Biosimilar Monoclonal Antibodies: Registration Requirements

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Introduction

• When the general Biosimilars Guideline was published in March 2012, it excluded vaccines and monoclonal antibody products.

• The reason being that these were very complex compared to well-characterized recombinant DNA-derived therapeutic proteins.
High complexity of monoclonal antibodies
Each monoclonal antibody is unique

**atorvastatin**
Molecular weight
= 558 Daltons
0 amino acids

**Interferon-alpha**
Molecular weight
= 19,625 Daltons
~165 amino acids

**Antibody (IgG)**
Molecular weight
= 150,000 Daltons
~1,300 amino acids

Source: [http://www.path.cam.ac.uk/~mrc7/mikeimages.html](http://www.path.cam.ac.uk/~mrc7/mikeimages.html)
Antibody Structure

- **Fab fragment**
- **Variable domain**
- **Constant domain**
- **Fc fragment**
Structural Complexity

Functional Complexity

- **CDC**: Complement binds to Fc → cell lysis
- **ADCC**: Fcg Receptor binds to Fc → cell lysis
- **Apoptosis**
- **Phagocytosis**
Manufacturing Complexity of MCB

Recombinant DNA Technology vs Hybridoma Technology

1. **Donor DNA**
   - **Digestion:** Cleaved with restriction enzyme.
   - **Overhangs:** Formed.
2. **Vector**
   - **Mixing:** With donor DNA.
   - **Ligation:** Using DNA ligase.
3. **Bacterial Chromosome**
   - **Introduction:** of DNA molecules.
   - **Replication:** and cell division.
4. **Recombinant DNA Molecules**
   - **Isolation:** From bacterial cells.
5. **Antigen**
   - **Isolation:** from spleen cells.
6. **Hybridization**
   - **Selection:** with hypoxanthine/aminopterin/thymidine (HAT medium).
7. **Polyclonal Antiserum**
   - **Clones:** Monoclonal antibodies.

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Evolution of mAbs

<table>
<thead>
<tr>
<th>Structure</th>
<th>% Human</th>
<th>Example</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0</td>
<td>Tositumomab, Ibritumomab</td>
<td>Radio-conjugates</td>
</tr>
<tr>
<td>Chimeric</td>
<td>65</td>
<td>Cetuximab, Rituximab</td>
<td></td>
</tr>
<tr>
<td>Humanized</td>
<td>95</td>
<td>Trastuzumab</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>100</td>
<td>Panitumumab</td>
<td>Transgenic mice</td>
</tr>
</tbody>
</table>
Data Requirements for Biosimilars- including for mAbs

CTD Module  | Originator  | Biosimilar
--- | --- | ---
3 Quality |  | |
4 Non-Clinical |  | Cross reference
5 Clinical |  | Cross reference

Cross reference – class specific Safety and Efficacy

Integrated Comparability Exercise – product specific Quality, Safety and Efficacy
Comparability Requirement

• Stepwise head-to-head comparison at the levels of quality, safety and efficacy to demonstrate that the biosimilar and the reference medicinal product have similar profiles in terms of physico-chemical properties (quality), safety and efficacy.

• Depending on the similarity profile, the extent of the nonclinical and clinical testing may be reduced compared to a stand-alone development.

• Any difference in quality attributes requires a satisfactory justification of the potential implications with regard to the safety and efficacy of the product.
General Biosimilars Guideline

This guideline outlines the quality, non-clinical and clinical requirements for Biosimilar medicines.

- The quality section addresses the physico-chemical, structural and functional requirements.
- The non-clinical section addresses the pharmacotoxicological assessments.
- The clinical section addresses the requirements for pharmacokinetic, pharmacodynamic, safety and efficacy studies as well as the pharmacotoxicological assessments with special emphasis on studying the immunogenicity of the Biosimilar medicines.
- The section on pharmacovigilance addresses the in-use safety of the medicine as well as the risk management plan.
Quality

• The annexure takes into account that different mAb products may share some properties, but may differ in other aspects such as mechanism of action and antigenicity.
  – MAbs may thus differ in terms of antibody-antigen binding regions and its secondary biological effects

• Molecular characterization should be as extensive as possible and be carried out in a head-to-head manner with the reference product.

• Primary, secondary and tertiary structure should be demonstrated as well as the composition and structure of post-translational modifications and additions – e.g. glycosylation.
Quality (cont.)

