PLANNING FOR SUCCESS: A CMC STRATEGY FOR BIOSIMILARS

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10th Biosimilars & Follow-On Biologics Congregation
9th May 2017
Overview

► Covance history with Biosimilars
► Importance of CMC
► What is required for successful Biosimilar development?
► Building a CMC Biosimilar strategy
► How to de-risk a Biosimilar drug development program (case study)
### Covance History with Biosimilars

#### CMC: Analytical characterisation
- **Continuous testing multiple batches**
- **Stability**
- **Batch release testing**

<table>
<thead>
<tr>
<th>CMC</th>
<th>Preclinical</th>
<th>Clinical</th>
<th>BioA</th>
<th>CCLS</th>
<th>Regulatory</th>
<th>CMA</th>
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<tbody>
<tr>
<td></td>
<td>Animal studies</td>
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<td>► Toxicology</td>
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<td>► PK/PD</td>
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<td>Structural and functional testing</td>
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<td>Phase I / Phase III</td>
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<td>► PK/PD</td>
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<td>► Immunogenicity</td>
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<td>► Efficacy (Similarity)</td>
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<tr>
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<td>BioA: PK and Immunogenicity (ADA, NAb)</td>
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<td>Biomarkers</td>
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<td>Input into clinical endpoints Policy re-imbursement advice</td>
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<td>Pharmacovigilance Patient reported outcomes</td>
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## Importance of CMC

<table>
<thead>
<tr>
<th>Pre-clinical phase</th>
<th>Clinical phase</th>
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</thead>
<tbody>
<tr>
<td>Analytic (physiochemical, structural and bioanalytical)</td>
<td>In vivo functional PK/PD (animal) Toxicology</td>
</tr>
<tr>
<td>In vitro functional (binding)</td>
<td>PK Efficacy Safety</td>
</tr>
</tbody>
</table>

**Amount of CMC data required**

**Biosimilar development program timeline**

PK = Pharmacokinetics  
PD = Pharmacodynamics

**Science driven; end in mind;**  
**Extensive innovator characterisation to minimize downstream risk**
Overview

► Our history with Biosimilars
► Importance of CMC
► What is required for successful Biosimilar development?
► Building a CMC Biosimilar strategy
► How to de-risk a Biosimilar drug development program (case study)
Know the Target Product Profile (TPP)

- Indications and Usage
- Dosage and Administration
- Dosage Forms and Strengths
- Contraindications
- Warnings and Precautions
- Adverse Reactions
- Drug Interactions
- Use in Specific Populations
- Drug Abuse and Dependence
- Overdosage
- Description
- Clinical Pharmacology
- Nonclinical Toxicology
- Clinical Studies
- References
- How supplied, Storage and Handling
- Patient Counselling Information

Safety, efficacy and quality of Innovator and Biosimilar must have no meaningful clinical difference
Understand Mechanism of Action (MOA)

Understanding MOA of product, and the clinical relevance of any observed structural differences, enables the design of an initial control strategy to monitor and control residual risk.

- Confirmation of **identical** amino acid sequence
- Assessment of post-translational modifications including glycosylation (high mannose, afucosylation)
- Product degradation (denature/ aggregation/ oxidation or deamidation)
- Product related impurities, sequence variants
- Process related impurities eg Host Cell Protein
Identify and Control Critical Quality Attributes (CQA)

Assess and control risk

TPP

MOA

CQA

Analytical and Manufacturing Controls

BIOSIMILARS DO NOT GET “MORE SIMILAR” DURING THE DRUG DEVELOPMENT PROCESS
Overview

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Covance Biosimilar Strategy

Assess Target Product Profile √
Determine Mechanism of Action √
Define Critical Quality Attributes √
Obtain Innovator Product and develop methods to support CQA √

Pre-clinical phase | Clinical phase

Amount of CMC data required

Continual Biosimilarity Assessment
Covance has developed Analytical Master Files

- Scientific rationale using TPP, MOA to justify CQA selection
- Portfolio of Developed Methods
- Extensive early characterization of Innovator

Risk-minimized, accelerated development approach
Overview

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Herceptin® Mechanism of Action
Her2 Binding and ADCC via FcγRIII

Herceptin® binds to HER2 receptor on target cell surface, and inhibits cell proliferation by blocking receptor dimerisation and hence downstream signaling

NK cell binding to Herceptin® Fc via FcγRIII triggers degranulation and cell death

► Lack of Fucose on Human IgG1 N-Linked oligosaccharide improves Binding to Human FcγRIII and Antibody-dependent Cellular Cytotoxicity (ADCC)*

► Quantification of % core glycan afucosylation (and % glycan occupancy and high mannose levels)

*Shields, RL et al, J Biol Chem 2002; 277: 26733-40

Herceptin® is a registered trademark of Genentech Inc

Covance Image
Case Study: A Biosimilar Solution

CLIENT

► A biotechnology company
► Developed a Herceptin® Biosimilar
► Ready to progress into clinical phase
► Structural characterisation and pre-clinical assessment complete, with no significant difference from the innovator identified
► Unexpected observation; limited in vitro ADCC activity

