Identification of multiple Ser to Asn sequence variation sites in an intended copy product of LUCENTIS® by mass spectrometry

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Analytical Development and Characterization NBEs
Mass spectrometry Lab

Intact/Native → Ab fragments → Peptide & N/O-glycan mapping

“Biosimilar” or “intended copy product”

Table 1 Lexicon of terms and definitions used to describe biosimilars

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biologic</td>
<td>An approved product composed of proteins, nucleic acids, or combinations of these, or living entities such as cells and tissues, which is isolated from natural sources (including humans, animals, and microorganisms) and produced by biotechnology methods and other cutting-edge technologies [34]</td>
</tr>
<tr>
<td>Biosimilar</td>
<td>A biological product developed such that there are “no clinically meaningful differences between the biological product and the reference [originator] product in terms of safety, purity, and potency” and “demonstrates similarity to the [originator] in terms of quality characteristics, biological activity, safety, and efficacy based on a comprehensive comparability exercise” [1, 2, 6]</td>
</tr>
<tr>
<td>Generic drug</td>
<td>Small (single molecule) or low molecular weight chemically synthesized compounds consisting of a simple, well defined structure that is independent of the manufacturing process and easy to characterize completely [35]</td>
</tr>
<tr>
<td>Extrapolation</td>
<td>A core concept for approval of biosimilars, extrapolation allows for the approval of a biosimilar for use in an indication held by the originator not directly studied in clinical trials of the biosimilar. It is based on sufficient scientific justification and the totality of the evidence [1, 2, 29]</td>
</tr>
<tr>
<td>Interchangeable biosimilar</td>
<td>The product is approved as a biosimilar; the biosimilar can be expected to produce the same clinical effects as the originator in any given patient, and the risk in terms of safety or diminished efficacy of alternating or switching between use of the product and its originator is not greater than the risk of using the originator without such alternation or switch [23]</td>
</tr>
<tr>
<td>Intended copy</td>
<td>Copies of an originator biologic that have not been evaluated using the stringent, specifically defined criteria of the EMA, FDA, or WHO guidelines for biosimilars [26]</td>
</tr>
</tbody>
</table>

NOTE: This designation is only granted by the FDA; the EMA and WHO do not provide any regulatory statement on whether or not a biosimilar is considered interchangeable [20, 21, 29]
Published work

Disclosure of potential conflicts of interest
All authors were employed by Novartis Pharma AG at the time they completed work described in this publication. Novartis Pharma AG funded all of this research, and some of the authors own Novartis stocks. LUCENTIS was developed by Genentech Inc. and Novartis. Genentech Inc. has the commercial rights to LUCENTIS in the United States. Novartis has exclusive rights in the rest of the world. LUCENTIS is a registered trademark of Genentech Inc.
Background information

- Intas has been producing a copy of LUCENTIS® since 2015
- Samples of two batches (3 additional batches at a later stage) of the Intas material have been analyzed in BPD labs in Basel.
- It was the intention to evaluate the similarity and potential differences between LUCENTIS® and RAZUMAB
- All the usual physico chemical testing and bioassay were performed
  - Potency
  - SEC
  - CE-SDS
  - CEX
  - CZE
Background information

• The formulation composition of RAZUMAB is identical with that of LUCENTIS®.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LUCENTIS®</th>
<th>Razumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active [mg/mL]</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Trehalose-Dihydrate [mg/mL]</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>L-His [mg/mL]</td>
<td>1.55</td>
<td>1.55</td>
</tr>
<tr>
<td>Polysorbate 20 [mg/mL]</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>pH</td>
<td>5.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>
Results of the physico-chemical testing

A. SEC

B. CE-SDS

C. CEX

D. CZE

## Results of the physico-chemical testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Quality attributes</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size Exclusion Chromatography (SEC)</td>
<td>Size variants</td>
<td>No differences</td>
</tr>
<tr>
<td>Ion Exchange Chromatography (IEC - CEX)</td>
<td>Charge variants</td>
<td>Slight differences</td>
</tr>
<tr>
<td>CZE</td>
<td>Charge variants</td>
<td>Slight differences and shoulder</td>
</tr>
<tr>
<td>SDS-Capillary Electrophoresis (CE-SDS)</td>
<td>Size variants under denaturing conditions</td>
<td>No differences</td>
</tr>
<tr>
<td>Activity/Inhibition of proliferation</td>
<td>Potency Assay</td>
<td>No differences</td>
</tr>
</tbody>
</table>
Results of the physico-chemical testing

CEX analysis

<table>
<thead>
<tr>
<th>Batch</th>
<th>Acidic variants [%]</th>
<th>Main [%]</th>
<th>Basic variants [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAZUMAB #1</td>
<td>0.9</td>
<td>98.0</td>
<td>1.1</td>
</tr>
<tr>
<td>RAZUMAB #2</td>
<td>1.1</td>
<td>97.9</td>
<td>1.0</td>
</tr>
<tr>
<td>LUCENTIS®</td>
<td>0.7</td>
<td>97.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

