Central Administration of biological and innovative products and clinical studies
General Administration of biological products

Guideline

Guideline for Registration of Biosimilar Products in Egypt

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I. Introduction

This guideline is a replacement of the published guideline for registration of biosimilar products in Egypt (2020) to keep up with the recent international guidelines on evaluation of biosimilar and should be read in conjunction with regulatory guide for mechanisms, procedures and rules of implementing decree of Egyptian Drug authority's president No. 343/2021 and Procedures for Registration of Biological products through Reliance pathways.

The biosimilar manufacturer should fulfill these regulations for registration of a biosimilar product.

This review would give the chance to assess recent advancements, pinpoint areas where the current guideline may be more flexible without compromising its fundamental principles, and provide more information on the potential for customizing the quantity of data required for regulatory approval.

The difference between the term generics used for description of a similar product to a reference pharmaceutical product and the term biosimilar used to describe the similar versions of a reference biological product should be clearly understood.

The guidelines for development, evaluation and registration of generic medicines are not suitable for biological products because biological products consist of relatively large, and complex proteins that are **a)** difficult to characterize/analyze all the quality attributes contributing to the safety and efficacy profile, **b)** highly dependent on manufacturing process that affects product quality, safety and tendency to induce an unwanted immune response as well as efficacy profile.

There are two approaches for registration of a biological product that can be applied:

1- Stand-alone approach: the manufacturer performs complete product development program (quality, pre-clinical and clinical studies) **(out of scope of this guideline)**.
2- Biosimilar approach: the manufacturer performs complete product CMC development process in addition to complete comparability quality exercise, and reduced preclinical and clinical comparability studies in order to demonstrate bio-similarity of the proposed biological medicinal product to a reference one.

II. Scope

This guideline is applied to well characterized biological products developed by means of biotechnology (including recombinant DNA technology). Some of the principles provided

in these Guidelines may also apply to low molecular weight heparins and recombinant analogues of plasma-derived products. Vaccines and plasma derived products and their recombinant analogues are excluded from the scope of these guidelines.

III. Definitions

Biological products (Biologicals): Products containing one or more active ingredient produced or derived from a biological source, including but not limited to human vaccines, serum, blood and plasma products and derivatives, also products manufactured using biotechnology and the like, as well as, any products or substances that may be created based on science update and/or international standard and reference.

Biosimilar: A biological product that is shown to be highly similar in terms of its quality, safety and efficacy to an already licensed reference product.

Imported products: It is the imported biological products weather fully manufactured overseas or manufactured overseas and packaged in factories within the Arab Republic of Egypt.

Locally manufactured products: They are biological products manufactured in factories inside the Arab Republic of Egypt or the products imported in bulk that are manufactured in the Arab Republic of Egypt.

Excipients: a constituent of a medicine other than the drug substance, added in the formulation for a specific purpose. While most excipients are considered inactive, some can have a known action or effect in certain circumstances.

Reference product (RP): A Product developed and registered on basis of complete dossier with full quality, preclinical and clinical data and used by the manufacturer for comparability studies versus a product supposed to be a biosimilar.

Comparability exercise: Direct head-to-head comparison of a biological product with a licensed reference product with the goal to establish similarity in quality, safety, and efficacy.

Pilot Scale batches: The production of the drug substance or drug product by a procedure fully representative of and simulating that to be applied at manufacturing scale. The methods of cell expansion, harvest, and product purification should be identical except for the scale of production.

Manufacturing scale batches: Batches of a finished product manufactured at production scale by using production equipment in a production facility as specified in the dossier

Pharmacovigilance: The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug related problems.

Reference Countries: An updatable list of countries approved by the technical committee

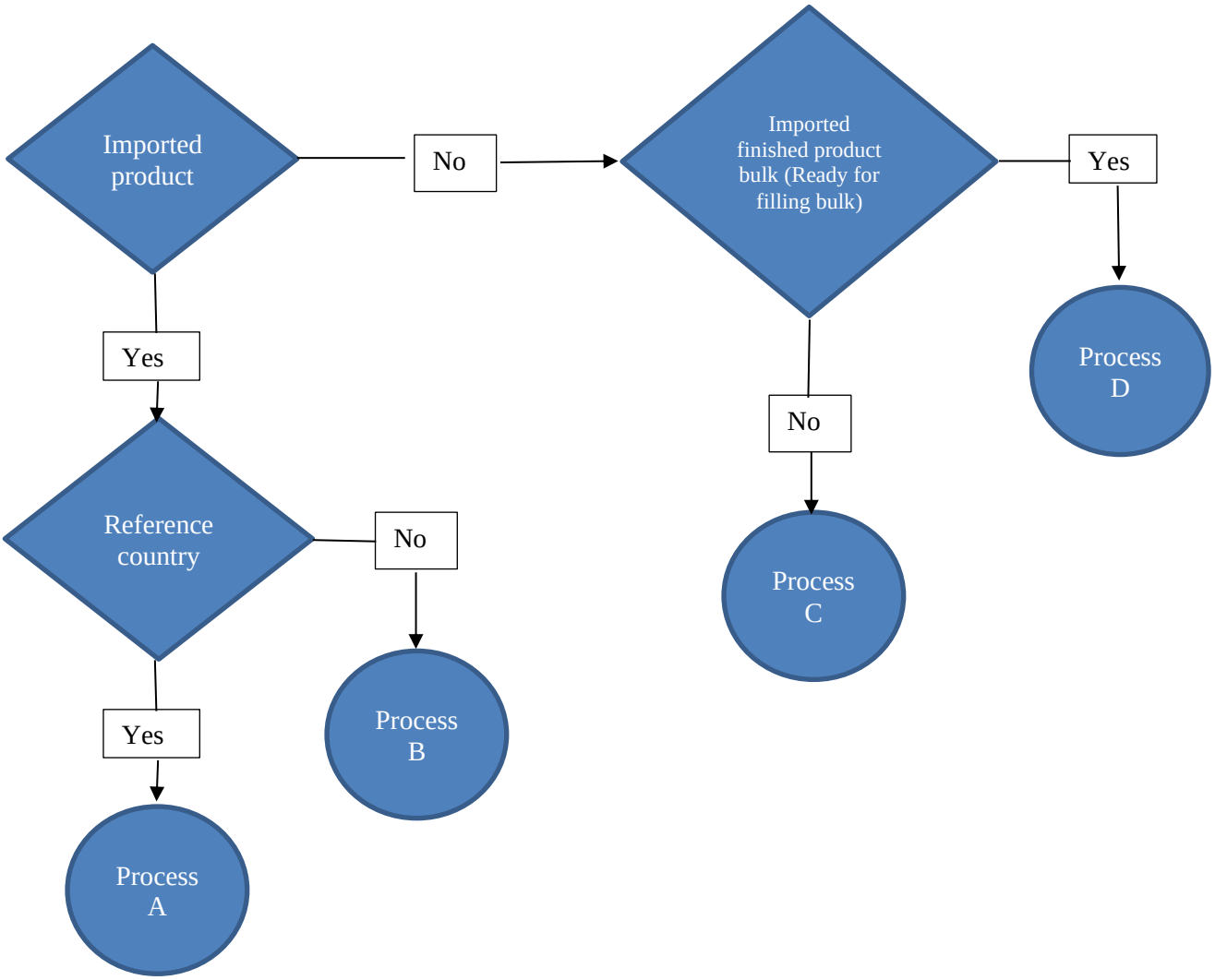
for drug control (published on EDA website).