THE CHALLENGES

► Client Biosimilar did not demonstrate the expected biological activity
► Client unable to progress to clinical studies
► 18 month addition to Biosimilar development program

RISK BASED CMC STRATEGY

► Early in Biosimilar development phase
► Link structure to function
► Extensive structural characterisation
► CQA

Herceptin® is a registered trademark of Genentech Inc

Image approved for use at Covance

# Understanding Critical Quality Attributes

<table>
<thead>
<tr>
<th>Attribute</th>
<th>CQA</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Intact mass</td>
<td>LC-MS</td>
</tr>
<tr>
<td>Identity</td>
<td>Amino Acid Sequence</td>
<td>Peptide map by LC-MS</td>
</tr>
<tr>
<td>Identity</td>
<td>Glycosylation</td>
<td>LC-MS</td>
</tr>
<tr>
<td>2 and 3 structure</td>
<td>Di-sulphide bonding</td>
<td>LC-MS</td>
</tr>
<tr>
<td>Purity</td>
<td>Monomer, HMWs</td>
<td>SE-HPLC</td>
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<tr>
<td>Purity</td>
<td>Deamidation</td>
<td>Peptide map</td>
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<tr>
<td>Purity</td>
<td>Methionine Oxidation</td>
<td>Peptide map</td>
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<tr>
<td>Purity</td>
<td>Charge Distribution</td>
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<tr>
<td>Potency</td>
<td>Concentration</td>
<td>UV-A280</td>
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<tr>
<td>Potency</td>
<td>Fc Effector function</td>
<td>Biacore</td>
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<tr>
<td>Potency</td>
<td>Fab binding</td>
<td>Cell based Bioassay</td>
</tr>
<tr>
<td>Potency</td>
<td>ADCC</td>
<td>Cell based Bioassay</td>
</tr>
</tbody>
</table>

Data: Covance
Proliferation Inhibition Assay

- BT474 - Her2 receptor expressing breast cancer cell line
- BT474 assay measures Fab binding to Her2
- Herceptin® serially diluted and added to BT474 cells
- Herceptin® Binds Her2 receptor and inhibits proliferation of cells
- Measured as a function of adenosine triphosphate (ATP) concentration (via luciferase luminescence)
- Relative Potency (Biological Activity) is calculated against a known standard

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Relative Potency Assessment by Proliferation Inhibition

Graph: Shows that the biological activity (binding to Her2) was the same for each product; within 70 – 130% criteria.

Table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Relative Potency, % (versus EU Innovator control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US Innovator</td>
<td>100</td>
</tr>
<tr>
<td>RG Trastuzumab</td>
<td>90</td>
</tr>
</tbody>
</table>

Data: Covance
Antibody Dependent Cell Cytotoxicity (ADCC) Assessment

- Herceptin® serially diluted and added to SKBR3 cells (which express Her2) and NK cells (enriched from PBMCs)

- Herceptin® binds Her2 receptor on SKBR3 cells (Fab) and FcyRIII on NK cells (Fc)

- NK cells release cytokines to lyse SKBR3 cells

- Relative Potency (Biological Activity) is calculated against a known standard

- Measure release of Lactate Dehydrogenase (LDH) as function of cell lysis

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Relative Potency Assessment by ADCC

- RG Trastuzumab significantly less potent in terms of ADCC function
- Consistent with the MS based structural characterisation and differences in afucosylation level

Herceptin® EU and US

Research grade Trastuzumab with less core glycan afucosylation

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Typical Fc-glycans of humanized IgG biopharmaceuticals

- Absence of the N-glycan core-fucose (afucosylation) can result in increased binding affinity of antibodies to FcγRIII and enhance ADCC
Intact Mass Herceptin®
Mass Spectra

Protein ion current is shared between multiple charge states, \([M+nH]^n+\) (typical of electrospray ionisation)

Glycan profile is defined at each charge state

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All data: Covance
Intact Mass Herceptin®
Deconvoluted MS data (MaxEnt1)

- Deconvolution converts MS data to a zero charge mass scale (Da)
- Permits characterisation and comparison of glycan profiles (pairing of glycosylated heavy chains)
- Mass accuracy in 150 kDa range typically within ±60 ppm

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Relative Potency % (versus EU Innovator)</th>
<th>N-Glycan Core Afucosylation % (excl. high mannose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU Herceptin®</td>
<td>N/A</td>
<td>14.7 (10.4)</td>
</tr>
<tr>
<td>US Herceptin®</td>
<td>94</td>
<td>10.2 (8.15)</td>
</tr>
<tr>
<td>RG Trastuzumab</td>
<td>45</td>
<td>4.35 (2.55)</td>
</tr>
</tbody>
</table>

Herceptin® is a registered trademark of Genentech Inc
Apply early and robust CMC testing to maximise success of a biosimilar development program.

A better understanding of the product leads to a higher predictability of the outcome.
About Us

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