No conclusive data. It was decided to perform MS analytics on LUCENTIS® and RAZUMAB:
- Intact analysis
- Analysis of released LC and HC after reduction // carbamidomethylation
- Peptide mapping
Overlaid UV Chromatograms of LUCENTIS® Intact
Main signal at 16 min of LUCENTIS® Intact
Main signal at 16 min (LUCENTIS® Intact)

Overlaid UV Chromatograms of released LC and HC after reduction // carbamidomethylation
**LC: Time resolved deconvolution around 22 min**

**HC: Time resolved deconvolution from 25 to 29 min**

Heavy chain (HC) species. HC, oxidized HC (HCox) and N-terminal pyroglutamate formation (HC(pE)) are annotated. *Major sample preparation artifact is overalkylation with iodoacetamide as shown by the addition of +57 Da.

Overlaid UV Chromatograms - Reduced Pepmap

- LUCENTIS®
- RAZUMAB # 2
- RAZUMAB # 1
Exploration of pepmap data

Exploration of pepmap data

RAZUMAB # 1    RAZUMAB #2    LUCENTIS®
Unbiased analysis of MS1 signals

Supplementary Figure S2

Quantification of each signal (charge state) observed
Quantification of each signal (charge state) observed

RAZUMAB # 1
Quantification of each signal (charge state) observed

More intense signal in LUCENTIS®

More intense signal in RAZUMAB

Two products have different Polysorbate fingerprints
Evidence for differences between samples

Evidence for differences between samples L-L10 DSTYSLSTLTLSK

Evidence for differences between samples

Identification of misincorporation in light chain peptides of RAZUMAB

<table>
<thead>
<tr>
<th>#</th>
<th>Range</th>
<th>Sequence</th>
<th>Ser→Asn [position]</th>
<th>Misincorporation (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>batch 1</td>
<td>batch 2</td>
</tr>
<tr>
<td>L-L1</td>
<td>1–39</td>
<td>DIQLTQSPSSLASVGDRVTITC</td>
<td>30</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>L-L1</td>
<td>1–39</td>
<td>DIQLTQSPSSLASVGDRVTITC</td>
<td>14</td>
<td>1.5*</td>
<td>2.1*</td>
</tr>
<tr>
<td>L-L4</td>
<td>46–103</td>
<td>VLYIFTSSLHSGVSPRFSGSGS</td>
<td>76 or 77</td>
<td>5.0</td>
<td>6.6</td>
</tr>
<tr>
<td>L-L6</td>
<td>108–126</td>
<td>RTVAAPSVFIFPPSDEQLK</td>
<td></td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>L-L7</td>
<td>127–145</td>
<td>SGTASWCCLNNFYPREAK</td>
<td></td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>L-L9</td>
<td>150–169</td>
<td>VDNALSQNSQESVTEQDSSK</td>
<td>156, 159, 162, and 168</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>L-L10</td>
<td>170–183</td>
<td>DSTYSSLSTTLTSK</td>
<td>176</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>L-L10</td>
<td>170–183</td>
<td>DSTYSSLSTTLTSK</td>
<td>177</td>
<td>2.9</td>
<td>4.0</td>
</tr>
<tr>
<td>L-L13</td>
<td>191–207</td>
<td>VYACEVTHQGLSSPVTK</td>
<td>202 or 203</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*potential overestimation due to the presence of substantial sodium adduct.

Confirmation with synthetic peptides

RAZUMAB #1

LUCENTIS® spiked with 5% S176→N176 and 5% S177→N177

LUCENTIS®

* Sodium adduct

Retention times and MS/MS spectra were confirmed
Estimation of the misincorporation in RAZUMAB LC

UV-based quantification: LC, after red/carboxy

MS-based quantification: LC, after red/carboxy

Misincorporation estimated in the range of 6-9% in the light chain of intended copy across batches analyzed

Summary

• The typical physico chemical analyses do not show a difference > 1% between the originator LUCENTIS® and intended copy RAZUMAB, although different peaks were observed.

• Only the MS data revealed a difference between RAZUMAB and LUCENTIS®, only in the LC of RAZUMAB.

• It is mainly due to the misincorporation of Asn instead of Ser (G/U base mismatching).

Hypotheses:

• Different codon optimization or expression systems for LC and HC or serine concentration is critical for LC expression.

• LC variant may be revealed by CZE main peak shoulder (pI (Ser) = 5.68, pI(Asn) = 5.41).

• Difference of 0.2-0.4% of acidic variants in CEX may be due to deamidated version of L-L 7 (SGTASVVCLL(N→D)NFYPREAK).
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