• Differences in critical product quality attributes (i.e. those that are known to have potential impact on clinical activity) will add to the clinical testing required for the Biosimilar.
  – For example, if differences are found in glycosylation patterns that alter the biodistribution of the product and thereby change the dosing scheme, dose-finding studies for the product would likely be required.
  – Differences of unknown clinical relevance, particularly regarding safety, may have to be addressed in additional pre- or post-marketing studies.
Quality (cont.)

• Other differences may be acceptable, and would not trigger the need for extra clinical evaluation.
  – For example, a biosimilar product with lower levels of protein aggregates will have a better safety profile.

• Due to the unavailability of the API of the reference, the biosimilar manufacturer will have to purchase the commercially available reference medicine for the comparison.
  – It should be verified that IPIs do not interfere with analytical methods and thereby impact the test results.
  – Purchase product from different batches to allow for batch-to-batch variability.

• If the reference API needs to be purified from a formulated reference medicine, it must be shown that the isolation method does not affect product integrity.
  – Comparative deformulation.
Quality:

*In vitro* Biostudies (Functional Assays)

- Data from at least three independent batches of the biosimilar mAb product used in the *in vitro* studies one of which must be a production batch should be provided. The studies should specifically include:
  - Binding of antibody to target antigen or antigens
  - Binding to isoforms of the relevant Fc gamma receptors
  - Fab-associated functions such as receptor activation or blockade
  - Fc-associated functions such as antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and complement activation.

- All such studies should be comparative.
Physico-chemical & Biological Characterisation

PHYSICOCHEMICAL CHARACTERISTICS

VARIABLE REGION
- Deamidation
- Oxidation
- N-term Pyro-Glu
- Glycosylation
- Glycation
...

CONSTANT REGION
- Deamidation
- Oxidation
- Acetylation
- Glycation
- Glycosylation (fucosylation, sialylation, galactosylation, mannosylation...)
- C-term Lys
- Di-sulfide bond shuffling/cleavage
- Fragmentation/clipping
...

BIOLOGICAL CHARACTERISTICS

BINDING
- Affinity
- Avidity
- Immunoreactivity/crossreactivity
- Unintentional reactivity
...

EFFECTOR FUNCTION
- Complement interaction
- EcRn, EcγR interaction
- Mannan binding ligand interaction
- Mannose receptor interaction
...

OTHER BIOLOGICAL PROPERTIES
- PK properties
- Epitope/Immunogenicity
- Modulatory region (Tregitope...)
...
Nonclinical

*In vivo* studies

• *When in vitro* studies cannot fully demonstrate biosimilarity, then *in vivo* studies must be performed.

• Comparative in nature – designed to detect differences.

• Must be conducted in appropriate species.
  – Pharmacodynamic study and at least one repeat dose study (Latter not recommended for non-human primates).

• *Since the biosimilar manufacturer will use a different production process, qualitative differences in impurities and product-related substances may be detected.*
  – These may have clinically important effects on the immunogenic potential of the biosimilars.

• **Studies on safety pharmacology and reproduction toxicology are not required for non-clinical testing of biosimilar mAbs.**
Clinical Studies

General:

• Comparative PK and PD studies are required.
• In certain cases comparative PK/PD studies may be sufficient but usually comparative efficacy trials are required.
• Pre-registration safety data should be obtained.
• One year follow-up immunogenicity data usually required pre-registration for long term treatment
Immunogenicity Assessment

• Immunogenicity data should be generated from head-to-head clinical trials using state-of-the-art assays with appropriate specificity and sensitivity.

• Animal studies are not predictive for immunogenicity in humans.
  – Needs to be assessed to ensure drug exposure and validity of study.
  – Explain irregular PK and PD results

• Immunogenicity data should be interpreted in conjunction with PK/PD, safety and efficacy results.
Pharmacovigilance/ Risk Management

- A Risk management Plan and Pharmacovigilance system must be in place.
- Any safety monitoring imposed on the reference product or product class should be considered in the RMP
Indications

• Each claimed indication should be justified or demonstrated separately.

• Extrapolation is possible, but depends on clinical experience, available literature data, same mechanism of action or receptor(s) involved in all indications.
Take Home Points on Biosimilar mAbs

• Monoclonal antibodies are complex molecules
  – High level of microheterogeneity, there will always be differences
  – The mode of action is complex and may involve contributions from multiple mechanisms.

• **The challenge**: To demonstrate that differences between the biosimilar and the reference do not have a significant impact on clinical efficacy and/or safety.
  – Even small differences may have significant effects.
  – Need to combine physico-chemical results with functional assays (e.g., antigen-antibody binding assays and cell based assays) and the qualification in preclinical and clinical studies.