Amgen has nearly four decades of specialization in biologics.1 Now, Amgen is leveraging this expertise to develop biosimilar therapies. Our current biosimilar and any future biosimilar(s) will be produced in the same state-of-the-art facilities that manufacture our innovator biologics.

Amgen applies the same level of robustness and rigour to the development of both innovator and biosimilar medicines.
INTRODUCTION

What are biologic medicines?
Biologics are produced through the metabolic activity of living cells and are often made using a process called “biotechnology.” They can include anything from blood components and cytokines to therapeutic proteins and monoclonal antibodies. Biologics are generally larger and more complex than chemically synthesized drugs.²,³

What are biosimilar medicines?
Biosimilars are medicines that are demonstrated to be highly similar to a biologic that has already been authorized for sale (also known as a reference biologic).²

BIOLOGIC/BIOSIMILAR MANUFACTURING IS COMPLEX

Biologic drugs, including biosimilars, are derived from the metabolic activity of living organisms and tend to be significantly more variable and structurally complex than chemically synthesized drugs.³

Biologics are up to 1 000 times the size of small-molecule drugs and can be far more complex, structurally.³–⁵

Monoclonal antibody (≈150 000 Da)
Small-molecule drug (≈180 Da)
Impact of changes in manufacturing processes

Unlike chemically synthesized drugs, biologic drugs are derived through the metabolic activity of living organisms and are variable and structurally complex. They tend to be labile and sensitive to changes in manufacturing processes. When changes are made to the manufacturing process, whether during development or commercialization, the manufacturer generally evaluates the relevant quality attributes of the product to demonstrate that modifications did not occur that would adversely impact the safety and efficacy of the drug product.

Manufacturing process considerations

A well-defined manufacturing process with its associated process controls ensures that an acceptable product is produced on a consistent basis.

Biologic manufacturers must establish methods to determine the impact of any process differences. These methods vary depending on:

- the product and its specific process;
- the extent of the manufacturer’s knowledge and experience with this process; and
- the development data generated.

Biosimilar manufacturers must provide Health Canada with information comparing the biosimilar to its reference biologic. Similarity is demonstrated using a step-wise approach beginning with structural and functional studies and continuing with human clinical studies. Because the purpose of these studies is to demonstrate similarity, the type of data required to support biosimilar authorization differs from that required for a stand-alone biologic drug. Health Canada evaluates all the information provided in order to confirm that the biosimilar and the reference biologic drugs are similar and that, in terms of safety and efficacy, there are no clinically meaningful differences between them.
BIOSIMILAR DEVELOPMENT: STEP BY STEP

Process customization\textsuperscript{3,7–8}

Pharmaceutical manufacturing information is proprietary. Therefore, a biosimilar manufacturer must develop an entirely new customized process.

The process begins by characterizing the reference biologic to quantify characteristics such as physicochemical properties, biological activity, immunochemical properties, purity, and impurities. A custom cell line is then created, and procedures are developed for all manufacturing stages, from cell cultivation and protein production through purification to formulation and packaging. Checkpoints are established at critical junctures during the manufacturing process to verify the similarity of critical quality attributes (CQAs) with respect to the reference product.

CQAs are physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality.\textsuperscript{8}
The quality target product profile forms the basis for the development of different pharmaceutical products. Characteristic considerations could include:

- intended use in clinical setting, route of administration, dosage form, and delivery systems;
- dosage strength(s);
- container closure system;
- release or delivery and attributes that affect pharmacokinetic characteristics; and
- drug product quality criteria (sterility, purity, stability, and drug release).

### Physicochemical properties
- Composition
- Primary structure
- Higher-order structure

### Biological activity

### Immunochemical properties
- Binding assays

### Purity, impurities, and contaminants
Chromatography

Recombinant cloning and expression technologies allow monoclonal antibody production in large-scale bioreactors.