Posology: Branch of medicine that is concerned by the determination of appropriate dose of medicine

IV. Procedures:

1. Steps of registration of a biosimilar product:

The following decision tree should be followed for determination of the application flow steps



Process A

- **Conditions:**
  1. This process is applied on imported finished products from reference countries (the reference countries list is published on EDA website)
  2. They can be either manufactured, primary and secondary packed in country of origin **OR** it can be imported as naked container (in its primary package) to undergo secondary packaging in local manufacturer.
  3. Must be registered & marketed in their Country of Origin (wavier from marketing in country of origin may be accepted if justified).

- **Flow chart:**

- **Steps:**

Step 1: The applicant submits an inquiry for inquiry approval, an inquiry approval or disapproval will be issued within 10 W.D for products submitted for registration through Ministerial Decree 343/2021.

Step 2: The applicant should submit the pricing dossier within 30 W.D from the date of issuing the inquiry approval. The pricing certificate is released within 60 W.D.

Step 3: The applicant is allowed to submit the MA File to registration administration of biological product during 30 W.D from the date of pricing certificate issuance, for products submitted for registration through Ministerial Decree 343/2021, MA file will be evaluated by all evaluation departments and analysis for registration will be performed within 120 W.D.

Note: In case of registration through reliance model, MA dossier will be evaluated within the specified W.D as mentioned in reliance guideline.

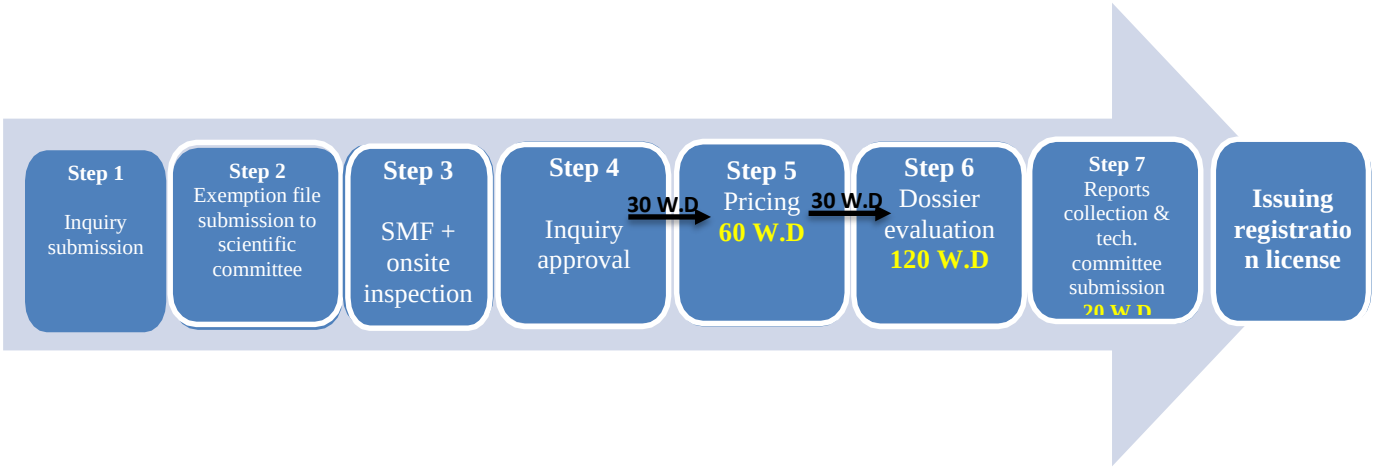
Step 4: Reports collection & submission to technical committee for drug control to issue

the registration license within 20 W.D.

Process B

- **Conditions:**
  1. This process is applied on imported finished products from non-reference countries
  2. They can be either manufactured, primary and secondary packed in country of origin **OR** it can be imported as naked container (in its primary package) to undergo secondary packaging in local manufacturer.
  4. Must be registered & marketed in their Country of Origin (wavier from marketing in country of origin may be accepted if justified).

- **Flow chart:**



- **Steps:**

Step 1: The applicant submits an application inquiry for inquiry approval, the applicant will be asked to contact scientific file examination unit to submit exemption file within 20 W.D or inquiry will be cancelled.

Step 2: After the approval of the scientific specialized committee for biological products, the applicant should submit the site master file (SMF) to be evaluated by biological inspection department; In case of approval of the submitted SMF, the inspection department shall inspect the site for compliance with GMP.

Step 3: Issue inquiry approval for the submitted product after the approval on the inspection

of the site.

Step 4: The applicant submits the pricing dossier within 30 W.D of receiving inquiry approval. Pricing license is issued within 60 W.D

Step 5: The applicant submits the MA dossier within 30 W.D of receiving pricing license, and evaluation of the submitted file and analysis for registration will be within 120 W.D in case of registration through Ministerial Decree 343/2021.

