in Extending IgG Half Life

- FcRn was first discovered as the receptor mediated IgG absorption in neonatal rats
  - Heterodimer: 50kD MHC1-like protein + 15kD β2 microglobulin
  - IgG elimination rate was increased 10-15 fold in FcRn knock-out mice

Ghetie et al., Eur J Immunol, 26:690-6
Martin WL et al., Mol Cell, 7:867-877
FcRn-Mediated Salvage Pathway for IgG – Proposed Mechanism

- Prolongs IgG half life by recycling endocytosed IgG back to circulation
  - Binding at low pH of the endosome resulting in recycling of IgG
  - Not binding at neutral pH resulting in release of IgG to extracellular compartment

Roopenian DC and Akilesh S. Nat Rev Immunol. 2007 7(9):715-25; Ghetie et al., Eur J Immunol, 26:690-6;
Target-Mediated Clearance – Common Clearance Mechanism for mAbs

PK profile of therapeutic mAb against EGFR

Lammerts van Bueren JJ et al., Cancer Res 66 (15):7630-8
Clinical Implications of Target-mediate Clearance

- High efficacious dose required to saturation of targets
- mAb exposure varies dependent on target expression levels
  - target expression dependent on disease stage and treatment

Lammerts van Bueren JJ et al., Cancer Res 66 (15):7630-8
### Target Mediated Clearance Plays Important Role at Low Doses

<table>
<thead>
<tr>
<th>Name</th>
<th>Target type</th>
<th>Backbone</th>
<th>Species</th>
<th>Dose (mpk, iv)</th>
<th>Clearance (mL/min/kg)</th>
<th>Terminal t1/2 (h)</th>
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</thead>
<tbody>
<tr>
<td>mAb1</td>
<td>Membrane</td>
<td>IgG1</td>
<td>Cyno</td>
<td>0.5</td>
<td>0.0210</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>0.0060</td>
<td>124</td>
</tr>
<tr>
<td>mAb2</td>
<td>Membrane</td>
<td>IgG2m4, kappa</td>
<td>Rhesus</td>
<td>3</td>
<td>0.0120</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>0.0065</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>0.0048</td>
<td>120</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>Soluble</td>
<td>IgG1</td>
<td>Cyno</td>
<td>2</td>
<td>0.0033</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>0.0039</td>
<td>210</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>0.0040</td>
<td>247</td>
</tr>
</tbody>
</table>

- Target-mediated clearance more commonly observed for mAbs with membrane bound targets.
Role of FcRn Vs Target-mediated Clearance on IgG PK, (Comparing to SM PK)

Typical compounds

- Saturation of Target – mediated uptake
- Saturation of FcRn-mediated protection

IgG

Small molecules

Saturation of CYP-mediated metabolism

Dose (mg/kg)

0.1 1 10 100

Clearance
Immunogenicity (Anti-Drug Ab): Effects on Drug Disposition

Effects of antilenercept Ab on the time-course of lenercept

### Immunogenicity – Common Observation for Biologics

**TABLE 3. Immunogenicity of Approved Therapeutic Monoclonal Antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Technology</th>
<th>Antigen</th>
<th>Comedication</th>
<th>Immunosuppression?</th>
<th>Immunogenicity incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT(^{\text{R}})(^{\text{3}})</td>
<td>Mu</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>80%</td>
</tr>
<tr>
<td>Zevalin(^{\text{TM}})</td>
<td>MuRC</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>3.8%</td>
</tr>
<tr>
<td>Bexxar(^{\text{R}})</td>
<td>MuRC</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>10–70%</td>
</tr>
<tr>
<td>ReoPro(^{\text{R}})</td>
<td>Ch</td>
<td>No</td>
<td>No</td>
<td></td>
<td>5.8% (1 dose)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25% (2+ doses)</td>
</tr>
<tr>
<td>Rituax(^{\text{R}})</td>
<td>Ch</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>1.1%</td>
</tr>
<tr>
<td>Simulect(^{\text{R}})</td>
<td>Ch</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>1.2–3.5% (2 doses)</td>
</tr>
<tr>
<td>Remicade(^{\text{R}})</td>
<td>Ch</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>10–61%</td>
</tr>
<tr>
<td>Erbitux(^{\text{TM}})</td>
<td>Ch</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>5%(^{1})</td>
</tr>
<tr>
<td>Zenapax(^{\text{R}})</td>
<td>Hz</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>8.4%</td>
</tr>
<tr>
<td>Synagis(^{\text{R}})</td>
<td>Hz</td>
<td>No</td>
<td>No</td>
<td></td>
<td>0.7–1.8%</td>
</tr>
<tr>
<td>Herceptin(^{\text{R}})</td>
<td>Hz</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td>Mylotarg(^{\text{TM}})</td>
<td>HzTC</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>0% HAHA 2 pts. HATA</td>
</tr>
<tr>
<td>Campath(^{\text{R}})</td>
<td>Hz</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>1.9% CLL patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63% RA patients</td>
</tr>
<tr>
<td>Xolair(^{\text{R}})</td>
<td>Hz</td>
<td>No</td>
<td>No</td>
<td></td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Raptiva(^{\text{TM}})</td>
<td>Hz</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>6.3%</td>
</tr>
<tr>
<td>Avastin(^{\text{TM}})</td>
<td>Hz</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td>ND(^{2})</td>
</tr>
<tr>
<td>Humira(^{\text{TM}})</td>
<td>HuPD</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>1% with MTX 12 % monotherapy</td>
</tr>
</tbody>
</table>

1. HAHA: Hypersensitivity associated with high antibody response. 
2. ND: Not determined.
Protein / Antibody PK (ADME)/PD Overview


Ab\textsubscript{plasma} \rightarrow Ab\textsubscript{tissue}

Renal filtration, catabolism

Catabolism within tissue

Absorption
Catabolism
Lymphatic transport

Ab-target complex

Catabolism of complex

Transduction

IV

SC

RESPONSE
Strategies to Improve Half-Life of Biologics

- Glycosylation
- Pegylation
- FcRn binding
  - Fc Engineer
  - Fc Fusion proteins
Impact of Glycosylation on Elimination of a Protein: Erythropoietic Agents (EPOs)

![Diagram showing biochemical and biological properties of rHuEPO and rHuEPO analogues containing four and five N-linked carbohydrate chains.](image)

**Figure 4** Biochemical and biological properties of rHuEPO and rHuEPO analogues containing four and five N-linked carbohydrate chains (Egrie et al, 1997)

*British Journal of Cancer (2001) 84(Supplement 1), 3–10*
Highly Glycosylated EPOs Has Extended Half-Life

**NESP is dosed weekly or less frequently**

Table 1. Comparison of intravenous pharmacokinetic parameters for NESP and Epoetin alfa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NESP</th>
<th>Epoetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2,z}$ (hours)</td>
<td>25.3 ± 2.2</td>
<td>8.5 ± 2.4</td>
</tr>
<tr>
<td>CL (ml/h per kg)</td>
<td>1.6 ± 0.3</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>AUC$_{(0-96)}$ (ng · h per ml)</td>
<td>291.0 ± 7.6</td>
<td>131.9 ± 8.3</td>
</tr>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>52.4 ± 2.0</td>
<td>48.7 ± 2.1</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM. NESP, novel erythropoiesis stimulating protein; $t_{1/2,z}$, terminal half-life; CL, clearance; AUC, area under the serum concentration–time curve; $V_d$, volume of distribution at steady state.

