Analytical similarity: Lessons from the first US biosimilar

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Agenda

- Biosimilars – Development principles
- Zarxio: analytical biosimilarity approach
- Zarxio: biosimilarity assessment
- Statistical tools for biosimilarity: considerations
Variability is inherent in biologics

**Batch-to-batch**

- Non-identicality is a normal principle in biologics
- No batch of any biologic is “identical” to the other batches
- Variability is natural even in the human body and usually not problematic

**Manufacturing changes**

- Manufacturing changes occur due to process improvements, scale up, etc
- Differences in attributes sometimes significantly larger than batch-to-batch variability


![Variability of major glycan variant in commercial mAB](image)
Variability is inherent in biologics

- Non-identicality is a normal principle in biologics.
- No batch is exactly the same as another batch.
- Variability is natural even in the human body and usually not problematic.

Batch-to-batch

- Manufacturing changes occur due to process improvements, scale up, etc.
- Differences in attributes sometimes significantly larger than batch-to-batch variability.

Safety and efficacy within this variability have been demonstrated in clinical studies and by real-life experience with the reference product.

Variability of major glycan variant in commercial mAB

Biosimilars are systematically developed to match the reference product

2. Target directed development

Recombinant cell line development

Bioprocess development

Purification process development

Drug product development

1. Target definition

Biological variability

No clinically relevant differences!

3. Confirmation of biosimilarity

Clinical

PK/PD

Preclinical

Biological characterization

Physicochemical characterization

Process development

Analytics

Target range

Reference product variability

Biosimilars are systematically developed to match the reference product
Agenda

1. Biosimilars – Development principles
2. Zarxio: analytical biosimilarity approach
3. Zarxio: biosimilarity assessment
4. Statistical tools for biosimilarity: considerations
Overview of Zarxio® (filgrastim)

- Zarxio is a biosimilar of the reference product Neupogen® (filgrastim)
  - Filgrastim (recombinant granulocyte-colony stimulating factor (G-CSF)), which stimulates the proliferation of white blood cells
  - Was first approved in the EU in 2009\(^1\) and subsequently developed for US marketing authorization
  - Since approval, has become the volume leader in Europe
  - Received marketing authorization by FDA in March 2015 as first biosimilar approved in USA; launched Sep. 2015 in USA

- Zarxio US Development Program
  - Analytical: Battery of structure and function analyses
  - Nonclinical: 5 animal studies to assess PD, toxicity, toxicokinetics, and local tolerance
  - Clinical (confirming similarity):
    - 1 pivotal and 4 supportive PK/PD studies to demonstrate similar PK/PD
    - Comparative safety and efficacy clinical study

\(^1\) Marketed as “Zarzio®” ex-US
Enabling technology: state-of-the-art analytics allow for thorough characterization of biologics

Attributes e.g.:
- Primary structure
  - Mass
- Disulfide bridging
- Free cysteines
  - Higher order structure
- N- and C-terminal heterogeneity
- Glycosylation
  - Glycation
- Fragmentation
  - Oxidation
  - Deamidation
- Aggregation
  - Particles
- Target-binding
  - Fc effector functions

Methods e.g.:
- MS
- Peptide mapping
  - Ellman’s
- CGE
- SDS-PAGE
- CD, FT-IR
- H-D exchange
- NMR, X-ray
  - HPLC
  - HPAEC
  - IEF
- 2AB NP-HPLC
- SE-HPLC
  - FFF
  - AUC
  - DLS
  - MALLS
  - Bioassays
  - SPR
## CQA assessment for Zarxio
### What Matters for Filgrastim Safety and Efficacy