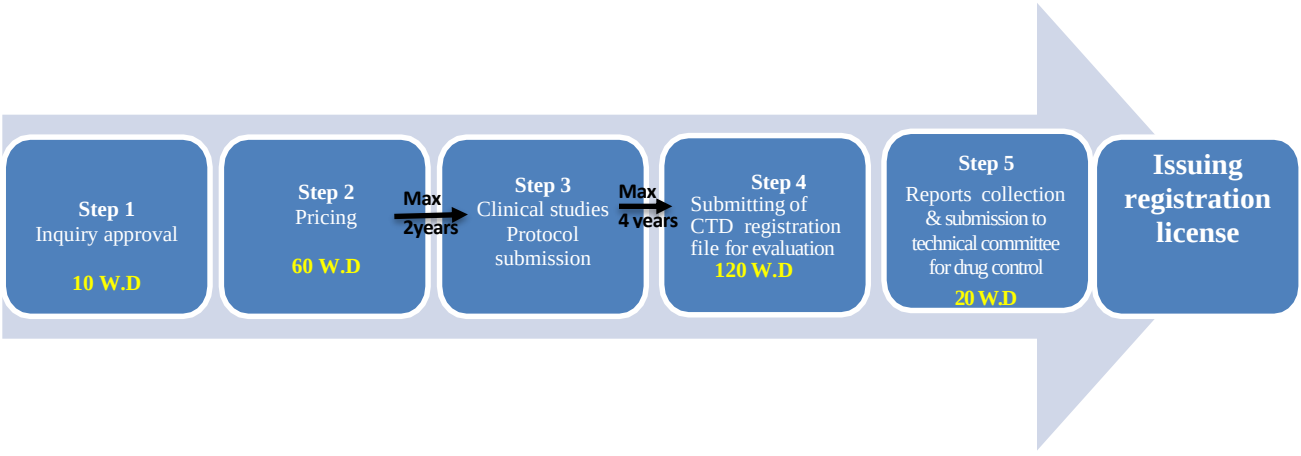
Step 6: Reports collection & submission to Technical Committee for Drug Control to issue the Registration License within 20 W.D.

Process C

- **Conditions:**

1. They are finished products manufactured in factories licensed in Egypt & include the following categories:
    - Manufacturing finished product starting from developing drug substance to the final finished product in local factory/factories.
    - Manufacturing finished product starting from imported drug substance.
    - Manufacturing finished product starting from imported bulk for further formulation in local manufacturer.

- **Flow chart:**



- **Steps:**

Step1: The applicant submits an application inquiry, the company will be informed with

the status of inquiry within 10 W.D.

Step 2: The applicant submits the pricing dossier within 30 W.D of receiving inquiry approval. Pricing license is issued within 60 W.D with 2 years validity period (can be extended by a justified request from the applicant).

During these 2 years:

- The applicant is allowed to purchase (in case of imported active substance) or produce (in case of locally manufactured active substance) specified amount of active substance required for manufacturing specified batch sizes for development.

Note: The applicant has to develop the biosimilar product, perform the quality and preclinical comparability studies along with the preparation of clinical studies protocol. At any stage, the results of quality and preclinical studies as well as the clinical studies protocol could be submitted for scientific advice. Also, scientific advice request could be conducted for the active substance master file & the site master file, if needed.

Step 3: the applicant submits the clinical studies protocol for evaluation, an approval to conduct clinical studies will be issued with 4 years validity period (can be extended by a justified request from the applicant).

After completion of the clinical studies, the applicant completes the MA dossier to be submitted as CTD format for assessment.

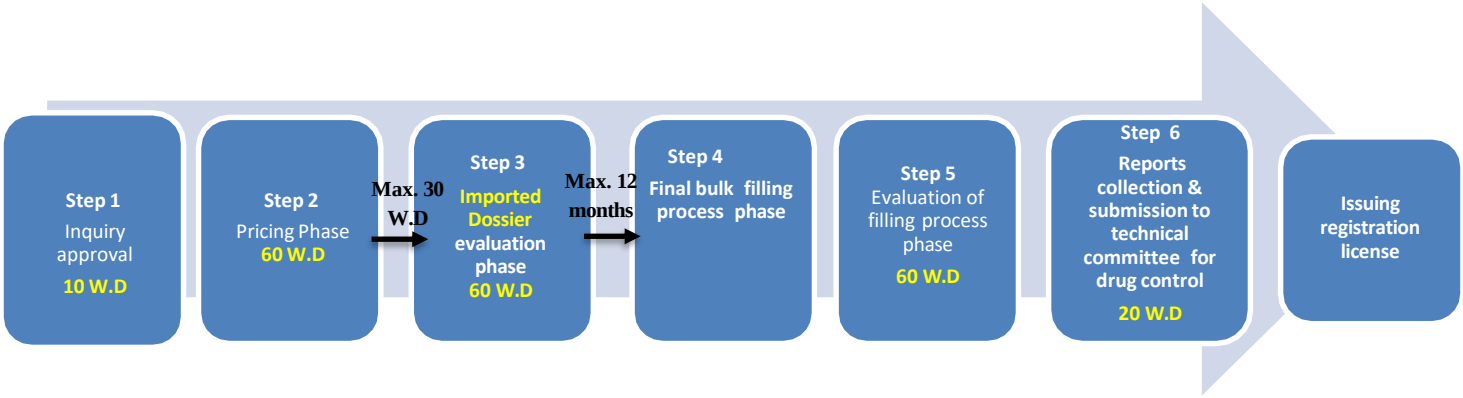
Step 4: An assessment of registration dossier and analysis for registration are performed during this phase within 120 W.D.

Step 5: Reports collection for submission to technical committee for drug control within 20 W.D.

Process D

- **Conditions:**
  1. They are finished products filled in factories licensed in Egypt starting from an imported finished product bulk (Ready for filling bulk)

➢ **Flow chart:**



➢ **Steps:**

Step 1: The applicant submits an inquiry for inquiry approval, an inquiry approval or disapproval will be issued within 10 W.D for products submitted for registration through Ministerial Decree 343/2021.

Step 2: The applicant should submit the pricing dossier within 30 W.D from the date of issuing the inquiry approval. The pricing certificate is released within 60 W.D.

Step 3: The applicant is allowed to submit the MA File to registration administration of biological product during 30 W.D from the date of pricing certificate issuance, MA file will be evaluated by all evaluation departments.

