Bioassays as an essential tool for Biosimilar Development

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Agenda

- Introduction
- EMA Guideline
- Biological Characterization
## What is a biosimilar?

<table>
<thead>
<tr>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Overview</strong></td>
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<tr>
<td>• <strong>Successor to a biologic</strong> medicine that has lost exclusivity</td>
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<tr>
<td>• <strong>Not a simple generic</strong> due to complexity: size, structure and manufacturing</td>
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<tr>
<td><strong>Regulatory definition</strong></td>
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<tr>
<td>• A biologic approved via a stringent regulatory defined pathway demonstrating comparability</td>
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<tr>
<td><strong>Comparability approach</strong></td>
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<tr>
<td>• <strong>Highly analogous structure</strong> (via robust analytical characterization)</td>
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<tr>
<td>• <strong>Comparable quality, safety and efficacy</strong> (via clinical trials)</td>
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Biosimilars address an unmet need and increase competition

**Biologics drugs have revolutionized modern medicine** and will continue to do so, but patient access is increasingly limited by high cost and growing demand. Access is also an unmet medical need.

**Originators should realize fair profit** and return on investment, but indefinite monopolies lead to stagnation. **Biosimilars will increase competition** and encourage the next wave of biologics innovation.
Biosimilars are recognized worldwide as safe and effective medicines

EU draft general guidelines adopted

Sandoz Omnitrope first biosimilar approved and launched in EU

Sandoz first EPO approved and launched in EU

Filgrastim* approved in EU

2004

2005

2006

2007

2008

2009

2010

Sandoz Omnitrope first biosimilar – type medicine approved in Australia

Sandoz Omnitrope first biosimilar-type medicine approved and launched in US

Sandoz Omnitrope first biosimilar approved and launched in Japan & Canada

US regulatory pathway

Japan regulatory guidelines

*S First competitor product (Sandoz product approved Feb 2009)
Biosimilars should NOT be confused with “Me-too” biologics

- “Me-too” are often not highly similar and not approved in highly regulated markets
- Labeling “Me-too” biologics as biosimilars induces unfounded concerns

<table>
<thead>
<tr>
<th>Isoelectric focusing gels</th>
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<tbody>
<tr>
<td>“Me-too” biologics ≠ biosimilar</td>
</tr>
<tr>
<td>NOT similar to Reference E</td>
</tr>
<tr>
<td>Approved biosimilar in EU</td>
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<tr>
<td>NO difference to originator</td>
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</table>

mAbs are large and complex molecules.....

Monoclonal antibodies: ~ 150kDa, ~ 1330 amino acids, 2 (1 per chain) glycosylation sites, 16 (7 per chain + 2) disulfide bridges
....but can be thoroughly characterized physico-chemically and biologically

<table>
<thead>
<tr>
<th>Molecular Parameter</th>
<th>Attribute</th>
<th>Methods for control and characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary structure</td>
<td>Sum formula: Mass of light chain, heavy chain</td>
<td>LC-ESI-MS</td>
</tr>
<tr>
<td></td>
<td>Sum formula: Mass of intact MAb</td>
<td>LC-ESI-MS</td>
</tr>
<tr>
<td></td>
<td>Amino acid sequence</td>
<td>Orthogonal peptide maps with high resolution MS and MS/MS sequencing</td>
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<tr>
<td></td>
<td>Free cysteines</td>
<td>Ellman’s, Peptide Map</td>
</tr>
<tr>
<td></td>
<td>Thiorester bridging</td>
<td>Peptide map, SDS-PAGE, CGE</td>
</tr>
<tr>
<td>Higher order structure</td>
<td>Secondary and tertiary structure</td>
<td>CD spectroscopy, DSC, FT-IR</td>
</tr>
<tr>
<td></td>
<td>Disulfide bridging</td>
<td>Non-reducing Peptide Map</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>C-terminal: ± Lys, truncation to Pro-amide</td>
<td>CEX with/without CBP-digest, Papain-IEX, Peptide Map, IEF</td>
</tr>
<tr>
<td>C- and N-terminal</td>
<td>N-terminal variants: (pGlu/Gln, pGlu/Glu)</td>
<td>CEX; Papain-IEX; RP-HPLC of LC, HC; Peptide Map, IEF</td>
</tr>
<tr>
<td>Heterogeneity: Glycosylation</td>
<td>Glycan isoforms:</td>
<td>NP-HPLC of 2AB-labeled glycans, coupled to ESI-MS, exoglycosidase digestion, MALDI TOF/TOF</td>
</tr>
<tr>
<td></td>
<td>• Major (G0, G1, G2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Minor (e.g. Unfucosylated, α-gal)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sialic Acids incl. NGNA</td>
<td>NP-HPLC, WAX, HPAEC; RP-HPLC after DMB-labeling</td>
</tr>
<tr>
<td></td>
<td>Aglycosylated MAb</td>
<td>CGE, Peptide map</td>
</tr>
<tr>
<td>Heterogeneity: Glycation</td>
<td>Glycation of Lys</td>
<td>Boronate affinity; LCMS; Peptide map</td>
</tr>
<tr>
<td>Other amino acid</td>
<td>Oxidation</td>
<td>RP-HPLC, Papain-HIC; Peptide map</td>
</tr>
<tr>
<td>modifications</td>
<td>Deamidation</td>
<td>CEX; Papain-IEX; Peptide map</td>
</tr>
<tr>
<td>Heterogeneity: Size</td>
<td>Aggregation</td>
<td>SEC, FFF, MALLS, DLS, AUC; imaging methods and particle characterization</td>
</tr>
<tr>
<td></td>
<td>Fragmentation in amino acid chain: p100, p50</td>
<td>CGE, SDS-PAGE, SEC, RP-HPLC</td>
</tr>
</tbody>
</table>
…with biosimilarity shown at different levels

