Regulatory requirements for production of monoclonal antibodies

Christian Bechon,
CEO,
LFB.
Pharmaceutical Industry Financials*

- Worldwide pharmaceutical market (2006) ~ $ 653 Billion

- Biopharmaceutical market (2006) ~ $93 Billion (~15%)

~ 2/3 of all biopharmaceuticals are produced using recombinant DNA technologies of which ~50% are monoclonal antibodies.

* Bioplan Associates 04/2008
<table>
<thead>
<tr>
<th>Product</th>
<th>Revenue</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enbrel</td>
<td>$ 5.982B</td>
<td>Rheumatoid Arthritis, Psoriatic Arthritis</td>
</tr>
<tr>
<td>Rituxan</td>
<td>$ 5.082B</td>
<td>Non-Hodgkin’s lymphoma, Rheumatoid Arthritis</td>
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<tr>
<td>Humira</td>
<td>$ 4.521B</td>
<td>Rheumatoid Arthritis, Psoriatic Arthritis</td>
</tr>
<tr>
<td>Avastin</td>
<td>$ 4.479B</td>
<td>Colorectal Cancer, Non-small-cell lung Cancer</td>
</tr>
<tr>
<td>Herceptin</td>
<td>$ 4.394B</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Remicade</td>
<td>$ 3.748B</td>
<td>Crohns Disease, Rheumatoid Arthritis</td>
</tr>
<tr>
<td>Gleevec</td>
<td>$ 3.700B</td>
<td>Chronic Myelogenous Leukemia, Gastr-intestinal Stromal Tumours</td>
</tr>
<tr>
<td>Neulasta</td>
<td>$ 3.318B</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>Lantus</td>
<td>$ 3.159B</td>
<td>Types I and II Diabetes</td>
</tr>
<tr>
<td>Aransep</td>
<td>$ 3.137B</td>
<td>Anaemia</td>
</tr>
<tr>
<td>Prevnar</td>
<td>$ 2.716B</td>
<td>Streptococcus pneumoniae vaccine</td>
</tr>
<tr>
<td>Taxotere</td>
<td>$ 2.622B</td>
<td>Breast cancer, Non-small cell lung cancer, prostate cancer, gastric cancer</td>
</tr>
<tr>
<td>Procrit</td>
<td>$ 2.460B</td>
<td>Anaemia</td>
</tr>
<tr>
<td>Epogen</td>
<td>$ 2.456B</td>
<td>Anaemia</td>
</tr>
<tr>
<td>Copaxone</td>
<td>$ 2.262B</td>
<td>Multiple sclerosis</td>
</tr>
</tbody>
</table>

* Bioworld Market Leading Biotechnology Drugs 2009
### Top Biotech Drugs (2008) – Expression Systems

<table>
<thead>
<tr>
<th></th>
<th>Product</th>
<th>Company</th>
<th>Expression system</th>
<th>Type of Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enbrel</td>
<td>Amgen/Wyeth/Pfizer</td>
<td>CHO cells</td>
<td>MAb Fusion Protein</td>
</tr>
<tr>
<td>2</td>
<td>Rituxan</td>
<td>Genentech/Roche</td>
<td>CHO cells</td>
<td>Chimeric Mab</td>
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<tr>
<td>3</td>
<td>Humira</td>
<td>Abbott</td>
<td>CHO cells</td>
<td>Human MAb</td>
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<tr>
<td>4</td>
<td>Avastin</td>
<td>Genentech/Roche</td>
<td>CHO cells</td>
<td>Humanised MAb</td>
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<tr>
<td>5</td>
<td>Herceptin</td>
<td>Genentech/Roche</td>
<td>CHO cells</td>
<td>Humanised MAb</td>
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<tr>
<td>6</td>
<td>Remicade</td>
<td>J&amp;J/Centocor</td>
<td>Murine Myeloma Cells</td>
<td>Chimeric MAb</td>
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<tr>
<td>7</td>
<td>Gleevec</td>
<td>Novartis</td>
<td>N/A - Chemical Synthesis</td>
<td>Chemical</td>
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<tr>
<td>8</td>
<td>Neulasta</td>
<td>Amgen</td>
<td>E.Coli</td>
<td>PEGylated GCSF</td>
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<tr>
<td>9</td>
<td>Lantus</td>
<td>Sanofi Aventis</td>
<td>E.Coli</td>
<td>Modified Insulin</td>
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<tr>
<td>10</td>
<td>Aransep</td>
<td>Amgen</td>
<td>CHO cells</td>
<td>Modified erythropoietin</td>
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<tr>
<td>11</td>
<td>Prevnar</td>
<td>Wyeth/Pfizer</td>
<td>N/A – Bacterial Culture</td>
<td>Streptococcus pneumoniae Vaccine conjugate</td>
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<td>12</td>
<td>Taxotere</td>
<td>Sanofi Aventis</td>
<td>N/A - Chemical Synthesis</td>
<td>Chemical</td>
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<td>13</td>
<td>Procrit</td>
<td>Ortho Biotech</td>
<td>CHO cells</td>
<td>Erythropoietin</td>
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<tr>
<td>14</td>
<td>Epogen</td>
<td>Amgen</td>
<td>CHO cells</td>
<td>Erythropoietin</td>
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<tr>
<td>15</td>
<td>Copaxone</td>
<td>Teva</td>
<td>N/A - Chemical Synthesis</td>
<td>Chemical</td>
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</table>
The top 6 biotech drugs are monoclonal antibodies or antibody fusion proteins.