Step 4: For (Final bulk filling process), the manufacturer should perform the comparability exercise before and after changing the filling site according to ICH Q5E guideline.

Step 5: Evaluation of quality part concerning filling process including stability study after filling, and analysis for registration will be performed within 60 W.D. Where a determination of comparability can be based on a combination of analytical testing, biological assays, and, in some cases, nonclinical and clinical data. If a manufacturer can provide assurance of comparability through analytical studies alone, nonclinical or clinical studies with the post-change product are not warranted. However, where the relationship between specific qualities attributes and safety and efficacy has not been established, and differences between quality attributes of the pre- and post-change product are observed, it is appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise.

Step 6: Reports collection and submission to technical committee for drug control to issue the registration license within 20 W.D

Note: EDA will conduct inspection for the manufacturing sites for both imported drug substance & finished product bulk (in case imported from non-reference countries or without stringent regulatory authority GMP certificate) through inspection administration.

2. General principles

2.1 Rational for choice of the reference biological product

- A single RP should be used as the comparator throughout the comparability program for quality, safety and efficacy studies during the development of a biosimilar in order to allow the generation of coherent data and conclusions.

- The RP used in the biosimilar comparability exercise at the quality level must be clearly identified (e.g., brand name, pharmaceutical form, formulation, strength, origin of the reference medicinal product, number of batches, lot number, age of batches, use). Where several strengths or presentations are available, their selection should be appropriately justified.
- Considering the inherent heterogeneity present in protein products and the expected lot-to-lot variability stemming from manufacturing processes, it is recommended that a sponsor includes multiple reference product lots throughout the development program and comparability assessment (acquired over a time frame that spans expiration dates of several years (i.e. shelf life), in the analytical assessment to ensure that specification limits capture not only the variability of the reference product manufacturing process but also variability due to product instability during storage.
- The following should be considered for RP:
  1) Random sampling of RP batches is desirable
  2) It is recommended that the RP batches are sourced over an extended time period
  3) These batches should also include the RP batches used in the clinical comparison studies of the biosimilar
  4) The RP batches should be transported and stored under the recommended conditions and tested within their approved shelf-life. Any exception to this would have to be fully substantiated with experimental data. The shelf-life of the RP at time of characterization should be considered and it is expected that RP batches of different ages will be included in the similarity assessment
  5) The biosimilar batches included in the comparability assessment should be manufactured using the intended commercial manufacturing process and should preferably originate from different drug substance batches to adequately represent the variability of attributes inherent to the drug substance manufacturing process.
  6) Small- or pilot-scale batches can be included if comparability between the small and commercial scale batches has been properly demonstrated. Usually all commercial scale batches produced – including process performance qualification batches and batches applied in the clinical trial(s) – should be included in the similarity assessment.

- Publicly available reference standards (e.g., Ph. Eur.) cannot be used as the RP for demonstration of bio-similarity. However, the use of these standards plays an important role in method qualification (e.g. potency determination) and standardization.
- Authorization is performed on basis of complete dossier (full quality, preclinical and clinical data). Therefore, an Approved biosimilar cannot be considered as a reference product.
- In case of using other version of the RP (i.e., licensed by other stringent authority than that of Egypt), it will be the applicant's responsibility to demonstrate that the comparator (i.e., the other version of the RP) is representative of the reference medicinal product.
- In case of using a RP that has not been registered in Egypt, the reference medicine must be approved and marketed in a reference country (for example, EU or US) before request submission. In addition, the applicant is permitted to import the RP with specific quantity for performing the comparability exercise. It is important to note that the acceptance of a RP (not registered in Egypt) for the evaluation of a biosimilar in a particular country does not imply that the EDA has approved the RP for use in the Egyptian market.
- In case of doubt, scientific advice is recommended to confirm choice of a suitable RP.

2.2. Biosimilarity principles (developmental aspects)

- Characterization of the quality attributes of the RP should be the first step in guiding the development of the biosimilar. The subsequent comparability exercise should demonstrate structural, functional and clinical similarity.
- Development of biosimilar product together with proving biosimilarity relies on the manufacturer of the drug product, whether the drug substance manufacturer is the same entity of the drug product manufacturer or a contract manufacturer. If the manufacturer of the drug substance differs from that of the drug product, it will be the applicant's responsibility to provide the regulatory authority with the active substance full data within CTD either by his own submission or directly by the manufacturer of the active substance.
- The manufacturing process of the biosimilar should be developed based on a comprehensive understanding of the RP gained through detailed characterization studies of a sufficient number of RP batches.
- It's recommended for the applicant during development process to monitor all the data regarding the safety and efficacy of the reference product.
- Particular attention should be given to quality attributes that might have an impact on immunogenicity or potency, or that have not been identified in the reference medicinal product.
- The use of enhanced approaches to pharmaceutical development, along with quality risk management, effective quality systems and implementing good manufacturing

practices, will facilitate the consistent manufacturing of a high-quality product.

- A biosimilar is manufactured and controlled according to its own development, taking into account state-of–the-art information on manufacturing processes and consequences on product characteristics.
- A comprehensive understanding of all steps in the manufacturing process for the proposed product should be established during product development. Information gained during process development including characterization tests, process controls and specifications must be specific for the proposed product and manufacturing process.

The development and documentation for biosimilar should cover two distinct aspects:

- Molecular characteristics and Quality Attributes (QA) of the target product profile should be comparable to the reference medicinal product;

  The Quality Target Product Profile (QTPP) of a biosimilar should be based on data collected on the chosen RP, including publicly available information and data obtained from extensive characterization of different batches of the RP. Since The biosimilar medicinal product is defined by the molecular composition of the active drug substance resulting from its manufacturing process, which may introduce its own molecular variants, isoforms or other product-related substances as well as process-related impurities. As a consequence, the manufacturing process should be appropriately designed to achieve the QTPP.

- Performance and Consistency of the manufacturing process of the biosimilar on its own.