**Expression vector**
The therapeutic protein’s gene sequence is cloned into mammalian expression vectors.

**Expression cell line**
The plasmid DNA carrying the gene is then delivered into the cell by transfection.

Chinese hamster ovary (CHO) cells are used for antibody-drug production.

Protein of interest is expressed. Cells are screened for growth, productivity, and clonal selection.

**Clonal cell selection**
The cell line that produces the protein of interest, with appropriate quality attributes, is selected and then expanded to establish a master cell bank.

**Expansion**
Each clonal cell line must be individually optimized for its suitability for large-scale manufacturing.

**Master cell bank/working cell bank**
The cells from the master cell bank are cultured and scaled up in large-scale bioreactors.

**2 Cell line development**
Recombinant cloning and expression technologies allow monoclonal antibody production in large-scale bioreactors.

**Expression vector**
The therapeutic protein’s gene sequence is cloned into mammalian expression vectors.

**Expression cell line**
The plasmid DNA carrying the gene is then delivered into the cell by transfection.

Chinese hamster ovary (CHO) cells are used for antibody-drug production.

Protein of interest is expressed. Cells are screened for growth, productivity, and clonal selection.

Clonal cell lines are unique to each manufacturing process or manufacturer; therefore, no two biologics will be identical.

**3 Amino acid sequence**
The biosimilar developer lacks access to any proprietary information about the reference product.

**Investigation of public sources of information to obtain basic aspects of the reference product:**
- amino acid sequence
- type of product (IgG antibody, fusion protein, enzyme, etc.)
- mechanism of action
- dosage
- formulation

A biosimilar is, by definition, related to the reference product by a common primary amino acid sequence. It is recommended that the biosimilar developer confirm the amino acid sequence of the reference product by direct analysis to circumvent any potential misrepresentations in the public information.

**4 Protein recovery/purification process**

**5 Formulation/fill-finish**

Stability studies should be conducted on the biosimilar and reference product to compare the stability of both using the same storage conditions and analytical methods.

**6**

**RECOMBINANT PROTEIN PRODUCTION PROCESS**

From amino acid sequence to biosimilar

9–10
Recombinant cloning and expression technologies allow monoclonal antibody production in large-scale bioreactors.

**Expression vector**
The therapeutic protein's gene sequence is cloned into mammalian expression vectors.

**Expression cell line**
The plasmid DNA carrying the gene is then delivered into the cell by transfection.

**Chinese hamster ovary (CHO) cells** are used for antibody-drug production.

**Protein of interest** is expressed. Cells are screened for growth, productivity, and clonal selection.

**Bioreactor**

**Clonal cell selection**
The cell line that produces the protein of interest, with appropriate quality attributes, is selected and then expanded to establish a master cell bank.

**Expansion**
Each clonal cell line must be individually optimized for its suitability for large-scale manufacturing.

**Master cell bank/working cell bank (one vial/batch)**
The cells from the master cell bank are cultured and scaled up in large-scale bioreactors.

**Cell line development**

**Protein recovery / purification process**
The protein of interest is purified from the bioreactor harvest fluid by affinity chromatography.

**Chromatography**

**Formulation, fill-finish**
Stability studies should be conducted on the biosimilar and reference product to compare the stability of both using the same storage conditions and analytical methods.3

1 Amino acid sequence

3 Cell line development

4 Protein recovery / purification process

5 Formulation, fill-finish
In addition to the typical chemistry and manufacturing data that is expected for a new biologic drug, biosimilars **must provide extensive data demonstrating similarity with the reference biologic.**

This includes analytical characterization studies conducted in a side-by-side format. The comparative structural and functional studies will determine the type and extent of data to be derived from non-clinical and clinical studies on the drug product.