It is expected by 2014 that the six best selling pharmaceutical drugs will be monoclonal antibodies as patent protections expire on existing best selling drugs.

Monoclonal antibodies will become an even more significant class of drug in the future.
A European biopharmaceutical group specialised in therapeutic proteins

1st October 2008
Presentation of LFB

What is LFB?

- One of the top European pharmaceutical companies manufacturing and commercialising human plasma-derived medicinal products
- A key player in the biotechnology field in France

A fully biopharmaceutical group focused on biological and biotechnological medicinal products
Figures in 2008

- 352 million € turnover: 9% growth
- 3rd pharmaceutical company serving hospitals in France
- 6th fractionator worldwide
- 1st French biotech company
- €66 million R&D budget: 19% of turnover
- 1531 employees
- €29.2 million industrial investments

500,000 patients treated each year with the 19 LFB plasma-derived medicinal products to care for 80 severe and sometimes rare pathologies
LFB S.A.

- **LFB BIOMÉDICAMENTS**
  - French subsidiary specialising in plasma fractionation and marketing plasma-derived medicinal products

- **LFB Hemoderivados e biotecnologia Brazil (2004)**
- **LFB GmbH Germany (2007)**
- **LFB Biopharmaceuticals Limited UK (2007)**
- **LFB Middle East (2007)**
- **CAF/DCF***

- **LFB BIOTECHNOLOGIES**
  - Subsidiary specialising in research & development and the Group’s biotechnology activities

- **LFB Biotehnologies, Inc. (USA)**
- **MAbgène**

- **GTC BIOOTHERAPEUTICS**

- **LFB / GTC LLC (USA)**

*Minority stakes*
2 monoclonal antibodies (MAbs) using EMABling® technology:

- Anti-Rhesus D (RhD) (human)
- Anti-CD20 (chimeric)

3 transgenic recombinant proteins with GTC:

- Factor VIIa
- Factor IX
- anti-CD20 monoclonal antibody
Regulatory aspects of monoclonal antibody development

Christian Bechon,
CEO,
LFB.
Evolution of monoclonal antibodies ("-mab"):

- Murine mAb: 100% mouse
  - Arcitumomab (CEA-Scan®) (1996)

- Chimaeric mAb: 33% mouse
  - Infliximab (Remicade®) (1999)

- Humanized mAb: 10% mouse
  - Trastuzumab (Herceptin®) (2000)

- Fully Human mAb: 100% human

New constructs:
- Bispecific antibodies
- Diabodies
- Single chain fragments
- Engineered Fc mAbs
- Conjugated mAbs
- ...

Immunogenicity

= murine
= human
<table>
<thead>
<tr>
<th>Regulatory Guidelines</th>
<th>Quality</th>
<th>Safety</th>
<th>Efficacy</th>
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<tr>
<td><strong>ICH</strong></td>
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<tr>
<td>Q5A : Viral safety evaluation of Biotechnology Products derived from cell lines of Human or Animal Origin (1997)</td>
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<td>Q5B : Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines used for Production of r-DNA derived Protein Products</td>
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<td>Q5C : Stability testing of biotechnological/Biological Products</td>
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<tr>
<td>Q5D : Derivation and characterisation of cell substrates used for Production of r-DNA derived Protein Products</td>
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<td>Q5E : NfG on biotechnological/Biological products Subject to changes in their Manufacturing Processes (2004)</td>
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<td>Q6B : Test procedures &amp; acceptance criteria for biotechnological &amp; biological products (1999)</td>
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<td>Q7A : GMP guidance for API clinical materials (2001)</td>
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<td><strong>EMEA</strong></td>
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<td>3AB1A : Production &amp; QC of medicinal products derived by recombinant DNA technology (1995)</td>
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<td>EMEA/CHMP/BWP/398498/05 : Virus safety evaluation of biotechnological investigational medicinal products (IMP)(2009)</td>
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<td>CPMP/BWP/268/95 : Virus validation studies(1996)</td>
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<td><strong>European Pharmacopeia</strong></td>
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<td>Monograph #2031 : Monoclonal antibodies for human use (2008)</td>
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<tr>
<td>Monograph #0784 : Products of recombinant DNA technology (2008)</td>
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<td><strong>FDA</strong></td>
<td></td>
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<tr>
<td>Points to consider in the manufacture &amp; testing of monoclonal antibody product for human use (1997)</td>
<td></td>
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</tr>
<tr>
<td>Guideline for submission of chemistry, manufacturing and controls information for a therapeutic recombinant DNA-derived product or a mAb for in-vivo use (1996)</td>
<td></td>
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</tr>
<tr>
<td>Guidance for Industry - Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs (draft, sept.2008)</td>
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</table>
Stages in the Clinical Development Process