Figure 1. Comparison of intravenous pharmacokinetic profiles of NESP ($n = 11$) and Epoetin alfa ($n = 10$). Mean ± SD.
**PEGylation of Proteins**

- Prolonged t1/2 allowing less frequent dosing
  - PEG polymers highly hydrated
    - increased effective molecular size
  - Decreased rate of kidney clearance
  - Increased protection from proteolytic degradation

Table 1. FDA Approved PEGylated Drugs

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Drug Name</th>
<th>Parent Drug</th>
<th>PEG Size (Da)</th>
<th>Indication</th>
<th>Year of Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adagen®</td>
<td>Pegadamase</td>
<td>Adenosine deaminase</td>
<td>5000</td>
<td>SCID&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1990</td>
</tr>
<tr>
<td>Oncasparg®</td>
<td>Pegasparagase</td>
<td>Asparaginase</td>
<td>5000</td>
<td>Leukaemia (ALL&lt;sup&gt;e&lt;/sup&gt;, CML&lt;sup&gt;f&lt;/sup&gt;)</td>
<td>1994</td>
</tr>
<tr>
<td>PEG-INTRON®</td>
<td>Peginterferon-α2b</td>
<td>IFN-α2B</td>
<td>12000</td>
<td>Hepatitis C</td>
<td>2000</td>
</tr>
<tr>
<td>PEGASYS®</td>
<td>Peginterferon-α2a</td>
<td>IFN-α2A</td>
<td>40000</td>
<td>Hepatitis C</td>
<td>2001</td>
</tr>
<tr>
<td>Neulasta®</td>
<td>Pegfilgrastim</td>
<td>GCSF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20000</td>
<td>Neutropenia</td>
<td>2002</td>
</tr>
<tr>
<td>Somavert®</td>
<td>Pegvisomant</td>
<td>GH&lt;sup&gt;b&lt;/sup&gt; antagonist</td>
<td>4–5 × 5000</td>
<td>Acromegaly</td>
<td>2003</td>
</tr>
<tr>
<td>Macugen®</td>
<td>Pegaptanib</td>
<td>Anti-VEGF&lt;sup&gt;c&lt;/sup&gt; aptamer</td>
<td>40000</td>
<td>Age-related macular degeneration</td>
<td>2004</td>
</tr>
</tbody>
</table>

<sup>a</sup>GCSF, granulocyte-colony stimulating factor.
<sup>b</sup>GH, growth hormone.
<sup>c</sup>VEGF, vascular endothelial growth factor.
<sup>d</sup>SCID, severe combined immunodeficiency disease.
<sup>e</sup>ALL, acute lymphoblastic leukemia, acute lymphocytic leukemia.
<sup>f</sup>CML, chronic myeloid leukemia.

J Pharm Sci, 97(10) 2008, 4167-4183
IgGs Engineering for Half-Life Enhancement: FcRn Binding

Increased binding to FcRn at acidic pH resulted in prolonged $t_{1/2}$

$K_d$ (nM)

- MEDI-524-YTE: 134
- MEDI-524: 1196

$0.5$ = 21 days

$0.5$ = 6 days
# Strategies to Improve Half-Life of Biologics

## Table 1. Strategies to improve half-lives of therapeutic proteins

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Modification</th>
<th>Increased hydrodynamic volume</th>
<th>FcRn-mediated recycling</th>
<th>Effect on half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing size</td>
<td>Di- or multimerization</td>
<td>+</td>
<td>−</td>
<td>Varying (size dependent)</td>
</tr>
<tr>
<td>PEGylation</td>
<td>Coupling of methoxy polyethylene glycol</td>
<td>+</td>
<td>−</td>
<td>Varying, depending on PEG size</td>
</tr>
<tr>
<td>Recombinant PEG mimetics</td>
<td>Fusion of flexible, hydrophilic amino acid chains (500–600 aa)</td>
<td>+</td>
<td>−</td>
<td>Limited data available (dependent on size and composition)</td>
</tr>
<tr>
<td>Polysialylation</td>
<td>Coupling of colominic acid</td>
<td>+</td>
<td>−</td>
<td>Moderate</td>
</tr>
<tr>
<td>HESylation</td>
<td>Coupling of hydroxyethyl starch</td>
<td>+</td>
<td>−</td>
<td>No data available</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Mutagenesis to introduce N-glycosylation sites</td>
<td>+</td>
<td>−</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>Modified expression systems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engineered IgG</td>
<td>Mutagenesis of the Fc region</td>
<td>−</td>
<td>++&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;IgG</td>
</tr>
<tr>
<td>Fc fusion</td>
<td>Fusion to the Fc region</td>
<td>+</td>
<td>+</td>
<td>≤IgG</td>
</tr>
<tr>
<td>IgG (Fc) binding</td>
<td>Fusion to antibodies, peptides, etc.</td>
<td>+</td>
<td>+</td>
<td>≤IgG</td>
</tr>
<tr>
<td>Albumin fusion</td>
<td>Fusion to albumin</td>
<td>+</td>
<td>+</td>
<td>Similar to albumin</td>
</tr>
<tr>
<td>Albumin binding</td>
<td>Fusion to an albumin-binding domain, peptide, antibody fragment</td>
<td>+</td>
<td>+</td>
<td>Similar to albumin</td>
</tr>
</tbody>
</table>

<sup>a</sup> For increased binding to FcRn at pH 6 and absent binding at neutral pH.

<sup>aa</sup> = amino acids; FcRn = neonatal Fc receptor.
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- Introduction on Biologics
  - Biologics landscape

- Overview of DMPK Properties of Large Molecules (LM)/Biologics
  - ADME processes
    - Approaches to increase half-life

- Overview of Large Molecules (LM) Drug Discovery and Development
  - High level DMPK support
  - Human PK prediction
    - First Human Dose prediction
  - Comparability exercise
  - Biosimilar development
  - Other DMPK studies
    - Linking to SM Experience
DMPK in Biologics Discovery and Development

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Preclin. Dev.</th>
<th>I</th>
<th>IIa</th>
<th>IIb</th>
<th>III</th>
<th>IV / V</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>BLA</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

PK, Human PK Prediction, FIH Dose Prediction

PK-PD, Distribution, Labeling

PK/PD Comparability

Focus
- Optimize, Minimize Development Risk
- Plan Development Program, Understand and Manage Risk

Key Customers
- Basic Research
- Safety Assessment, Clin Pharm, PR&D, Clinical Research

MERCK
Be well
Human PK Prediction

- Approaches to Species Scaling
  - Allometry
    \[ \text{CL} = a(BW)^b \]
    - Simple
    - Fixed exponent
    - Allometry with Adjustments

- Physiological Models (PBPK)
  - Somewhat limited; need additional mechanistic understanding

- References
First publication on human PK prediction for Biologics: Allometric Scaling of 4 Therapeutic Proteins


\[
\text{CL} = 0.1 W^{0.74} \quad \text{V}_1 = 40 W^{1.05} \quad \text{V}_{SS} = 60 W^{1.01}
\]
Simple Allometry Gave Systemic Biases

Included non-mAb therapeutic proteins (TP), mAbs and Fc fusions based on data over the dose range where its PK is linear.

<table>
<thead>
<tr>
<th>Fixed exponent</th>
<th>0.70</th>
<th>0.75</th>
<th>0.80</th>
<th>0.85</th>
<th>0.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mAb/Fc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compounds with prediction accuracy within 2-fold (%)</td>
<td>82%</td>
<td>75%</td>
<td>91%</td>
<td>83%</td>
<td>95%</td>
</tr>
<tr>
<td>Compounds with prediction accuracy within 3-fold (%)</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Allometry with Fixed Exponent Yielded Better Results

Included non-mAb therapeutic proteins (TP), mAbs and Fc fusions based on data over the dose range where its PK is linear

![Graph showing predicted vs observed values for different species and drug combinations](image-url)
Summary: Approach for Human Clearance Prediction

- **Scope**
  - Only applicable to compounds over the dose range where its PK is linear
  - Not applicable to compounds with target-mediated clearance mechanism at low doses where non-linear PK is expected

- **Recommended method: Allometric scaling with fixed exponent**
  \[
  \text{CL}_{\text{human}} = \text{CL}_{\text{animal}} \times (\text{BW}_{\text{human}}/\text{BW}_{\text{animal}})^{\exp}
  \]

  - **For TP (non-mAb)**
    - Use fixed exponent = 0.75
    - Predictions from >= 2 species desired
    - Average predictions from individual species if necessary

  - **For mAb and Fc fusion**
    - Use monkey CL data and fixed exponent = 0.85
    - Use together with complementary tools
      - Determine in vitro human FcRn binding and PK in human FcRn, and compare to benchmarking mAbs to determine a range
First In Human Dose Selection