Similarity ranges establishment:

- Where possible, quantitative similarity ranges should be established for the biosimilar comparability exercise.
- The established similarity range should tightly reflect.
- The quality profile of the marketed RP batches.
- Different statistical intervals can be used to establish similarity ranges. (Commonly used approaches include mean ± x SD, the min-max range and tolerance intervals).
- Different statistical intervals can be used to establish similarity ranges. Commonly used approaches include mean ± x SD, the min-max range and tolerance intervals:

  ▪ **Mean ± x SD:** is the most commonly applied approach for establishing similarity ranges is the x-sigma interval, that is, mean ± x SD of the RP batch data. The multiplier used (x) should be scientifically justified and could be linked to the criticality of the quality attribute tested, with a smaller multiplier applied for high criticality quality attributes.

▪ **Min-max range:** is a conservative approach in which establishing the similarity ranges is directly based on the min-max quality attribute data obtained from the characterization studies of RP batches. Such similarity ranges could be viewed as clinically qualified (since the RP batches are on the market and taken by patients). However, compared to other approaches the min-max approach is often associated with high risk of a false negative conclusion (that is, a high risk of concluding non-similarity even though the underlying data distributions for the RP and biosimilar would support a similarity claim).

▪ **Tolerance intervals:** similarity ranges based on tolerance intervals would usually require a high number of RP batches for establishing meaningful ranges. With a limited number of RP batches characterized and/or inappropriate parameterization, the tolerance interval approach can result in an estimated range that is much wider than the actual min max quality attribute ranges of the RP. The risk of a false-positive conclusion of similarity (that is, the risk of concluding similarity where the underlying data distributions do not support such a claim) may therefore be unreasonably high when the similarity ranges are based on inappropriately applied tolerance intervals. The most frequently applied overall similarity criteria require that a certain percentage of the biosimilar batches (usually between 90% and 100%) fall within the similarity range.

2.3. Characterization of biosimilar

- Biosimilarity is evaluated using a scientifically tailored approach, with approval based on the "totality of the evidence," including analytical, (structural and functional), animal toxicity, pharmacokinetic (PK), pharmacodynamic (PD), immunogenicity, and clinical safety and effectiveness.
- Collecting data from publicly available information and data from extensive analytical characterization for different batches of the reference product, will enable the applicant to:
  - Achieve the quality target product profile (QTPP) of the proposed biosimilar.
  - Detect batch to batch variation within batches of the same reference product.
  - Specify the acceptance criteria for biosimilarity with justification.
- For differences in quality attributes with higher criticality, functional assays to thoroughly address their possible clinical impact are generally expected. Where there are confirmed differences in the most critical quality attributes it will be more challenging to justify the conclusion that the product is a true biosimilar.
- Confirmed differences in low criticality quality attributes also need to be adequately considered, but in the case of such differences reference to available information (which could, for example, originate from scientific publications) is usually sufficient.
- Lower impurity levels in the biosimilar (for example, of aggregates) or differences in

quality attributes present at very low levels in both the RP and the biosimilar would in most cases be predicted to have no clinical relevance, and could therefore be accepted without further assessment.

2.4. Comparability assessment

- An extensive head-to-head comparability exercise will be required to demonstrate that the biosimilar has a highly similar quality profile when compared to the RP. This should include comprehensive analyses of the proposed biosimilar and RP using sensitive and orthogonal methods to determine not only similarities but also potential differences in quality attributes. Any differences detected in the quality attributes will have to be appropriately justified with regard to their potential impact on safety and efficacy.
- The aim of the biosimilar comparability exercise is to demonstrate that the biosimilar product and the RP chosen by the applicant are similar at the level of the finished medicinal product as well as adequate characterization of the proposed product and understanding of manufacturing variability.
- Demonstration of similarity of a biosimilar to an RP in terms of structural and functional aspects is a prerequisite for establishing comparability, with a tailored clinical data package required as needed.

- A clinical bioequivalence trial with pharmacokinetic (PK) and pharmacodynamic (PD) parameters (if available), and including an assessment of immunogenicity in human subjects, will typically be a core part of the clinical comparability assessment, unless scientifically justified.

- Complete CMC data in CTD format according to ICH guidelines, preclinical and clinical comparative studies with the same reference product used in the quality comparability exercise should be submitted.
- The decision to license a biosimilar should be based on evaluation of the whole data package generated during the overall comparability exercise.
- If relevant differences between the proposed biosimilar and the RP are detected at any stage (structural, functional, nonclinical or clinical level) the reasons should be justified. If this is not possible, the product is unlikely to qualify as a biosimilar and a full licensing **(standalone)** application should be considered.

- Some minor differences between the RP and the biosimilar are expected. Nevertheless, any quality attributes not fulfilling the established similarity criteria should be considered as a potential signal for non-similarity and should be assessed for possible impact on clinical safety and efficacy.

3. Content of biosimilar applications

3.1. Quality

3.1.1. Manufacturing process

❖ **Expression System:**

- Therapeutic protein products can be produced in microbial cells (prokaryotic or eukaryotic), cell lines (e.g., mammalian, avian, insect, plant), or tissues derived from animals or plants. It is expected that the expression construct for a proposed product will encode the same primary amino acid sequence as its reference product. However, minor modifications, such as N- or C terminal truncations (e.g., the heterogeneity of C-terminal lysine of a monoclonal antibody) that are not expected to change the product performance, may be justified and should be explained by the manufacturer.
- Possible differences between the chosen expression system (i.e., host cell and the expression construct) of the proposed product and that of the reference product should be carefully considered because the type of expression system will affect the types of process- and product-related substances, impurities, and contaminants (including potential adventitious agents) that may be present in the protein product. In this case, more extensive comparability exercise should be employed to assure quality, efficacy and safety of the biosimilar product. Using different expression system will be evaluated on case-by-case basis.

3.1.2. Analytical considerations

- The biosimilar manufacturer will usually be using a commercial drug product for the similarity exercise due to the unavailability of drug substance for the RP. The commercial drug product will be formulated with excipients. It should be verified that these excipients do not interfere with the analytical methods used and thus have no impact on test results.
- If the drug substance in the RP needs to be purified from a formulated reference drug product in order to be suitable for characterization then studies must be carried out to demonstrate that product heterogeneity and relevant attributes of the active moiety are not affected by the isolation process.

A) Structural and Conformation Characterization:

- A comprehensive set and combination of analytical methods should be, generally characterization tests include but not limited to:

➔ Primary Structures, such as amino acid sequence, N and C-terminal sequence. The target amino acid sequence of the biosimilar should be confirmed and is expected to be the same as for the reference medicinal product. The N- and C-terminal amino

acid sequences, free SH groups and disulfide bridges should be compared, as appropriate.