<table>
<thead>
<tr>
<th>Quality Attribute</th>
<th>Criticality</th>
<th>Relevant for</th>
<th>Methods Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid sequence</td>
<td>Very High</td>
<td>Efficacy, Safety, Immunogenicity</td>
<td>Edman, peptide mapping, MS</td>
</tr>
<tr>
<td>Potency</td>
<td>Very High</td>
<td>Efficacy, Safety</td>
<td>Bioassay</td>
</tr>
<tr>
<td>Target binding</td>
<td>Very High</td>
<td>Efficacy, Safety</td>
<td>Surface plasmon resonance</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>Very High</td>
<td>Efficacy</td>
<td>Content determination</td>
</tr>
<tr>
<td>Higher order structure</td>
<td>High</td>
<td>Efficacy, Immunogenicity</td>
<td>CD and NMR spectroscopy</td>
</tr>
<tr>
<td>High-Molecular Weight Variants/Aggregates</td>
<td>High</td>
<td>Immunogenicity</td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td>Oxidized Variants</td>
<td>High</td>
<td>Efficacy</td>
<td>Reversed phase chromatography</td>
</tr>
<tr>
<td>Subvisible particles</td>
<td>High</td>
<td>Immunogenicity</td>
<td>Light obscuration</td>
</tr>
<tr>
<td>Truncated Variants</td>
<td>Low</td>
<td>None</td>
<td>RP-HPLC-MS</td>
</tr>
<tr>
<td>Norleucine</td>
<td>Very Low</td>
<td>None</td>
<td>Reversed phase chromatography</td>
</tr>
<tr>
<td>Deamidation</td>
<td>Very Low</td>
<td>None</td>
<td>Cation exchange chromatography</td>
</tr>
</tbody>
</table>
Tiered approach for analytical similarity assessment for a biosimilar

Summary of FDA Advice on Statistics for Analytical Similarity Assessment for a Proposed Biosimilar

- Evaluate quality attributes consistent with the risk assessment principles the ICH Quality Guidelines Q8, Q9, Q10, and Q11.
- Consider criticality risk ranking of quality attributes with regard to their potential impact on activity, PK/PD, safety, and immunogenicity
- Use a tiered approach for assessment
  - Equivalence testing for some high risk attributes
  - Quality ranges (mean ± X SD) for other high to low risk attributes
  - Raw/graphical comparisons for other attributes
- For advice on individual development programs submit proposal to Agency for feedback
- FDA is considering these issues further and intends to develop guidance for industry as appropriate

Source: CMC Strategy Forum Japan Dec 8, 2014, Marjorie Shapiro
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Biosimilarity assessment Zarxio with Neupogen
Highly similar prim., sec., tertiary structure

- Primary structure (amino acid sequence): same for Zarxio and Neupogen
  - Edman Sequencing
  - Peptide Map
  - Mass Spectrometry
  - Amino Acid Analysis
- Secondary structure: CD (circular dichroism) → overlay
- Tertiary structure: 2D NMR → overlay

→ Biosimilarity tool: Qualitative / Visual comparison
Both Zarxio and Neupogen have Very Low Levels of Aggregates, Oxidised and other Variants

Size Exclusion HPLC (SEC)

Reversed Phase HPLC (RPC)

High resolution, high sensitivity methods

→ Biosimilarity tool: Quantitative comparison
→ Min-max or equal/less than reference product
Sensitive Biological Assay Confirms Highly Similar Biological Activity

<table>
<thead>
<tr>
<th>Product</th>
<th>Zarxio</th>
<th>Neupogen</th>
<th>Neupogen Product Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity in U/mg x 10^8</td>
<td>1.0 – 1.1</td>
<td>0.9 – 1.2</td>
<td>0.4 – 1.6</td>
</tr>
</tbody>
</table>

Source image: Sandoz
Zarxio and Neupogen are Highly Similar Regarding All Molecular attributes

### Analytical Similarity Summary


<table>
<thead>
<tr>
<th>Quality Attribute</th>
<th>Assessment</th>
<th>Quality Attribute</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary structure</td>
<td>Same amino acid sequence</td>
<td>Sequence variants:</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Bioactivity</td>
<td>Highly Similar</td>
<td>His→Gln</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Protein content</td>
<td>Highly Similar</td>
<td>Asp→Glu</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Receptor binding</td>
<td>Highly Similar</td>
<td>Thr→Asp</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Clarity</td>
<td>Highly Similar</td>
<td>Succinimide species</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Sub-visible particles</td>
<td>Highly Similar</td>
<td>Phosphogluconeoylation</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Secondary and tertiary structure</td>
<td>Highly Similar</td>
<td>Acetylated species</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>High molecular weight variants/aggregates</td>
<td>Highly Similar</td>
<td>N-terminal truncated variants</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Oxidized species</td>
<td>Highly Similar</td>
<td>Norleucine species</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Covalent dimers</td>
<td>Highly Similar</td>
<td>Deamidated species</td>
<td>Highly Similar</td>
</tr>
<tr>
<td>Partially reduced species</td>
<td>Highly Similar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fMet1 species</td>
<td>Highly Similar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For product-related species, "highly similar" means same type and levels of the species under evaluation

In addition, the three products have highly similar stability profiles.