- In Concept, similar approach to SM
  - Based on preclinical safety studies (NOEAL), efficacy studies and human PKPD prediction
    - NOAEL – No Observed Adverse Effect Level

- For high risk compounds (based on MOA and nature of target)
  - MABEL approach recommended
    - MABEL – Minimal Anticipated Biological Effect Level
  - Introduced after the TGN1412 Case


EMEA/CHMP: Guideline on Strategies to Identify and Mitigate Risks for First-In-Human Clinical Trials with Investigational Medicinal Products. 2007.
Approach to Select First In Man Dose: NOEAL or MABEL

MRSD = Maximum Recommended Starting Dose
HED = Human Equivalent Dose
# Steps to Estimate MSRD

<table>
<thead>
<tr>
<th><strong>Toxicological Assessment</strong></th>
<th><strong>Pharmacological Assessment</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HED@NOAEL</strong></td>
<td><strong>MABEL</strong></td>
</tr>
<tr>
<td>Step 1: Determine NOAEL in all toxicology species</td>
<td>Step 5:</td>
</tr>
<tr>
<td>Step 2: Convert NOAEL to HED* for all toxicology species</td>
<td>(a) Estimate human MABEL based on:</td>
</tr>
<tr>
<td>Step 3: Select HED* for most sensitive toxicology species</td>
<td>(i) <em>In vitro</em> pharmacology studies with target cells from humans and toxicology species</td>
</tr>
<tr>
<td>Step 4: Apply ≥ 10-fold safety factor</td>
<td>(ii) Concentration-effect data from <em>in vitro</em> and <em>in vivo</em> studies</td>
</tr>
<tr>
<td></td>
<td>(b) Integrate data into PK/PD model, if feasible, in order to predict pharmacological response in humans at multiple dose levels</td>
</tr>
<tr>
<td></td>
<td>(c) Account for:</td>
</tr>
<tr>
<td></td>
<td>(i) Animal-human differences in affinity / potency</td>
</tr>
<tr>
<td></td>
<td>(ii) Animal-human differences in exposure / distribution</td>
</tr>
<tr>
<td></td>
<td>(iii) Anticipated duration of effect(s)</td>
</tr>
</tbody>
</table>

## Maximum recommended safe starting dose (MRSD)

Define anticipated safety window based on NOAEL and MABEL and apply additional safety factor**, if necessary, based on

1. Potential risk / hazard and uncertainty thereof
2. Degree of uncertainty in MABEL calculation
Comparability Exercise: Unique to Biologics

Critical product quality highly process dependent
Biologics Product Quality Highly Process Dependent
Comparability Exercise: Regulatory Guidance

Unique to Biologics – Critical product quality highly process dependent

Box 1. Regulatory guidance pertaining to the comparability of biological products

ICH Q5E, Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process (CPMP/ICH/5721/03) [2]:

- Provides guidance regarding approaches for comparing the post-change product to the pre-change product following manufacturing changes and for assessing the impact of differences in quality attributes between pre-change and post-change materials on safety and efficacy.
- Does not prescribe any particular analytical, nonclinical or clinical strategy.

ICH Q6B, Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (CPMP/ICH/365/96) [3]:

- Provides general principles on the setting and justification of a uniform set of international specifications for biotechnological/biological products.
- Does not recommend specific test procedures or acceptance criteria.

Guideline on Comparability of Biotechnology-Derived Medicinal Products after a Change in the Manufacturing Process, Non-clinical and Clinical Issues (EMEA/CHMP/BMWP/101695/2006) [4]:

- Addresses the requirements for non-clinical and/or clinical bridging studies to demonstrate that a manufacturing change by a single manufacturer, including those by a contract manufacturer, does not impact safety or efficacy.

- Describes a risk-based approach for determining the need for non-clinical and/or clinical comparability studies.

Guidance for Industry: Comparability Protocols – Protein Drug Products and Biological Products – Chemistry, Manufacturing and Controls Information [5]:

- Provides recommendations on preparing and using comparability protocols, which are comprehensive plans describing tests, validation studies and acceptable limits for assessing the effects of post-approval changes in chemistry, manufacturing and controls on the safety and efficacy of products.

FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products [6]:

- Describes categories of comparability tests that might allow manufacturers to make manufacturing changes without performing additional clinical efficacy and safety studies, subject to FDA evaluation of test results and approval of changes.

(Abbreviations: BMWP, Biosimilar Medicinal Products Working Party; CHMP, Committee for Medicinal Products for Human Use; CPMP, Committee for Proprietary Medicinal Products; EMEA, European Medicines Agency; FDA, United States Food and Drug Administration; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.)
Glycosylation Pattern of mAbs

- Qualitative differences dependent on cell lines
- Quantitative differences within same cell line
# Glycosylation Variations Impacting PKPD (and Potentially Safety/Efficacy)

<table>
<thead>
<tr>
<th>Glycan</th>
<th>PK</th>
<th>Effector functions&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ADCC</th>
<th>CDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aglycosylation</td>
<td>⇔</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Afucosylation</td>
<td>⇔</td>
<td>↑</td>
<td>⇔</td>
<td></td>
</tr>
<tr>
<td>Man5 (Man6-9) (terminal Man)</td>
<td>↑ at 100%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑</td>
<td>⇔</td>
<td></td>
</tr>
<tr>
<td></td>
<td>⇔ at 5–10%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>⇔</td>
<td>⇔</td>
<td></td>
</tr>
<tr>
<td>Agalactosylation (terminal GlcNAc)</td>
<td>⇔</td>
<td>↑</td>
<td>⇔</td>
<td></td>
</tr>
<tr>
<td>Asialylation (terminal Gal)</td>
<td>⇔</td>
<td>⇔</td>
<td>⇔</td>
<td></td>
</tr>
<tr>
<td>Bisecting GlcNAc</td>
<td>ND</td>
<td>⇔</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Increased sialylation (terminal sialic acid)</td>
<td>⇔</td>
<td>⇔</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>⇔, no difference; ↑, increase; ↓, decrease; ND, not determined.

<sup>b</sup>Effector functions have been evaluated using <i>in vitro</i> assays.

<sup>c</sup>Glycans in the mAb comprised 100% Man5 or 5–10% Man5.

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**Trends in Biotechnology** 2010 (28) 509–516

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[MERCK Logo]
PK of a Fusion Protein: Impact of Glycosylation

**Lenercept – IgG-Fc/TNFR_{55} Fusion Protein**

![Diagram of Lenercept structure showing glycosylation sites](image)

Fig. 1. Cartoon of lenercept structure showing glycosylation sites.

Process related glycosylation pattern changes resulted altered PK in animals and humans

**Fig. 3.** Cynomolgus monkey pharmacokinetic profiles. Animals were injected (5 mg/kg) with one of three lots of lenercept. Data are presented as means ± SD, see Table I.

**M11-1/3 – Process A; original; low yield**

**M11-72/73 – Process C; highest tGlcNAc and high yield**

Selective clearance of glycoforms

Presence of terminal N-acetylglucosamine (tGlcNAc) on TNFR increased clearance via mannose receptor

Jones et al., *Glycobiology* vol. 17 no. 5 pp. 529–540, 2007
Sources of mAb Variants Potentially Impacting PKPD, Efficacy/Safety

Fig. 6. Sources of heterogeneity in monoclonal antibodies. The functional utility of antibodies depends on programmed variation in the six hypervariable complementarity determining regions (CDRs) in $V_L$ and $V_H$. Other undesired heterogeneity arises from either biological post-translational modifications or from chemical degradation and aggregation (Kozlowski and Swann, 2006).
Methionine Oxidation Impacts mAb PK and FcRn Binding

Table 1
Levels of Met oxidation in AbM samples tested in animal studies. The fraction of oxidized and unoxidized Met 252 and Met 428 were determined by LC-UV peptide mapping. Results are reported as mean ± SD.