- ➔ Higher order structures, including secondary, tertiary, and quaternary structure (including aggregation).
- ➔ Enzymatic post-translational modifications, such as glycosylation and phosphorylation. If present, carbohydrate structures should be thoroughly compared; including the overall glycan profile, site-specific glycosylation patterns as well as site occupancy. The presence of glycosylation structures or variants not observed in the RP may raise concerns and would require appropriate justification, with particular attention to non-human structures (non-human linkages, sequences or sugars).
- ➔ Other potential variants, such as protein deamidation and oxidation.
- ➔ Intentional chemical modifications, such as pegylation sites and characteristics.

- Appropriate analytical methods such as, mass spectrometry, circular dichroism, spectroscopy etc. should be used for comparing products structure and variants

B) Physicochemical Properties:

- A physicochemical characterization program should include determination of the composition, physical properties, primary, and higher order structures of the active substance of the biosimilar product.
- The amino acid sequence of a biosimilar should be conformed to be the same as that of its RP. Low-level sequence variants may occur due to transcription and translation errors (through amino acid disincorporation during high level expression) this should be described and controlled to reasonable level. Assessment of the potential clinical impact of such variants must be considered.
- An inherent degree of structural heterogeneity occurs in proteins due to the biosynthetic process; therefore, the biosimilar product can contain a mixture of post-transnationally modified forms. Appropriate efforts should be made to investigate and identify these forms.
- To address the full range of physicochemical properties or biological activities adequately, it is often necessary to apply more than one analytical procedure to evaluate the same quality attribute. Methods that use different physicochemical or biological principles to assess the same attribute are especially valuable because they provide independent data to support the quality of that attribute (e.g., orthogonal methods to assess aggregation). In addition, the use of complementary analytical techniques in series, such as peptide mapping or capillary electrophoresis combined with mass spectrometry of the separated molecules, should provide a meaningful and sensitive method for comparing products
- Some techniques provide information on multiple characteristics. It is expected that appropriate analytical test methods will be selected based on the nature of the protein being characterized and knowledge regarding the structure and heterogeneity of the reference product and the proposed product, as well as characteristics critical to product performance.

C) Biological activity

- An important property is the biological activity that describes the specific ability or capacity of a product to achieve a defined biological effect. A valid biological assay (animals, cell culture, and/or ligand binding) to measure this activity shall be used by the manufacturer.
- It should be noted that many biological assays may have relatively high variability that might preclude detection of small but significant differences between the biosimilar and RP. Therefore, it is recommended that assays be precise and can detect changes in the intended biological activities of the product to be evaluated with adequate accuracy.
- For some drug substances or drug products, the protein molecule may need to be examined using immunochemical procedures (e.g., ELISA, Western-blot) utilizing antibodies which recognize different epitopes of the protein molecule. Immunochemical properties of a protein may serve to establish its identity, homogeneity or purity, or serve to quantify it.
- When binding is part of the activity attributed to the protein product, analytical tests should be performed to characterize the proposed product in terms of its specific binding properties (e.g., if binding to a receptor is inherent to protein function, this property should be measured and used in comparative studies) (see ICH Q6B for additional details).
- For product with multiple biological activities, manufactures should perform, as part of the product characterization, a set of relevant function assays designed to evaluate the range activities of the product.
- In case of mAb, further information on Fc-mediated Functions should be provided and compared for example, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC), where relevant
- The results of relevant biological assay(s) should be provided and expressed in units of activity calibrated against an international or national reference standard, where available and appropriate.
- Various methods such as surface plasmon resonance, microcalorimetry, or classical scatchard blot can provide information on the kinetics and thermodynamics of binding. Such information can be related to the functional activity and characterization of the proposed product's higher order structure.
- These assays should comply with appropriate international pharmacopoeia requirements for biological assays, as applicable.

D) Purity and impurities:

- Identification of product and process impurities should be performed using orthogonal testing.
- The purity and impurity profiles of the proposed biosimilar product and RP should be compared both qualitatively and quantitatively by a combination of analytical procedures.
- By a combination of analytical procedures. Level of impurities should be comparable and any new impurities should be well characterized, justified and its effect on product quality

and safety should be discussed

- Appropriate state-of-the art methods should be used to compare the product-related substances and impurities. This comparison should take into account specific degradation pathways (for example: oxidation, deamidation, aggregation, truncation, charge variants, visible, sub-visible and sub-sub visible particle, etc.…) of the biosimilar product and potential post-translational modifications of the proteins. The manufacturer should characterize, identify, and quantify in the proposed biosimilar product and the reference product, to the extent feasible.
- If a comparative physicochemical analysis reveals comparable product-related impurities at similar levels between the two products, pharmacological/toxicological studies to characterize potential biological effects of specific impurities may not be necessary, however, if the manufacturing process used to produce the proposed product introduces different impurities or higher levels of impurities than those present in the reference product, additional pharmacological/toxicological or other studies may be necessary.
- To obtain sufficient information of the product-related substances and impurities it is recommended that comparative stability studies under accelerated and/or stress conditions are conducted
- Process-related impurities arising from cell substrates (e.g., host cell DNA, host cell proteins), cell culture components (e.g., antibiotics, media components), and downstream processing steps (e.g., reagents, residual solvents, leachable, endotoxin, bioburden) should be evaluated. The process-related impurities in the proposed product are not expected to match those observed in the reference product and are not included in the comparative analytical assessment. Nevertheless, State-of-the-art analytical technologies following existing guidelines and compendial requirements should be applied, and the potential risks related to these newly identified impurities (for example, immunogenicity) have to be appropriately documented and justified.
- The chosen analytical procedures should be adequate to detect, identify, and accurately quantify biologically significant levels of impurities. In particular, results of immunological methods used to detect host cell proteins depend on the assay reagents and the cell substrate used. Such assays should be validated using the product cell substrate and orthogonal methodologies to ensure accuracy and sensitivity.

