Analytical Tools for Higher Order Structure Assessment in Comparability and Biosimilarity

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How good are analytical methods?

Worthless copy or something more valuable?

Original Mona Lisa  Mona Lisa Del Prado

How good are analytical methods?

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Ana Gonzalez Mozo
Expert Art Curator

Mona Lisa Del Prado

How good are analytical methods?

Mona Lisa Del Prado is the oldest known replica

Painted alongside Da Vinci in his studio as he painted the original

How good are analytical methods?

The Discovery
Infrared reflectography reveals drawing lines under the paint, invisible to the naked eye. Every adjustment that Leonardo made on his underlying drawing was repeated in the copy, indicating that the two pieces were painted in tandem. The orange dots show where adjustments were made in both paintings. The arrows point to adjustments made to the head by the two painters.
Current state: What analytical data can and can’t tell us

Far UV CD

But they cannot predict safety/efficacy?

FTIR

Reliance on prior knowledge: supportive data

Can rely on manufacturer product/process understanding, control strategies, and a risk-based approach to comparability design

Biosimilar manufacturers will not have access to the knowledge base possessed by the reference product manufacturer and must design and implement their own control strategies

Knowledge gap

Comparability compares pre- and post-change product taking into account prior knowledge.

Comparability is a stand-alone event after approval.

Manufacturers understand product attributes and implement appropriate control strategies.

In many cases supportive data may be enough.
Comparability uses risk-based approaches based on type and extent of process changes

Understanding of product and process allows risk-assessment

- Cell line changes
- Cell culture process changes
- Purification process changes
- Formulation or drug Process changes
- Equipment or raw material changes
- Scale or site changes
- Primary container changes
- Device change

- Clinical safety and efficacy
  - Confirmation of comparable efficacy, safety, and immunogenicity
  - Post change surveillance

- Nonclinical or clinical PK-PD
  - Animal studies (rodent or primates)
  - Human studies

- Biologically comparable
  - Potency and target binding
  - Additional biological activities such as effector functions
  - Other known mechanism of action of the product

- Analytically comparable
  - Physicochemical properties including higher order structure (HOS)
  - Stability profiles
  - Impurities and contaminants
Similarity assessment plan is based on risk ranking of reference product attributes

High Risk Quality Attributes
- Ranges defined by reference product; May be narrowed based on assay variability; Statistical approach should be justified
- Known or probable interactions in product performance may limit the ranges of certain attribute combinations
  - The use of one attribute to compensate for a difference in another may be challenging as the attributes may have other effects

Moderate Risk Quality Attributes
- Ranges defined by reference product ranges or other appropriate scientific justification
- Known or probable interactions that have a higher risk of impacting product performance should be treated as a higher risk attribute

Low Risk Quality Attributes
- May not have target ranges
- Known or probable interactions that have a higher risk of impacting product performance should be treated as a higher risk attribute
- Even though these attributes may not need to match the reference product, the closer the overall match the greater the confidence that unmeasured attributes are not different

1. Slide concept from Kozlowski, S (CDER) presentation at CASSS WCBP, Washington DC, January 31, 2013
Similarity uses tiered approach for risk ranking and statistical analysis of quality attributes

Relative Binding to Soluble TNFα

High Risk QA
Tier 1

Moderate Risk QA
Tier 2

Low Risk QA
Tier 3

Equivalence
(± 1.5 σ)

Quality Range

Visual Inspection

Analytics can establish high similarity but even current techniques leave ‘residual uncertainty’

**Clinical safety and efficacy**
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Clinical studies in a sensitive patient population can address residual uncertainty

**ICH Q5E**

Although the pre- and post change product appear highly similar, the analytical procedures used are not sufficient to discern relevant differences that can impact the safety and efficacy of the product. The manufacturer should consider employing additional testing (e.g., further characterisation) or nonclinical and/or clinical studies to reach a definitive conclusion;

### The Value of Clinical Data

**Case Study: Epoetin alfa process change**

<table>
<thead>
<tr>
<th>Structural and Functional Attribute</th>
<th>Comparability Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Structure (peptide mapping and sequencing)</td>
<td>Comparable AA seq and S-S linkage</td>
</tr>
<tr>
<td>Higher Order Structure (CD, FT-IR)</td>
<td>Comparable HOS</td>
</tr>
<tr>
<td>Carbohydrate Profile (sialo and asialo N-glycan mapping, peptide map, isoform distribution)</td>
<td>Similar qualitative profile – within specs</td>
</tr>
<tr>
<td></td>
<td>• Slightly higher acidic isoforms – but within spec</td>
</tr>
<tr>
<td></td>
<td>• Slightly higher N-glycans with N-acetyllactosamine extensions</td>
</tr>
<tr>
<td></td>
<td>• Reduced N-glyco neuraminic acid</td>
</tr>
<tr>
<td></td>
<td>• Higher bisialylated O-glycans</td>
</tr>
<tr>
<td></td>
<td>• Comparable N-glycan sialylation</td>
</tr>
<tr>
<td>Purity and Impurities</td>
<td>Comparable and within spec</td>
</tr>
<tr>
<td><em>In vitro</em> bioassays</td>
<td>Comparable and within spec</td>
</tr>
<tr>
<td>Exhypoxic polycythemic mouse bioassay</td>
<td>Comparable potency</td>
</tr>
</tbody>
</table>

### Clinical Bridging Study

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<th>Comparability Finding</th>
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<tr>
<td>30 patient, single-dose crossover study (16-20 week titration)</td>
<td>90% CI, 94.8%-104.3% - comparable PK</td>
</tr>
</tbody>
</table>

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The value of clinical data

Case Study: Epoetin alfa process change

Clinical Evaluation: Hemoglobin Maintenance

- 462 patient hemoglobin maintenance study
- Equivalence in hemoglobin levels demonstrated (Delta of -0.07 g/dL)
- Log dose ratio over the last 8 weeks of the study demonstrated the product from the new process to be more potent than pre-change product

Analytics were not able to accurately predict this clinical observation

The value of clinical data
Case Study: Epoetin alfa process change

In vitro potency assays can help understand differences

- Mouse hemoglobin accumulation model to assess in vitro effects
- Pairwise comparison of $S^+, L^+$ samples and $S^-, L^-$ demonstrated statistically significant differences in hemoglobin levels due to additive impact of sialylation and lactosamine content
- Lactosamine content can offset effects of increased sialylation

Orthogonal methods are needed to support analytical tools

Could better analytics have predicted this?

FDA Definition of Fingerprint-like similarity:

“integrated, multi-parameter approaches that are extremely sensitive in identifying analytical differences.”

More sensitive techniques allow detection of differences not previously observed.

PROFILE NMR

Far UV CD

80% IgG1/20% IgG2

90% IgG1
10% IgG2

Mats Wikström, CASSS HOS 2018. “NMR represents a superior method for the assessment of higher order structure (HOS) of biopharmaceuticals.”
Advancements in technology will allow lower detection limits and additional structural insights. ‘Fingerprint-Like’ HOS may reduce residual uncertainty, but some clinical data will likely be needed to inform safety and efficacy.

Deeper understanding of process and products will further reduce residual uncertainty.
Summary

• Analytical techniques for characterizing higher order structure have evolved and can assist in reducing residual uncertainty

• Analytical data are *not* a substitute for clinical data

• Fingerprint-like similarity, when a reality, may better inform the type and degree of clinical data needed

• Greater understanding of product, process, and control strategy reduces residual uncertainty
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