<table>
<thead>
<tr>
<th>AbM samples</th>
<th>Met 252 oxidized (%)</th>
<th>Met 428 oxidized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AbM_frozen</td>
<td>5.2 ± 0.5</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>AbM_refrig</td>
<td>13.1 ± 0.1</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>AbM_refrig_nonox</td>
<td>1.8 ± 0.2</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>AbM_refrig_ox</td>
<td>43.2 ± 2.2</td>
<td>8.4 ± 0.6</td>
</tr>
</tbody>
</table>

Table 2
Pharmacokinetics parameters in human FcRn mice for AbM and AbH containing different amounts of oxidized Met. Half-life results are reported as mean ± SD. The relative variation of the binding affinity to FcRn is also shown for corresponding levels of oxidation in AbH and AbM.

<table>
<thead>
<tr>
<th>hFcRn mice</th>
<th>Samples</th>
<th>Serum half-life</th>
<th>$K_{D\text{ctt}}/K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hours</td>
<td>% of control</td>
</tr>
<tr>
<td>Het276</td>
<td>AbH_0h</td>
<td>56 ± 11</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>AbH_1h</td>
<td>59 ± 5</td>
<td>106%</td>
</tr>
<tr>
<td></td>
<td>AbH_3h</td>
<td>13 ± 2</td>
<td>22%</td>
</tr>
<tr>
<td></td>
<td>AbH_23h</td>
<td>9.4 ± 0.3</td>
<td>17%</td>
</tr>
<tr>
<td>Homo32</td>
<td>AbM_frozen</td>
<td>134 ± 54</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>AbM_refrig</td>
<td>121 ± 8</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>AbM_refrig_nonox</td>
<td>128 ± 5</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>AbM_refrig_ox</td>
<td>96 ± 15</td>
<td>72%</td>
</tr>
</tbody>
</table>
## Comparability Studies Reported by Various Companies


<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Stage of Development</th>
<th>Type of Change</th>
<th>Type of Product</th>
<th>Data</th>
<th>Presenter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centocor</td>
<td>Pre–phase 3</td>
<td>Formulation</td>
<td>Monoclonal antibody</td>
<td>Analytical Primate PK</td>
<td>Honghui Zhou</td>
</tr>
<tr>
<td></td>
<td>Post–phase 3, premarket</td>
<td>Device</td>
<td>Monoclonal antibody</td>
<td>Analytical Clinical PK</td>
<td></td>
</tr>
<tr>
<td>Eli Lilly</td>
<td>Pre–phase 2</td>
<td>Cell line</td>
<td>Monoclonal antibody</td>
<td>Analytical Primate PK</td>
<td></td>
</tr>
<tr>
<td>Agen</td>
<td>Pre–phase 3</td>
<td>Cell line</td>
<td>Monoclonal antibody</td>
<td>Analytical Animal PK/PD Immunogenicity</td>
<td></td>
</tr>
<tr>
<td>Novartis Biologics</td>
<td>Pre–phase 3</td>
<td>Cell line</td>
<td>Monoclonal antibody</td>
<td>Animal pharmacology Mouse xenograft Animal PK Monkey Animal toxicology Clinical PK</td>
<td></td>
</tr>
<tr>
<td>Genzyme</td>
<td>Method for design of PK and PD comparability studies</td>
<td>PK/PD transgenic vs NHP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genentech</td>
<td>Paradigm for evaluating comparability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyeth</td>
<td>Postmarketing</td>
<td>Site change, media change</td>
<td>Recombinant enzyme</td>
<td>Analytical Rat PK Recovery Clinical PK efficacy</td>
<td>Garvin Warner</td>
</tr>
<tr>
<td>Amgen</td>
<td>Phase 3 extension</td>
<td>Process</td>
<td>Fusion protein</td>
<td>Analytical Clinical PK and PD</td>
<td></td>
</tr>
<tr>
<td>Seattle Genetics</td>
<td>End of phase 1</td>
<td>Scale-up, media change, process change</td>
<td>Monoclonal antibody</td>
<td>Analytical Clinical PK</td>
<td></td>
</tr>
<tr>
<td>Novartis</td>
<td>Cell line, process, scale-up, site</td>
<td>Monoclonal antibody</td>
<td></td>
<td>Analytical Primate PK</td>
<td></td>
</tr>
</tbody>
</table>

Program Committee: Joy Cavagnaro, Barry Chemey, Gregory C. Davis, Armida Joslin, Richard Lit, Sara Radcliffe, Mark Rogge, Yuan Xu, and Hong Zhao. Additional discussants included Amy Rosenberg, Anne Pilaro, and Patrick Swan of CBER, FDA.
Summary of DIA Workshop: Comparability Challenges: Regulatory and Scientific Issues in the Assessment of Biopharmaceuticals

<table>
<thead>
<tr>
<th>Hierarchy of Sensitivity for Potential Comparability Testing Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hierarchy of Testing</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Analytical</td>
</tr>
<tr>
<td>Newer testing methods</td>
</tr>
<tr>
<td>Stability testing</td>
</tr>
<tr>
<td>• Biological potency assays</td>
</tr>
<tr>
<td>• Animal PK/PD (relevant vs nonrelevant)</td>
</tr>
<tr>
<td>Animal immunogenicity (to detect changes)</td>
</tr>
<tr>
<td>• Animal toxicology</td>
</tr>
<tr>
<td>• Human PK/PD</td>
</tr>
<tr>
<td>• Human safety and efficacy</td>
</tr>
<tr>
<td>&lt;sup&gt;a&lt;/sup&gt;Some studies may be conducted in parallel.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Categories of Consideration for the Design of Comparability Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Type and scope of changes</td>
</tr>
<tr>
<td>Cell line</td>
</tr>
<tr>
<td>Process changes</td>
</tr>
<tr>
<td>Site changes</td>
</tr>
<tr>
<td>Formulation changes</td>
</tr>
<tr>
<td>Major vs minor</td>
</tr>
<tr>
<td>Novel vs platform</td>
</tr>
<tr>
<td>• Stage of development or postmarketing</td>
</tr>
<tr>
<td>• Patient population/disease</td>
</tr>
<tr>
<td>• Product risk</td>
</tr>
</tbody>
</table>

### Table 5: Top 20 Antineoplastic Drug Expenditures in Outpatient Clinics

<table>
<thead>
<tr>
<th>Rank</th>
<th>Drug</th>
<th>Biologic or Nonbiologic</th>
<th>2010 Total Expenditure (in Millions of Dollars)</th>
<th>Top 20 Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bevacizumab</td>
<td>Biologic</td>
<td>1884</td>
<td>22.1%</td>
</tr>
<tr>
<td>2</td>
<td>Rituximab</td>
<td>Biologic</td>
<td>1466</td>
<td>17.2%</td>
</tr>
<tr>
<td>3</td>
<td>Trastuzumab</td>
<td>Biologic</td>
<td>931</td>
<td>10.9%</td>
</tr>
<tr>
<td>4</td>
<td>Docetaxel</td>
<td>Nonbiologic</td>
<td>688</td>
<td>8.1%</td>
</tr>
<tr>
<td>5</td>
<td>Pemetrexed</td>
<td>Nonbiologic</td>
<td>579</td>
<td>6.8%</td>
</tr>
<tr>
<td>6</td>
<td>Oxaliplatin</td>
<td>Nonbiologic</td>
<td>508</td>
<td>6.0%</td>
</tr>
<tr>
<td>7</td>
<td>Gemcitabine</td>
<td>Nonbiologic</td>
<td>463</td>
<td>5.4%</td>
</tr>
<tr>
<td>8</td>
<td>Cetuximab</td>
<td>Biologic</td>
<td>329</td>
<td>3.9%</td>
</tr>
<tr>
<td>9</td>
<td>Bortezomib</td>
<td>Nonbiologic</td>
<td>327</td>
<td>3.8%</td>
</tr>
<tr>
<td>10</td>
<td>Leuprolide</td>
<td>Nonbiologic</td>
<td>220</td>
<td>2.6%</td>
</tr>
<tr>
<td>11</td>
<td>Paclitaxel–albumin</td>
<td>Nonbiologic</td>
<td>212</td>
<td>2.5%</td>
</tr>
</tbody>
</table>
# Biosimilar vs SM Generics

<table>
<thead>
<tr>
<th>Area</th>
<th>Biosimilars</th>
<th>Small-Molecule Generics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td>The amino acid sequence is the same, but there is expected to be slight differences in terms of protein folding and glycosylation</td>
<td>The active drug is chemically identical to the reference product</td>
</tr>
<tr>
<td><strong>Analytical characterization</strong></td>
<td>The final structure cannot be fully defined based on current analytical techniques; therefore, the degree of structural similarity to the reference product is unknown</td>
<td>Current techniques are available to ensure that the active drug in the generic product is identical to the reference product</td>
</tr>
<tr>
<td><strong>Manufacturing</strong></td>
<td>Very complex; produced in living cells and involves several stages of purification, production, and validation of the final product</td>
<td>Relatively simple, uses organic medicinal chemistry reactions</td>
</tr>
<tr>
<td><strong>Impact of a change in manufacturing process</strong></td>
<td>Small changes in process may alter the final structure and function of the protein</td>
<td>Likely to be negligible because the end product is identical</td>
</tr>
</tbody>
</table>
Biosimilar

- Due to the complexity of biological/biotechnology-derived products the standard generic approach (demonstration of bioequivalence with a reference medicinal product by appropriate bioavailability studies) normally applied to chemically derived medicinal products is scientifically not appropriate for these products.