3.1.3. Comparative analytical assessment

- The number of RP batches needed for the comparative analytical assessment will be influenced by the criticality of the quality attributes under investigation and the approach chosen for demonstrating similarity.
- It is the responsibility of the applicant to demonstrate that the selected methods used in the comparability exercise would be able to detect slight differences in all aspects pertinent to the evaluation of quality. Methods used in the characterization studies form an integral part of the quality data package and should be appropriately qualified for the purpose of comparability (e.g., ability to detect relevant variants with high sensitivity).

- The analytical limitations of each technique (for example, limit of detection or resolving power) should be considered when determining the similarity of a biosimilar to its RP.
- For some analytical techniques, a direct or side-by-side analysis of the biosimilar and RP may not be feasible or give limited information (e.g. due to the low concentration of active substance and/or the presence of interfering excipients such as albumin). Thus, samples could be prepared from the finished product (e.g., extraction, concentration, and/or other suitable techniques). In such cases, the techniques used to prepare the samples should be outlined, and their impact on the samples should be appropriately documented and discussed (e.g., comparison of active substances before and after formulation/de-formulation preparation).
- Quantitative ranges should be established for the biosimilar comparability exercise, where possible. These ranges should be based primarily on the measured quality attribute ranges of the RP and should not be wider than the range of variability of the representative RP batches, unless otherwise justified. The relevance of the ranges should be discussed, taking into account the number of RP lots tested, the quality attribute investigated, the age of the batches at the time of testing and the test method used.
- It should be noted that acceptable ranges used for the biosimilar comparability exercise versus the RP should be handled separately from release specifications.
- The Age/Shelf Life of the RP at the time of testing should be mentioned, and its potential effect on the quality profile should be discussed where appropriate, taking into consideration that it is recommended that the reference product batches used in the comparability have similar age as the proposed biosimilar product.
- Comparison of relevant quality attributes, tested at selected time points and storage conditions (for example, accelerated or stress conditions), could be used to further support the similarity of the degradation pathways of the RP and the biosimilar.
- A sponsor considering manufacturing changes after completing the initial comparative analytical assessment or after completing clinical studies may need to conduct additional comparative analytical studies of the proposed product (before and after change) and the reference product. The nature and extent of the changes may determine the extent of these additional analytical studies

3.1.4. Specifications

- Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval to ensure product quality and consistency. They should focus on those molecular and biological characteristics found to be useful in ensuring the safety and efficacy of the product.
- The selection of tests to be included in the specifications is product specific and should be performed according to the ICH guidelines Q6B.
- Specifications for a biosimilar may not be the same as for the RP since the manufacturing process will be different and different analytical procedures and laboratories will be used

for the assays. Nonetheless, the specifications should capture and control important known product quality attributes.

- Each acceptance criterion should be established and justified based on data obtained from lots used in preclinical and/or clinical studies, and by data from lots used for the manufacturing process validation, data from stability studies, relevant development data and data obtained from the quality, safety and efficacy comparability exercise.
- The setting of specifications should be supported by global reasoning based on the manufacturer experience of the biosimilar product (quality, safety and efficacy) and own experimental results obtained by testing the reference product.
- Methods used for setting specifications may or may not be the same as analytical methods used for product characterization and for establishing product comparability.
- The setting of specifications should be based on:

  (a) The manufacturer's experience with the biosimilar (for example, with regard to its manufacturing history, assay capability and the quality profile of batches used for establishing similarity).

  (b) The experimental results obtained by testing and comparing the biosimilar and RP.

  (c) Attributes with potential impact on product performance.

  (d) Available monograph (Where this exist)

- The manufacturer should take into consideration that the limits set for a given specification should not, unless properly justified, be significantly wider than the range of variability of the RP over the shelf-life of the product.

3.1.5. Formulation / Container closure system

- The formulation of the biosimilar should be selected taking into account state-of-the-art technology and, regardless of the formulation selected, the suitability of the proposed formulation with regards to stability, compatibility (i.e., interaction with excipients, diluents and packaging materials), integrity, activity and strength of the active substance should be demonstrated.
- Sponsors should clearly identify excipients used in the proposed product that differ from those in the reference product. The acceptability of the type, nature, and extent of any differences between the finished proposed product and the finished reference product should be evaluated and supported by appropriate data and rationale. Additionally, different excipients in the proposed product should be supported by existing toxicology data for the excipient or by additional toxicity studies with the formulation of the proposed product. Excipient interactions as well as direct toxicities should be considered.
- The acceptability of the type, nature, and extent of any differences between the proposed finished biosimilar product and the finished reference product should be evaluated.
- Proteins are very sensitive to their environment. Therefore, differences in excipients or

primary packaging may affect product stability and/or clinical performance.

- Differences in formulation and primary packaging between the proposed product and the reference product are among the factors that may affect whether or how subsequent clinical studies may take a selective and targeted approach.
- If a different formulation and/or container/closure system to the RP is selected (including any material that is in contact with the medicinal product), its potential impact on the safety and efficacy should be appropriately justified.

3.1.6. Stability

- Stability studies should be summarized in an appropriate format (such as tables) and should include results from accelerated degradation studies and studies under various stress conditions (for example: high temperature, oxidation, freeze-thaw, light exposure, humidity and mechanical agitation).
- The stability data should support the conclusions reached on the recommended storage and shipping conditions and on the shelf life and storage period for the drug substance, drug product and process intermediates which might be stored for significant periods of time.
- Stability studies should be carried to show which release and characterization methods are stability indicating for the product.
- Real time/real temperature stability studies should be performed compared with the RP to determine the storage conditions and shelf life for the biosimilar (which may or may not be the same as those for the RP). Results from studies conducted under accelerated and stress conditions may also show that additional controls should be used in the manufacturing process, and during shipping and storage, in order to ensure the integrity of the product.
- Comparative stability studies conducted under accelerated, and/or in some cases stress conditions (for example, freeze-thaw, light exposure and mechanical agitation), can be valuable in determining the similarity of the products by showing a comparable degradation profile and rate, with formulation, volume, concentration and/or container differences taken into account.
- Stability studies on both drug substance and drug product should be carried out using containers and conditions that are representative of the actual storage containers and conditions.
- Drug Product with different container orientations should be included in the stability study to evaluate potential impact of protein/container interactions.
- Typically, vials are stored in both inverted and upright positions while syringes are stored horizontally.
- At time of submission, stability data is on at least 3 pilot scale batches can be provided with a commitment to place the first 3 manufacturing scale batches into the long-term stability program after approval.
- Any claims with regard to stability and compatibility cannot be extrapolated from the reference product and must be supported by data

- Data should be collected for such orientations early in product development and potentially can be used to justify the use of worst-case scenarios only for later studies
- The minimum sterility testing generally performed as a component of stability protocol for sterile products is at initial time point (release) and final testing interval (expiration).
- Alternatives to sterility testing as part of stability protocol such as replacing the sterility test with container closure integrity.
- Container closure integrity can replace sterility testing as a part of sterility protocol.