Proving biosimilarity with comparability to reference product at all stages

- **Appropriate clinical trials** to show safety / efficacy
- **Design manufacturing processes** to ensure comparability
- **Science-based process development** to deliver target quality
- **Characterization** to prove that product is safe and efficacious

SAFETY - EFFICACY - QUALITY
Biosimilar mAbs can be systematically developed to match the quality attributes of the reference product.
Targeting ADCC-activity and fucosylation

Targeting ADCC activity by targeting unfucosylated glycan structures during cell line development
Development targets are defined by the originator range...but what if originator changes QAs?

- Manufacturing process changes are tightly regulated (see ICH Q5E)
- Change of quality attributes only acceptable if they do not alter safety/efficacy

For demonstrating analytical comparability to originator product it is therefore acceptable to use the upper and the lower limit of the pre and post shift material

However, the Biosimilar/SBP release specification should be as tight as the current originator specification but need not to be the same values.
Monitoring batches of MabThera® and Rituxan® revealed a shift in glycosylation profile and ADCC potency.

Differences/shift in glycosylation pattern results in different potency in cell-based assays.

Agenda

- Introduction
- EMA Guideline
- Biological Characterization
The growing importance of biological characterization in biosimilar development

- Draft Guideline on similar biological medicinal products containing monoclonal antibodies released (18 Nov 10) for consultation by the EMA
- Guideline is covering preclinical and clinical studies and states:

  "In vitro non-clinical studies should include relevant studies on:
  - 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)"
The growing importance of biological characterization in biosimilar development

“Together these assays should cover all functional aspects of the mAb even though some may not be considered necessary for the mode of action in the clinic. As these assays may be more specific and sensitive than studies in animals, these assays can be considered fundamental in the non-clinical comparability exercise.”

- Extensive biological characterization package
  - can greatly reduce the need for *in vivo* animal studies
  - is a pre-requisite for an abbreviated clinical package
  - is a fundamental pillar in supporting extrapolation of indications
Summary EMA guideline regarding binding and functional bioassays

1. Target binding

2. Binding to:
   - all Fcgamma receptors
   - FcRn
   - complement

3. Fab-associated functions, e.g.:
   - neutralization
   - receptor activation
   - receptor blockade

4. Fc-associated functions:
   - ADCC
   - CDC
   - complement activation
Agenda

- Introduction
- EMA Guideline
- Biological Characterization
Classification of therapeutic antibodies based on their putative mechanisms of action

Case study:

- Biosimilar mAb
- Type I antibody
Current biological characterization strategy for a biosimilar mAb

1. Target binding:
   - Cell based target binding assay

2. Binding to:
   - FcγRlla, FcγRllla, FcγRlllb, FcγRllllla\textsuperscript{158F}, FcγRllllla\textsuperscript{158V}, FcγRlllllb
   - FcRn
   - C1q

3. Fab-associated functions
   - Apoptosis

4. Fc-associated functions:
   - ADCC
   - CDC
   - Whole blood assay
Cell based target binding assay

- Competitive assay using A488-labeled mAb and unlabeled mAb as sample
- Detection of binding to cell surface by flow cytometry
- Parallel line assay data evaluation

A488-labeled mAb
Cell based target binding assay

- Binding activity of biosimilar mAb within originator binding activity range
- Binding assay capable of detecting relevant changes in potency

* Error bars in column diagram indicate Min-Max values

* Sandoz
Binding to FcγR and FcRn

- SPR based assays
- measure binding of mAb to extracellular domains of a panel of FcγR (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIla\textsuperscript{158F}, FcγRIIla\textsuperscript{158V}, FcγRIIib) and FcRn
- Originator and biosimilar mAb show comparable binding kinetics to all FcR
C1q Binding