- **In the US**
  - Biosimilar pathway in 2010
  - Specific guidance still under development
    - See FDA Position paper - [PDF](http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf)

- **In the EU (see next few slides)**
  - Specific guidance for recombinant products (e.g. Insulin, GCSF) in 2004
  - Draft guidance on mAbs issued November 2010

- **WHO**
  - General guidance issued in 2009; applicable to well characterized proteins (e.g. recombinant DNA derived) only

[PDF](http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf)
### Table 1: EMA approved biosimilars*

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Active Substance</th>
<th>Therapeutic Area</th>
<th>Manufacturer/Company Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abseamed</td>
<td>epoetin alfa</td>
<td>Kidney Failure Chronic Anemia Cancer</td>
<td>Medice Arzneimittel Pütter GmbH &amp; Co</td>
</tr>
<tr>
<td>Binocrit</td>
<td>epoetin alfa</td>
<td>Kidney Failure Chronic Anemia</td>
<td>Sandoz GmbH</td>
</tr>
<tr>
<td>Biograstim</td>
<td>filgrastim</td>
<td>Hematopoietic Stem Cell Transplantation Neutropenia CT</td>
<td>Arzneimittel GmbH</td>
</tr>
<tr>
<td>Epoetin alfa Hexal</td>
<td>epoetin alfa</td>
<td>Kidney Failure Chronic Anemia Cancer</td>
<td>Hexal AG</td>
</tr>
<tr>
<td>Filgrastim Hexal</td>
<td>filgrastim</td>
<td>Neutropenia Cancer Hematopoietic Stem Cell Transplantation</td>
<td>Hexal AG</td>
</tr>
<tr>
<td>Nivestim</td>
<td>filgrastim</td>
<td>Hematopoietic Stem Cell Transplantation Cancer Neutropenia</td>
<td>Hospira UK Ltd</td>
</tr>
<tr>
<td>Omnitrope</td>
<td>somatropin</td>
<td>Turner Syndrome DwarfismPituitary Prader-Willi Syndrome</td>
<td>Sandoz GmbH</td>
</tr>
<tr>
<td>Ratiograstim</td>
<td>filgrastim</td>
<td>Neutropenia Hematopoietic Stem Cell Transplantation</td>
<td>Ratiopharm GmbH</td>
</tr>
<tr>
<td>Retacrit</td>
<td>epoetin zeta</td>
<td>Hematopoietic Stem Cell Transplantation Cancer Neutropenia</td>
<td>Hospira UK Limited</td>
</tr>
<tr>
<td>Silapo</td>
<td>epoetin zeta</td>
<td>Chronic Anemia Blood Transfusion Autologous Cancer Kidney Failure</td>
<td>Stada R &amp; D AG</td>
</tr>
<tr>
<td>Tevagrastim</td>
<td>filgrastim</td>
<td>Neutropenia Cancer Hematopoietic Stem Cell Transplantation</td>
<td>Teva Generics GmbH</td>
</tr>
<tr>
<td>Valtropin</td>
<td>somatropin</td>
<td>Dwarfism Pituitary Turner Syndrome</td>
<td>BioPartners GmbH</td>
</tr>
<tr>
<td>Zarzio</td>
<td>filgrastim</td>
<td>Cancer Hematopoietic Stem Cell Transplantation Neutropenia</td>
<td>Sandoz GmbH</td>
</tr>
</tbody>
</table>

*Data collected on 12 May 2011

---

No mAbs Biosimilar approved yet

---

http://www.gabionline.net/Biosimilars/General/Biosimilars-approved-in-Europe
European Biosimilar Guideline

McCamish and Woollett
mAbs 3:2, 209-217; March/April 2011

http://www.who.int/biologicals/areas/biological_therapeutics/BIOThERAPEUTICS_FOR_WEB_22APRIL2010.pdf
**WHO:** similar biotherapeutic products (SBPs)

- **Full dossier** (Stand alone approach)
- **Similar Biotherapeutic Products (SBPs)**
  - Existing knowledge, full, comparative characterization, plus comparative but reduced non-clinical, clinical data
  - Applicable to well characterized biologicals only
- **Generic**
  - For chemical entities only
  - Not applicable to biologicals

Fig. 1. Applicability of licensing requirements to different types of medicinal products.
General Hierarchy Approach for Biosimilar Development

Proving “highly similar” to reference product often requires multiple iterations of process change and physicochemical characterization.

- Clinical Trials
- PK/PD
- Preclinical
- Biological characterization
- Physicochemical characterization

Process development

Analytics
Pharmacokinetics Is A Critical Component for Establishing Product Similarity (similar to Comparability)

- **EU** - Focus on demonstration of similar efficacy and safety compared to the reference product, not patient benefit per se, which has already been shown for the reference product.

- **Non-clinical data**
  - *In vitro* studies
    - Binding to the target antigen
    - Binding to all Fcgamma receptors, FcRn and complement
    - Fab-associated functions (e.g. neutralization, receptor activation or receptor blockade)
    - Fc-associated functions (ADCC and CDC assays, complement activation)
  - *In vivo* studies
    - PK and/or PKPD
    - Limited Safety study in relevant species

- **Clinical studies**
  - PK, (PD) and PK/PD in homogenous population for sensitivity
  - Efficacy/Safety
    - Extrapolation of other indications not studied possible
  - post-authorisation follow-up - pharmacovigilance

Draft Guideline on similar biological medicinal products containing monoclonal antibodies; 18 November 2010
Figure 4. Biosimilarity goal posts. The “goal posts” of biosimilarity are established by the biosimilar sponsor by their analysis of the distribution of product attributes present in the reference product pre- and post-manufacturing change. They then use these to select the design space for their biosimilar candidate. While the complete quality range may be quite broad for the life time of the reference product, the biosimilar sponsor will select a tighter range of control for their biosimilar product.
Biologics DMPK Supports: Linking to SM Experience

- From DMPK Perspective, the current state for LM support paradigm is similar to where we were with SM >15 yrs ago
  - Mind set/paradigm - DMPK primarily involved after candidate selection
    - PK not known to be major success limiting factor
      - Relatively low attrition rate vs SMs, thus far
    - PK of Biologics believed to be well behaved/predictable
  - DMPK approach limited to in vivo PK
    - ADME processes less well understood
    - Heavily dependent on single species, NHP
  - Inadequate tools
    - Bioanalytical challenges

Prueksaritanont, Presented at SUNY Buffalo, Aug 2011
Highly specific off-target binding identified and eliminated during the humanization of an antibody against FGF receptor 4.

Early Development PKPD; Genentech, Inc.; South San Francisco, CA USA

Complex pharmacokinetics of a humanized antibody against human amyloid Beta Peptide, anti-abetab2, in nonclinical species.

Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Inc., Andover, Massachusetts, 01810, USA
Pharmacokinetic (ADME) Concept Analogy

Small molecules

Oral Dose

Gut wall

Portal Vein

Liver

Metabolism/biliary excretion

Absorption

Intestinal first Pass; $F_g$

Hepatic first Pass; $F_h$

Systemic ($F$)

$F = f_a \times F_g \times F_h$

Urine

Tissues

Distribution/elimination

Perfusion, tissue binding, CYPs; renal filtration...