Each stage brings its own regulatory challenges

- Drug discovery
  - In vivo, in vitro
  - Tox, PK, etc.
- Clinical testing (CTA/IND)
  - Ph. I
  - Ph. II
  - Ph. III
  - Product development
  - Process development
- MAA/BLA
  - Phase IV

Good Lab Practices (GLPs)

Good Clinical Practices (GCP)

Good Manufacturing Practices (cGMP)

CTA: Clinical Trial authorisation
IND: Investigational New Drug
BLA: Biologics License Application
MAA: Marketing Authorisation
Manufacturing Process for mAbs

Upstream Processing:
- Thaw vial of WCB
- Cell Expansion
- Production Bioreactor

Downstream Processing:
- Clarify harvest
- Protein A Chromatography
- Virus Inactivation
- Chromatography
- Virus Removal
- Filtration

Aseptic Filling:
- Formulation
- Filling
Master and Working Cell Banks (MCB & WCB)

- MCB and WCB required as per guidelines ICH Q5B & Q5D
  - MCB derived from the selected cell clone containing the expression construct
  - WCB derived by expansion of one or more ampoules from MCB

- This two-tiered cell banks system ensures consistent starting material for each lot of product

- Testing required for MCB and WCB characterisation
  - Identity (phenotypic and/or genotypic characteristics)
  - Purity (absence of adventitious agents: viruses, mycoplasma, bioburden; no contamination by other cell lines)

- Necessary to determine & document stability of MCB and WCB.
Potential Changes to process during development:

- Modify the cell line (e.g. to improve stability of productivity)
- Modify the culture medium (e.g. to improve productivity)
- Modify process steps (e.g. to reduce HCP contamination)
- Scale-up of process (e.g. to reduce unit costs)
- Change of facility (e.g. for process scale-up)
- Change of technology (e.g. stainless steel to disposable bioreactor)

Each modification must be evaluated for regulatory impact and comparability demonstrated by means of thorough analytical testing.
Comparability during clinical development

Pre-change product  

Post-change product  

Comparability  

Non clinical / Clinical development

✓ Pre- and post-change products not identical but comparable
✓ Sequential comparability plan

Analytical > In vitro bioactivity > In vivo bioactivity > non clinical > clinical

⇒ Good knowledge of the product, process and of relationship between the quality attributes and safety and efficacy of mAbs essential
Viral Safety testing

Guidelines: ICH Q5A & EMEA guideline on investigational products

Potential sources of contamination
- Nature of cell line used (mammalian cells) ⇒ endogenous viruses
- Raw material used ⇒ viral contaminants
- Manufacturing ⇒ introduction of viruses into the product

Biological safety based on complementary measures
- Virus testing of production cell line & viral safety of biological raw materials
- Virus testing of unprocessed bulk harvest
- Capacity of the production process to inactivate and/or eliminate viruses
  (Need to have at least two complementary dedicated viral elimination/inactivation steps, e.g. solvent-detergent treatment + nanofiltration)
EU Guideline on immunogenicity assessment of biotech-derived proteins

(Guideline on immunogenicity of mAbs currently evaluated)

Immunogenic response to mAb influenced by
- Patient and disease-related factors
- Product-related factors (human or not, post-translational modifications, impurities, formulation, immunomodulatory and functional properties of the mAb, administration scheme)

Can affect key functional parameters
- Safety (risk of anaphylactic reactions or delayed hypersensitivity)
- Bioavailability (PK/PD profile) as immune complexes enhance clearance
- Efficacy, in case of neutralizing anti-drug antibodies (ADA)
- Physiological function of endogenous counterparts, in case of cross-reacting ADA ("high risk products" e.g. refractory anemias with rEPO)

Immunogenicity assessment
- Not easy to predict from animal models
- Must be part of the clinical monitoring
Future Challenges

- The greater use of disposable systems – validation of extractables and leachables

- Emergence of the Biosimilars’ market to compete with existing monoclonals
Regulatory requirements for production of monoclonal antibodies

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