Source: FDA presentations for the January 7, 2015 Meeting of the Ologic Advisory Committee
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Protein content as tier 1 quality attribute...

Source: FDA presentations for the January 7, 2015 Meeting of the Ocologic Advisory Committee
Statistical equivalence testing for protein content

Statistical Equivalence Test for Protein Content

Protein content of the three products is statistically equivalent (mean values)

EP2006 vs. US-Neupogen
(-1.87, 0.15)

EP2006 vs. EU-Neupogen
(-2.98, -0.85)

EU-Neupogen vs. US-Neupogen
(0.27, 2.09)

Results indicate that the products have the same strength and also support analytical similarity and the analytical bridge

Source: FDA presentations for the January 7, 2015 Meeting of the Ocologic Advisory Committee
Why statistics…?

Statistics could have a potential to...

- Describe large and complex sets of data in a clear manner (many QAs, different outputs, different criticality)
- Deduce hard facts out of “soft” data
- Develop an objective basis for decisions (define the statistical hypothesis which can support a biosimilar decision – link to the scientific understanding)
- Support the background of a decision in a convincing and traceable manner

How much of this is actually applicable in real life?
Selecting the statistical tool to compare populations

Quality Ranges

<table>
<thead>
<tr>
<th>Raw data originator</th>
<th>Min/Max</th>
<th>3 Stdev</th>
<th>Tolerance Interval (90/99)</th>
<th>Raw data biosimilar</th>
</tr>
</thead>
</table>

Source: adapted from FDA presentations for the January 7, 2015 Meeting of the Oncologic Advisory Committee

Source: adapted from FDA presentations for the January 7, 2015 Meeting of the Oncologic Advisory Committee
Equivalence testing to assess a practical difference in the means

Concluding equivalence by rejecting the null hypothesis $H_0: |\mu_1 - \mu_2| \geq \delta$

The EAC interrelates strongly to sample sizes, allowable difference ($\delta = \mu_1 - \mu_2$), significance level ($\alpha$) and power (1-$\beta$)
The conceptual & theoretical flaws of equivalence testing

All biosimilar batches are within variability of originator
means are different → not equivalent

Some biosimilar batches are outside of the variability of originator
means are the same → equivalent
The practical limitations of equivalence testing

Very low sample sizes & analytical variability

- A biosimilar program may not require more than 5 – 10 large scale batches
- Disproportionate costs – little scientific value – for statistical reasons only

Non-normal distributions

- Actual distributions are rarely normal, normality is often only a coarse approximation
- Special cause variability in manufacturing processes: deviations, changes, raw material variability
The practical limitations of equivalence testing

Undesired quality attributes:
Less is better

More than one reference product population
Final thoughts on statistical tools for comparability / biosimilarity evaluation...

- First be clear about your scientific question, then choose the statistical tool, and be aware of the limitations

- The statistical test parameter needs to be carefully chosen
  - Too tight: a product may be statistically not comparable to itself
  - Too loose: a non-comparable product may fit it

- Statistics should not be a self-contained claim for biosimilarity on the quality level, it always should be just a contributor to the totality of evidence

- Statistical tools may be able to flag those quality attributes which need further evaluation
  - May speed up final evaluation if statistics is set up in the right way
  - However, the incremental knowledge gain is very little compared to a descriptive but critical raw data comparison
Zarxio analytical biosimilarity learnings

Assess analytical similarity considering

• **Extensive data package** of reference product and biosimilar: set of methods, number of batches

• **Quality attribute criticality assessment** of impact on safety & efficacy as well as uncertainty

• Tiered approach:
  • **Select proper tool(s) to assess similarity**, including statistics, min-max, graphical comparison

→ Statistics as one tool, supportive, but not as exclusive tool
→ Align with agency early on analytical similarity approach
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