- **Analytical characterization studies**
- **Non-clinical studies**
  - Where extensive in vitro mechanistic studies are indicative of similarity, in vivo non-clinical studies may not be necessary.
- **Clinical pharmacology**
  - Pharmacokinetic (PK) studies
  - Pharmacodynamic (PD) studies
- **Clinical studies**
  - Clinical efficacy
  - Safety evaluation
  - Immunogenicity

The purpose of the clinical program is to show that there are no clinically meaningful differences between the biosimilar and the reference biologic.
A side-by-side characterization should be performed to directly compare the biosimilar and the reference biologic. The significance of any differences should be evaluated. When demonstrating biosimilarity, appropriate techniques are used to compare the following properties:3,7

- Physicochemical properties
  - Primary (amino acid sequence) and higher-order structures (i.e., secondary, tertiary, and quaternary)
  - Enzymatic post-translational modifications (glycosylation, phosphorylation)
  - Intentional chemical modifications (PEGylation sites)
- Biological activity
- Immunochemical properties
- Purity, impurities, and contaminants
  - Potential variations (i.e., oxidation)

Protein heterogeneity

Because biologics are produced by living organisms, an inherent degree of structural heterogeneity occurs. Consequently, the desired biologic product can be a mixture of anticipated post-translationally modified forms that do not negatively impact the desired product’s safety and efficacy. The manufacturer should define the pattern of heterogeneity of the desired product and demonstrate consistency with that of the lots used in preclinical and clinical studies. If a consistent pattern of product heterogeneity is demonstrated, an evaluation of the activity, efficacy, and safety (including immunogenicity) of individual forms may not be necessary.3,7

Examples of protein heterogeneity include:

- Terminal amino acid sequence variants
- Aggregation
- Glycosylation
- Truncation/fragmentation
Frequently encountered molecular variants of the desired product include:

- Truncated forms
- Other modified forms
  - Deaminated
  - Isomerized
  - Mismatched S-S linked
  - Oxidized
  - Conjugated (glycosylation, phosphorylation)
- Aggregates
  - Dimers
  - Higher multiples

COMPARATIVE STRUCTURAL AND FUNCTIONAL STUDIES

To be considered a biosimilar, the weight of evidence should be provided by structural and functional studies.³

The demonstration of similarity does not signify that the quality attributes of the two products being compared are identical, but that they are highly similar. This means that:

- the existing knowledge of both products is enough to predict that any difference in quality attributes should have no impact upon safety or efficacy; and
- the reference biologic’s clinical and non-clinical data are also relevant to the biosimilar.
CLINICAL STUDIES

The purpose of the clinical studies is to show that there are no clinically meaningful differences between the biosimilar and the reference biologic.³

Pharmacokinetic and pharmacodynamic (PK/PD) studies

Comparative PK studies should be conducted to rule out differences in pharmacologic characteristics between the biosimilar and the reference biologic. They should be carried out in healthy subjects when appropriate as they are usually considered to be a homogeneous and sensitive population. Comparative PD studies should be combined with PK studies, in which case the PK/PD relationship should be characterized.³

AUTHORIZATION OF INDICATIONS

A biosimilar manufacturer can request authorization for all indications held by the reference biologic. The decision to authorize the requested indications is dependent on the demonstration of similarity between the biosimilar and reference biologic drug based on data derived from comparative structural, functional, non-clinical, and clinical studies.³

Where similarity has been established, indications may be granted even if clinical studies are not conducted in each indication, sometimes referred to as “extrapolation.” A detailed scientific rationale for each indication request is necessary.
Immunogenicity studies

The purpose of comparative immunogenicity studies is to rule out clinically meaningful differences in immunogenicity between the biosimilar and the reference biologic.\(^3\)

Clinical trials

A comparative clinical trial is important to rule out clinically meaningful differences in efficacy and safety between the biosimilar and the reference biologic. The trial should be adequately sensitive to rule out clinically meaningful differences within predefined comparability margins.\(^3\)

In line with the principle of similarity, equivalence trials are generally preferred.\(^3\)