Biologic analogy

SC Absorption:

ECM interactions aggregation

Injection site:

Catabolism/Metabolism

Lymphatic:

Lymph node uptake metabolism

Distribution/elimination:

Convection/transcytosis Target mediated/ADA/FcRn..

Prueksaritanont, Presented at SUNY Buffalo, Aug 2011
Bioanalytical Aspects for Biologics

- **Assays of biologic concentrations (PK)**
  - Immunoassays (ELISA, RIA) — *most commonly used for Biologics*
    - Highly sensitive
    - Lack of specificity (active versus inactive, isoforms, endogenous versus exogenous)
    - Interference from binding proteins (e.g., IGFs), anti-drug antibodies
    - Cross-reactivity (e.g., rheumatoid factor)
  - LCMS — *most commonly used for SM*
    - Currently not as sensitive, but highly specific

- **Assays for anti-drug antibodies (ADA) — Biologics specific**
  - Binding assay
    - Titer
    - Specificity
  - Neutralizing assay/bioactivity assay
Key Focus Examples: Following SMs Path

- Establishing better understanding of key ADME determinants
  - IgG Clearance - FcRn Platform (in vitro and in vivo)
    - FcRn proposed in 1964 and shown to be a key determinant of IgG pharmacokinetics in 1996
    - Drug Metabolism and Disposition – Wang et al., Monoclonal Antibodies with Identical Fc Sequences Can Bind to FcRn Differentially with Pharmacokinetic Consequences
    - Molecular Immunology – Wang et al., Impact of methionine oxidation in human IgG1 Fc on serum half-life of monoclonal antibodies
  - Absorption/disposition of Therp protein after Sc
    - 2011 AAPS Biotechnology meeting - Sunrise session

- Establishing Tools
  - LCMS

- Regulatory consideration
  - In Vitro DDI – PhRMA/FDA initiative

Prueksaritanont, Presented at SUNY Buffalo, Aug 2011
Current Understanding of FcRn Impact on mAb PK

- FcRn is a key determinant of mAb PK
  - FcRn prolongs mAb half life by rescue it from lysosomal degradation.
  - Modulated FcRn interaction can lead to changes in mAb half-life
  - Multiple binding parameters in IgG-FcRn interaction are important
    - Binding at slight acidic pH
    - No binding at neutral pH

- Desired *in vitro* FcRn binding parameter predictive of *in vivo* PK have not been identified.
  - Various methods were used to quantify FcRn-IgG interaction
    - BIAcore, FACS, ELISA
    - Equilibrium binding vs. kinetics measurement

Wang, Wibio presentation, 2008
Not All Human IgGs Bind FcRn Equivalently

Binding of mAbs with identical Fc sequences to immobilized human FcRn at pH 6.0 and 7.3

TABLE 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MK-1</th>
<th>MK-2</th>
<th>MK-3</th>
<th>MK-4</th>
<th>MK-5</th>
<th>MK-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotype</td>
<td>IgG1</td>
<td>IgG2</td>
<td>IgG1</td>
<td>IgG2</td>
<td>IgG1</td>
<td>IgG2</td>
</tr>
<tr>
<td>Fab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>C</td>
<td>C'</td>
</tr>
<tr>
<td>$K_D$ (nM) at pH 6.0</td>
<td>56.3</td>
<td>73.0</td>
<td>18.5</td>
<td>24.7</td>
<td>25.7</td>
<td>33.2</td>
</tr>
<tr>
<td>%bound at pH 7.3 (%)</td>
<td>0.4</td>
<td>0.0</td>
<td>2.5</td>
<td>2.9</td>
<td>12.9</td>
<td>9.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> MK-1 and MK-2 have an identical Fab domain A, MK-3 and MK-4 have an identical Fab domain B, and MK-5 and MK-6 have closely related, but slightly different, Fab domains C and C'.

Half life of mAbs with Identical Fc Sequence Appeared to Correlate Better with Dissociation at Neutral pH

Note: we have confirmed literature data that for mAbs with different Fc sequences, t1/2 correlates with FcRn binding at pH 6.

Case Example: FcRn Tool Aided in mAb Lead Optimization

- Based on in-house database established: mAbs with the same wild-type human Fc sequences but different Fab domains, showed correlation between in vitro FcRn dissociation at neutral pH and PK

- Issues: Short t1/2
- The FcRn tool enabled rapid/effective identification of leads with significantly improved PK t_{1/2} in hFcRn mice and NHP

Wang et al., Drug Metab Dispos, 39:1469-1477, 2011
Factors Capable of Influencing SC Absorption

**Physiological Factors**
- Body weight/BMI
- Age
- Gender
- Activity level

**Drug Factors**
- Injection site
- Anesthesia, message
- Hydrostatic pressure
- Blood flow

- Intrinsic stability
- Dose
- Dosing volume/conc
- Isoelectric point
- Formulation

- Lymphatic absorption/transport
- Catabolism

%F and variability

McDonald et al., (2010) 74Curr Opin Mol Ther 12:461-470
Challenges to Predict Subcutaneous (SC) Absorption of Macromolecules in Humans

<table>
<thead>
<tr>
<th>Therapeutic protein</th>
<th>Name</th>
<th>Description</th>
<th>Bioavailability (%F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>rat</td>
</tr>
<tr>
<td>Aranesp</td>
<td>darbepoetin alfa</td>
<td></td>
<td>37%</td>
</tr>
<tr>
<td>Betaseron</td>
<td>IFNβ-1b</td>
<td></td>
<td>16%</td>
</tr>
<tr>
<td>Cimzia</td>
<td>PEG-anti-TNFα Fab</td>
<td>24-34%</td>
<td></td>
</tr>
<tr>
<td>Epogen</td>
<td>epoetin</td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Kineret</td>
<td>rhIL-1R antagonist</td>
<td></td>
<td>62%</td>
</tr>
<tr>
<td>Mirsela</td>
<td>PEG-EPO</td>
<td></td>
<td>31-45%</td>
</tr>
<tr>
<td>Neulasta</td>
<td>PEG-GCSF</td>
<td></td>
<td>5-23%</td>
</tr>
<tr>
<td>Somavert</td>
<td>PEG-hGH</td>
<td></td>
<td>70-81%</td>
</tr>
<tr>
<td>Pegasys</td>
<td>PEG-IFNα-2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG-Intron</td>
<td>PEG-IFNα-2b</td>
<td>43-51%</td>
<td>57-89%</td>
</tr>
<tr>
<td>Amevive</td>
<td>LFA3-Fc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcalyst</td>
<td>(IL-1RI/IL-1RAcP)-Fc</td>
<td>60%</td>
<td>70%</td>
</tr>
<tr>
<td>Embrel</td>
<td>TNFR-Fc</td>
<td></td>
<td>73%</td>
</tr>
<tr>
<td>Humira</td>
<td>Anti-TNFα mAb</td>
<td></td>
<td>89%</td>
</tr>
<tr>
<td>Ilaris</td>
<td>Anti-Ill1β mAb</td>
<td></td>
<td>60%</td>
</tr>
<tr>
<td>Raptiva</td>
<td>Anti-CD11a mAb</td>
<td></td>
<td>36%</td>
</tr>
<tr>
<td>Simponi</td>
<td>Anti-TNFα mAb</td>
<td></td>
<td>77%</td>
</tr>
<tr>
<td>Stelara</td>
<td>Anti-p40 mAb</td>
<td>97%</td>
<td>24-95%</td>
</tr>
<tr>
<td>Xolair</td>
<td>Anti-IgE mAb</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>

- Wide range of bioavailability (%F) and high degrees of variability
  - Especially for therapeutic proteins
- Lack of preferred animal model or scaling strategy


Wang, AAPS Biotech meeting, May 2011
SC Absorption of Macromolecules in Commonly Used Preclinical Species

- Experimental evidence of the lymphatic absorption of macromolecules following SC dosing mostly came from a sheep lymphatic cannulation model
  - Not a commonly used preclinical species