- ELISA based assay
- Titrated mAb is immobilized on ELISA plate
- Assay measures binding of purified C1q to the Fc part of immobilized mAb
- Coating efficiency control included
- Parallel line assay data evaluation

(method see also: Idusogie, E. et al., J. of Immunology 2000, 164: 4178-4184)
Apoptosis assay

- mAb induces phosphatidylserine externalization on target cell
- Staining of cells with Annexin V-FITC and propidium iodide
- Detection of apoptotic cells by flow cytometry

(method see also: Mössner, E. et al., Blood 115, 4393-4402, 2010)
ADCC assay

- NK cell line lyses calcein-loaded target cells by ADCC
- Lysis of target cells quantified by release of calcein into supernatant
- Parallel line assay data evaluation
ADCC assay

- Wide originator range due to quality shift
- ADCC activity of biosimilar mAb within originator ADCC activity range
- ADCC assay capable of detecting relevant changes in potency

* Error bars in column diagram indicate Min-Max values
CDC assay

- mAb mediates complement binding, activation and finally lysis of target cells
- Complement source: rabbit complement
- Read out: ATP-dependent chemiluminescence
- Parallel line assay data evaluation
CDC assay

- CDC potency of biosimilar mAb within originator CDC potency range

* Error bars in column diagram indicate Min-Max values
Whole blood assay

- Represents all mechanisms of action present in peripheral blood (e.g. ADCC, CDC, apoptosis)
- *Ex vivo*, whole blood autologous cell depletion assay
- Mix fresh blood with sample and measure B-cell depletion by flow cytometry

(method see also: Mössner, E. *et al.*, *Blood* 115, 4393-4402, 2010)
Current biological characterization strategy for a biosimilar mAb

**Binding Assays**

1. Target binding:
   - Cell based target binding assay

2. Binding to:
   - FcγRIa, FcγRIIa, FcγRIIIa\(^{158F}\), FcγRIIIa\(^{158V}\), FcγRIIib
   - FcRn
   - C1q

**Functional Assays**

3. Fab-associated functions:
   - Apoptosis

4. Fc-associated functions:
   - ADCC
   - CDC
   - Whole blood assay
Influence of EMA biosimilar mAb draft guideline on Biosimilar Development

Proving biosimilarity with comparability to reference product at all stages

PK/PD

Preclinical

Biological characterization

Physicochemical characterization

PK/PD

Preclinical

Biological characterization

Physicochemical characterization

EMA Biosimilar mAb draft guideline
EMA draft guideline: remaining questions

- EMA requests functional *in vitro* data on effector functions for ALL biosimilar mAbs

- These data may be hard to impossible to obtain for antibodies binding exclusively soluble targets (class III)

- Highly artificial assay setups may be needed

  → relevance of generated data for safety and efficacy of the drug may be low
EMA draft guideline: remaining questions
ADCC for Class III antibody

- **Option 1:**
  - Target cell expresses soluble antigen
  - No ADCC mediated lyses of the target cell

- **Option 2:**
  - Soluble antigen is artificially expressed on the cell surface by adding recombinant membrane domain
  - Antibody induces ADCC mediated lyses

- **Option 3:** others

- **Option 4:** nothing
EMA draft guideline: remaining questions

Complement Assays

**Binding Assays**

1. Target binding
2. Binding to:
   - all Fcgamma receptors
   - FcRn
   - complement

**Functional Assays**

3. Fab-associated functions, e.g.:
   - neutralization
   - receptor activation
   - receptor blockade
4. Fc-associated functions:
   - ADCC
   - CDC
   - complement activation
Adressing complement: redundancy or added value?

1. C1q binding assay
   • Detection using e.g. ELISA based format

2. Complement activation assay
   • Formation of terminal complement complex TCC (= membrane attack complex)
   • Detection of TCC using e.g. ELISA based format

3. CDC assay
   • Insertion of membrane attack complex into membrane and subsequent killing of cell
   • Detection by measuring amount of remaining living cells

Question: Does the complement activation assay add value?
Conclusions

- Growing importance of biological characterization
  - Needed as tool to direct cell line and process development
  - Pre-requisite for an abbreviated clinical package and a fundamental pillar in supporting extrapolation of indications
  - Essential to create product understanding by elucidating structure-function relationships

- Biological characterization package is defined in EMA Draft Guideline on similar biological medicinal products containing monoclonal antibodies
  - This draft guideline leaves questions open
    - Testing for effector functions for class III antibodies
    - Redundancy of complement testing
Thanks for listening

Thanks for contributing
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