This scientific rationale should consider the mechanism(s) of action, pathophysiological mechanism(s) of the disease(s) or conditions involved, safety profile, dosage regimen, clinical experience with the reference biologic drug, and any case-by-case considerations. A biosimilar may be authorized for use in more than one indication because of the rigorous demonstration of similarity between the biosimilar and the reference biologic drug.\(^2,3\)

Extrapolation refers to a biosimilar that is marketed for indications where clinical studies were not done. Because a biosimilar is very similar in structure and function to a reference biologic with well-established safety and efficacy, clinical studies do not need to be repeated for each indication.
Biologics and biosimilars tend to be labile and sensitive to changes in the manufacturing process. Biological source materials, production cells, or their fermentation media can present risks, such as the presence of pathogens or the growth of outside agents such as viruses. Due to these risks, careful attention is paid to raw material controls, viral/bacterial inactivation, or clearance during product purification, and product testing. Changes to source materials, manufacturing processes, equipment, or facilities can result in significant unexpected changes to the intermediate and/or final product.

A well-defined manufacturing process with its associated process controls helps ensure that an acceptable product is produced on a consistent basis. Following manufacturing process changes, the manufacturer generally evaluates the relevant quality attributes of the product to demonstrate that modifications did not occur that would adversely impact the safety and efficacy of the drug product.\textsuperscript{3,6}

Manufacturing process critical control points are identified to detect process changes that may affect product characteristics.\textsuperscript{3}

Elements of a total control strategy designed to help ensure product quality and consistency include the following:\textsuperscript{3,7}

- Product characterization
- Adherence to good manufacturing practices (GMP)
- Validated manufacturing process
- Raw materials testing
- In-process testing
- Stability testing
QUALITY CONTROL IN MANUFACTURING

What is **Quality by Design**?

A systematic approach to development that begins with predefined objectives and emphasizes **product and process understanding** and **process control**, based on sound science and quality risk management.⁸

A **Quality by Design** approach to biosimilar development involves:⁸
- identifying the **material attributes and process parameters** that can have an effect on product CQAs;
- determining **functional relationships** that link material attributes and process parameters to product CQAs; and
- using the enhanced product and process understanding in combination with quality risk management to establish an appropriate **control strategy**.

Amgen has adopted a **Quality by Design** approach to biosimilar development.
Manufacturers of biologic or biosimilar products frequently make changes to manufacturing processes both during development and once in the market. Reasons for such changes include:• improving the manufacturing process;• increasing scale;• improving product stability; and• complying with changes in regulatory requirements.

A biosimilar is a new drug with all the associated regulatory requirements. For changes to the manufacturing process that warrant a demonstration of comparability, the products to be compared will be the pre- and post-change versions of the biosimilar.

Comparisons with the original reference biologic are not required.3

The goal of the comparability exercise is to ensure that the pre- and post-change product is comparable in terms of quality, safety, and efficacy.3,6
Comparative structural and functional studies

No access to the reference product’s history or manufacturing process.

Clinical studies

Non-clinical studies

Comparative quality studies

Comparability bridging studies (when quality studies are insufficient)

Extensive knowledge about:
- the product
- the manufacturing process
- the established controls
- acceptance parameters

MANUFACTURING PROCESS DEVELOPMENT VS. PROCESS CHANGES

Biosimilar entering the market
(during the development of a manufacturing process)

The biosimilar must demonstrate similarity to a reference product.  

Each has a different manufacturer. The biosimilar is compared to the reference biologic.

Marketed biologic/biosimilar
(following manufacturing process changes)

The biologic/biosimilar must demonstrate comparability between the pre- and post-change product.  

Both have the same manufacturer. The same product, biologic or biosimilar, is tested pre- and post-change.

Clinical studies

Non-clinical studies
OUR MISSION IS TO SERVE PATIENTS
We have committed vast resources to the development and manufacturing of biosimilar medicine because it is our goal to ensure a reliable and consistent supply of medicine for every Amgen patient, every time.

References