- Controversial evidence reported in commonly used preclinical species, e.g. rats, rabbits
  - Minimal serum exposure reduction in thoracic lymph duct cannulated animals
  - Low drug recovery in thoracic duct lymph


Wang, AAPS Biotech meeting, May 2011
Case Example: SC Absorption of Macromolecule in the LDC Rat Model

- **Evidence of lymphatic absorption in our LDC rat model**
  - $^{125}$I-labeled, pegylated proteins, F = ~40%
  - Injection site = lower hind leg

Serum exposure reduction (~70%)

Cumulative lymph recovery

---

Wang, AAPS Biotech meeting, May 2011
Case Example: Catabolism During SC Absorption and Lymphatic Transport

- Catabolic activity observed in rat SC tissue homogenate and lymph node cell suspension

**SC tissue homogenate**

```
<table>
<thead>
<tr>
<th>Comp A</th>
<th>Comp B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>24 (hr)</td>
</tr>
</tbody>
</table>
```

**Lymph node cell suspension**

```
<table>
<thead>
<tr>
<th>Comp A</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>24 (hr)</td>
</tr>
<tr>
<td>Cell #</td>
<td>2x10^6</td>
<td>2x10^6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comp B</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>24 (hr)</td>
</tr>
<tr>
<td>2x10^6</td>
<td>2x10^6</td>
<td>17x10^6</td>
</tr>
</tbody>
</table>
```

Wang, AAPS Biotech meeting, May 2011
Case Example: LCMS Key Enabler in Candidate Selection: *In Vivo* Transformation of mAb

- Isomerization of a single Asp in the CDR region completely loses its target binding ability
- Immunoassay measured both active and inactive components

![Graph showing drug levels and metabolite ratios](chart)

**parent/metabolite ratio**

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>2:1</td>
<td>1:1</td>
</tr>
</tbody>
</table>

Wang et al., Poster presented at SUNY Buffalo, Aug 2011
Summary

- Over the last several years, there has been a tremendous increase in interest in the development of proteins and peptides as drugs.

- Proteins and peptides often show complex disposition kinetics due, in part, to: elimination by cells throughout the body, target-mediated distribution and elimination, processing by specialized transporters (e.g., FcRn), the development of host anti-drug immune responses, etc.
  - Limited understanding in ADME process and limited availability of tools, as compared to SMs.

- Role of DMPK in Biologics discovery and development could follow a similar path for SMs.
  - Enhance POS of Biologics discovery and development.
Acknowledgements

- Weirong Wang
- Azher Hussain
- Lora Hamuro
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- Michelle Groff
- Lorrain Lipfert

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  - Jennifer Adelsberger
  - Christine Bowman
  - Scott Faulty
  - Kimberly Michel
  - Tamara Pittman
  - Xiaolan Shen

- Jiunn Lin
- Jane Harrelson
- Lisa Shipley
Backups
Toxicology Studies Needed for Licensure Biologics vs. Small Molecules

<table>
<thead>
<tr>
<th>Toxicity Testing</th>
<th>BLA Approval</th>
<th>NDA Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Toxicity</td>
<td>Relevant species (one species acceptable)</td>
<td>2 species (one rodent, one non-rodent)</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td>Not required (unless linker, contamination, direct interaction with DNA)</td>
<td>3 assays</td>
</tr>
<tr>
<td>Safety Pharmacology</td>
<td>Often incorporated into general toxicology study</td>
<td>Often conducted with standard models</td>
</tr>
</tbody>
</table>
# Toxicology Studies Needed for Licensure

## Biologics vs. Small Molecules

<table>
<thead>
<tr>
<th>Toxicity Testing</th>
<th>BLA Approval</th>
<th>NDA Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive Toxicity</td>
<td>- Relevant species</td>
<td>Performed in at least 2 species, standard models of rat and rabbit</td>
</tr>
<tr>
<td></td>
<td>- Embryo-fetal development</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Select species closest to human physiology</td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>- Interested in effects on cell proliferation, tumor promotion</td>
<td>- Interested in direct interaction with DNA</td>
</tr>
<tr>
<td></td>
<td>- In vitro and in vivo studies (alternative models)</td>
<td>- Performed in standard models (rodent), sometimes alternative models</td>
</tr>
<tr>
<td>Immunotoxicity</td>
<td>Interested in systemic exposure of biologic and consequence of anti-product antibodies</td>
<td>Centers on hypersensitivity and modification of immune system as an adverse event</td>
</tr>
</tbody>
</table>
mAbs, Fragments and Others...

**a**

- **IgG**
  - $V_H$, $C_H^1$
  - $C_H^2$, $C_H^3$
  - 150 kDa

- **scFv-Fc**
  - $V_H$, $V_L$
  - $C_H^1$, $C_H^2$, $C_H^3$
  - 110 kDa

- **scFv-C$_H$3 (minibody)**
  - $V_H$, $V_L$
  - $C_H^1$, $C_H^2$, $C_H^3$
  - 80 kDa

- **dAbs-Fc**
  - $V_H$
  - $C_H^1$, $C_H^2$, $C_H^3$
  - 85 kDa

**b**

- **Fab**
  - $V_H$, $V_L$
  - 28 kDa

- **scFv**
  - $V_H$, $V_L$
  - 11–13 kDa

- **dAbs**
  - $V_H$
  - 15 kDa

- **V$_{HH}$**
  - $C_H^1$, $C_H^2$

**c**

- **Diabodies**
  - 50 kDa

- **scDb**
  - 55 kDa

- **taFv**
  - 55 kDa

*BioDrugs 2009; 23 (2)*
There are five main categories of conduits in the lymphatic systems:

- Lymph capillaries, collecting vessels, lymph nodes, trunks and ducts

The movement of lymph is due to skeletal muscle contraction, respiratory movement and contraction of smooth muscle in vessel wall:

- Exercise or heat can cause a 2 to 10 times increase in lymph flow rate
- Anesthesia can significantly slow down the lymph flow

The collective lymph through the thoracic duct enters the blood system at the junction of the jugular and subclavian veins.
IgG-Fc sugars

-Gln-Tyr-Asn$_{297}$-Ser-Thr-

GlcNAc  -  Fuc

GlcNAc

α(1-6)  Man

Man  GlcNAc

α(1-3)

GlcNAc  Man

GlcNAc

Gal

G0, G0F, G1, G1F, G2, G2F

IgG-Fab sugars

-Gln-Tyr-Asn$_{297}$-Ser-Thr-

GlcNAc  -  Fuc

GlcNAc

α(1-6)  Man

Man  GlcNAc

α(1-3)

GlcNAc  Man

GlcNAc

Gal

Neu5Ac

Neu5Ac

### Table 1 Approved biosimilars in the EU

<table>
<thead>
<tr>
<th>Brand (generic name; date of MAA in the EU)</th>
<th>Company (location)</th>
<th>Biosimilar clinical development for each biologic type</th>
<th>Pharmacokinetics/pharmacodynamics (PK/PD) and efficacy/safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin alfa</td>
<td>Medice Arzneimittel Pütter (Berknehe, Germany)</td>
<td>Reference product: Eprex/Erypo (erythropoietin alfa; Janssen-Cilag, Beerse, Belgium)</td>
<td>PK/PD studies; only comparative PK/PD studies using the reference product were considered relevant. These established biosimilarity as regards PK/PD. Efficacy/safety: The biosimilar development for the subcutaneous (s.c.) route could not be performed according to the EMEA guideline because, at that time, the reference product Eprex/Erypo was temporarily contraindicated in the EU for the s.c. use in chronic renal failure due to the occurrence of immune-mediated pure red cell aplasia (PRCA). Because of the lack of immunogenicity data in this clinical scenario with the biosimilar, a restriction to intravenous (i.v.) use in this population was included in the Summary of Product Characteristics (SPC). The companies committed to provide a comparative s.c. study according to EU guidance post-marketing.</td>
</tr>
<tr>
<td>Anabranm (recombinant human erythropoietin alfa; August 28, 2007)</td>
<td>Sandoz (Kundl, Austria)</td>
<td>Five PK/PD studies in healthy volunteers (four comparative; comparator: erythropoietin alfa and epoetin beta; single and multiple dose). One comparative pivotal therapeutic equivalence phase 3 study (comparator EpoPro) in intravenous use in patients with renal anemia. One supportive phase 3 noncomparative study assessing efficacy and safety in the subcutaneous treatment of chemotherapy-associated anemia (EpoPro group included as a measure of internal validity).</td>
<td></td>
</tr>
<tr>
<td>Epoctit alfa Hexal (recombinant human erythropoietin alfa; August 28, 2007)</td>
<td>Hexal Biotechnik (Holzkirchen, Germany)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythropoietin zeta</td>
<td>Hospira Enterprises (Hoofddorp, The Netherlands)</td>
<td>Reference product: Eprex/Erypo (erythropoietin alfa; Janssen-Cilag) Two PK studies in healthy volunteers (single dose, comparative). Two phase 3 studies (one correction phase study, one maintenance phase study), both for i.v. administration. One supportive uncontrolled safety trial in cancer patients with chemotherapy-induced anemia.</td>
<td>PK studies were in line with EMEA guidance for biosimilar erythropoietins. PK evaluation was lacking, but the EMEA guideline on biosimilar erythropoietins was at the time of development not available. Efficacy/safety: Study results indicated that both biosimilar and reference product control hemoglobin levels to a similar extent. No anti-erythropoietin antibodies emerged during i.v. treatment. Immunogenicity of s.c. use of the biosimilar was not evaluated in patients with renal anemia and, therefore, a respective warning and restriction to i.v. use in this population was included in the SPC.</td>
</tr>
<tr>
<td>Retacrit (epoetin-zeta; December 18, 2007)</td>
<td>STADA Arzneimittel (Bad Vilbel, Germany)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silapo (epoetin-zeta; December 18, 2007)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Sandoz</td>
<td>Reference product: Genotropin (human growth hormone; Pfizer, Kent, UK, formerly Pharmacia) Three PK/PD studies in healthy volunteers One pivotal efficacy study (comparative, open label) with an extension study One pivotal safety study (noncomparative)</td>
<td>First biosimilar to be approved in the EU PK/PD: PK profiles were considered comparable. No PD evaluation was performed as requested in the EMEA guidance, but this document was not yet available at the time of clinical development. Efficacy/safety: The clinical studies demonstrated similar clinical efficacy for the biosimilar and the reference product. The incidence of anti-somatropin antibodies was higher in the biosimilar group. However, these antibodies did not affect efficacy or safety of the biosimilar. Their occurrence was most likely related to the presence of an increased level of host cell proteins. After introduction of additional purification steps, antibody frequency dropped to the expected range.</td>
</tr>
<tr>
<td>Omnitrope (somatropin growth hormone; April 12, 2006)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valtropin (somatropin, human growth hormone; April 24, 2006)</td>
<td>Bio Partners (Röschelsheim, Germany)</td>
<td>Reference product: Humatrope (human growth hormone; Eli Lilly, Houston, The Netherlands) One PK study in healthy volunteers (comparative) One phase 3 study (randomized, double-blind) One supportive open-label uncontrolled study in women with Turner syndrome</td>
<td>PK/PD: PK profiles were similar for the biosimilar and the reference product. No PD markers were measured in the PK study, but some were in the phase 3 pivotal trial, which suggested similarity. Efficacy/safety: The primary objective of the phase 3 study was to demonstrate noninferior efficacy, whereas for biosimilars usually therapeutic equivalence is requested. From further analyses, however, it was concluded that the biosimilar is also therapeutically equivalent to the reference product. A special feature of Valtropin is the use of yeast as an expression system. Overall, only low anti-yeast protein-antibody titers were found in patients treated with the biosimilar, which were considered not to be of clinical relevance. Furthermore, unlike bacterial cell expression systems, the components have not been described</td>
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<tr>
<td>I. Process development</td>
<td>II. Analytical development</td>
<td>III. Preclinical/clinical development</td>
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<tr>
<td>Cloning of the gene</td>
<td>Definition of standards</td>
<td>Preclinical studies (two mammalian species)</td>
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<tr>
<td>Construction of the</td>
<td>Bioassays (cell-based in vitro expression vector</td>
<td>Toxicology in a rodent species (acute, chronic, subchronic)</td>
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<tr>
<td>Transfection of the</td>
<td>SDS-polyacrylamid gel</td>
<td>Toxicology in a nonrodent species (acute, chronic, subchronic)</td>
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<tr>
<td>host cell</td>
<td>electrophoresis (SDS-PAGE)</td>
<td>Safety pharmacology (cardiovascular, respiratory, renal, gastrointestinal, CNS, depending on drug)</td>
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<td>Selection of stable</td>
<td>Western blot (WB)</td>
<td>Pharmacokinetic studies</td>
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<td>Capillary zone</td>
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<tr>
<td>Optimization of</td>
<td>electrophoresis</td>
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<tr>
<td>expression, culture</td>
<td>(CZE)</td>
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<td>media selection</td>
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<td>Master cell bank (MCB)</td>
<td>Reversed phase HPLC (RP-HPLC)</td>
<td>Phase I studies (healthy volunteers):</td>
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<tr>
<td>Working cell bank (WCB)</td>
<td>Size-exclusion HPLC (SEC-HPLC)</td>
<td>Safety/Tolerance/Pharmacokinetic/Pharmacodynamic</td>
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<td>Characterization and</td>
<td>Product-specific ELISA</td>
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<td>safety of cell banks</td>
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<td>Upstream procedures:</td>
<td>Host-cell-protein ELISA</td>
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<td>Fermentation process</td>
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<td>(USP)</td>
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<td>Downstream procedures.</td>
<td>Residual DNA detection</td>
<td>Phase II studies (patients):</td>
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<td>Purification scheme</td>
<td>(picogreen, threshold)</td>
<td>Safety, proof of efficacy, dose</td>
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<td>(DSP)</td>
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<td>finding</td>
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<td>Optimization of</td>
<td>N-terminal sequencing</td>
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<td>individual process</td>
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<td>steps</td>
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<tr>
<td>Stability and robustness of the</td>
<td>C-terminal amino acid</td>
<td>Phase III studies (patients):</td>
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<td>composition</td>
<td>Controlled safety and efficacy in specific indications</td>
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<td>Introducing of GMP</td>
<td>Peptide mapping</td>
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<td>Consistency batches</td>
<td>Total amino acid content (upon hydrolisis)</td>
<td>Multiple arms, vs. reference therapy or placebo controlled.</td>
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<tr>
<td>Validation</td>
<td>Carbohydrate analyses (total sugar, antennarity, sialic acids)</td>
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</tbody>
</table>

Walter Hinderer

Biogeneric Drugs
# Table 2  The pros and cons for biosimilar mAbs

<table>
<thead>
<tr>
<th>Pros for biosimilar approach</th>
<th>Cons for biosimilar approach</th>
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</thead>
<tbody>
<tr>
<td>The structural characterization, manufacture and regulatory history of mAbs are reasonably well established</td>
<td>Every mAb is unique and small structural changes can have significant functional consequences. Even the same expression system and similar culture conditions might lead to a distinct product profile (e.g., impurities or microheterogeneity). Some methods for physicochemical characterization might not be sufficiently sensitive to establish similarity conclusively</td>
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<tr>
<td>Readily available potency assays, most of which are relevant (that is, they correlate with the rationale of the product)</td>
<td>Potency assays might not be able to discriminate differences (see above)</td>
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<td>In most cases, understanding of mAb function is reasonably well established, facilitating the planning of nonclinical studies as regards endpoints and other criteria</td>
<td>The efficacy and safety of mAbs are in most cases highly species specific, which makes performing nonclinical studies more difficult and potentially expensive</td>
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<td>Safety profile is generally reasonably well established</td>
<td>Safety profile might differ due to factors like differences in impurity profile, immunogenicity and others</td>
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<td>Efficacy profile is generally reasonably well established</td>
<td>Efficacy from one indication might not be transferable to other indications if the reference product is licensed for several clinical conditions. Equivalence/noninferiority study against reference product might require many more patients than stand-alone trials</td>
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