3.2. Nonclinical evaluation

- In general, this section addresses the pharmaco-toxicological studies needed to support a demonstration of biosimilarity between a proposed product and a reference product, including an assessment of the effects of any observed differences between the products, but not to independently establish the safety and effectiveness of the proposed product.

- To support biosimilarity, relevant comparative non-clinical studies should be performed before initiating clinical trials. The stepwise approach should start with extensive structural and functional characterization of both the proposed product and the reference product (i.e., in vitro studies should be conducted at first and then a decision made on whether or not additional in vivo animal studies are required. At each step, the sponsor should evaluate the extent to which there is residual uncertainty about the biosimilarity of the proposed product and identify next steps in order to address that uncertainty. Where possible, studies conducted should be designed to maximize their contribution to demonstrating biosimilarity.

- It is important to evaluate these differences. Hence, in order to design an appropriate non-clinical study programme a clear understanding of the characteristics of the reference product is required.

- Non clinical evaluation mainly includes **in vitro and/or in vivo functional assays**. In vitro assays may include, but are not limited to, biological assays, binding assays, and enzyme kinetics. In vivo assays may include the use of animal models of disease (e.g., models that exhibit a disease state or symptom) to evaluate functional effects on pharmacodynamic (PD) markers or efficacy measures.

- Batches used for the analyses should support the biosimilarity of both the clinical material used in the clinical study(ies) intended to demonstrate biosimilarity, and the to-be-marketed proposed product, to the reference product. In addition, the applicant should **justify the selection of the representative batches** of biosimilar and reference product. Importantly, the number tested should be sufficient to draw meaningful conclusions on the variability of a given parameter for both the biosimilar and the reference product.

- Differences in formulation between the biosimilar and reference product are among factors that affect the extent and nature of subsequent animal or clinical testing. Therefore, the applicant considering manufacturing changes after completing initial analytical similarity or after completing clinical testing that mean it should perform an additional analytical similarity assessment with batches manufactured by new process and establish comparability of proposed product manufactured by old and new manufacturing processes. The nature and extent of the changes may determine the extent of analytical similarity and comparability studies and any necessary additional studies.

- The following approach to non-clinical evaluation may be considered and should be tailored on **a case by-case basis to the biosimilar concerned**. In all cases, the approach taken will need to be fully justified in the non-clinical overview

3.2.1. Step 1: In vitro studies

- In order to assess any potential difference in biological activity between the biosimilar and the reference product, data from a number of comparative in vitro studies – some of which may already be available from the quality-related assays – should be provided. In light of this data overlap, it is suggested that the in vitro nonclinical studies related to characterization of the biological activity of the biosimilar be addressed alongside the related quality data in the corresponding quality module. Any other nonclinical in vitro studies should then be addressed in the relevant nonclinical modules of the dossier where they should be reviewed and discussed from the point of view of potential impact on the efficacy and safety of the biosimilar.

- As the in vitro assays may be more specific and sensitive than in vivo studies for detecting differences between the biosimilar and reference product, these assays can be considered paramount in the nonclinical comparability exercise. Moreover, available information about these assays, including sensitivity, specificity, and extent of validation, can affect the amount and type of additional animal or clinical data that may be needed to establish biosimilarity.

❖ **For such in vitro studies, the following general principles apply:**

- The studies should be **comparative** and designed to be sufficiently sensitive, specific and discriminatory to allow for the detection of (clinically) relevant differences in pharmaco-toxicological activity between the biosimilar and reference product – or, conversely, to provide evidence that any observed differences in quality attributes are not clinically relevant.
- The studies should **compare the concentration–activity/binding relationship** of the biosimilar and the reference product at the pharmacological target(s), covering a concentration range within which potential differences are most accurately detectable.

- The studies should **cover the whole spectrum** of pharmaco-toxicological aspects with potential clinical relevance for biosimilar and reference product. The applicant should discuss to what degree the in vitro assays used can be considered representative/predictive of the clinical situation according to current scientific knowledge.

❖ **The Non-Clinical in vitro program for biosimilars should usually include relevant assays for the following:**

- **Binding Assays:**

Evaluation of the primary binding events (e.g., binding to receptors, antigens, enzymes) known to be involved in the pharmaco/toxicological effects and/or pharmacokinetics (PK) of the reference product in the clinically approved indications.

- **Functional studies/determination of biological activities:**

Studies should evaluate signal transduction and/or functional activity/viability of cells or isolated tissues known to be of relevance for the pharmaco-toxicological effects of the reference product in the clinically approved indications.

N.B.: If the quality and nonclinical in vitro comparability exercises indicate relevant differences between the biosimilar and the reference product (thus making it unlikely that biosimilarity would eventually be established), then standalone development to support a full marketing authorization application should be considered instead.

3.2.2. Step 2 Determination of the need for In-Vivo Studies:

- It is important to note that, the decision of the EDA on whether or not to require such studies will be taken into account the following:

- If the quality comparability exercise and the nonclinical in vitro studies have shown high similarity and the level of residual uncertainty is considered acceptable to move to the clinical phase of the similarity exercise then an additional in vivo animal study is not considered necessary
- If a need is identified to reduce remaining uncertainties concerning the similarity (including drug safety) of a biosimilar and its reference product before the initiation of clinical evaluations then additional in vivo animal studies may be considered if a relevant animal species or other relevant models (e.g., transgenic animals, transplant models) is available – however this should only occur:
  (a) When it is expected that such studies would provide relevant additional information; and
  (b) If the needed additional information cannot be obtained using an alternative approach that does not involve in vivo animal studies.

- In this respect, **the factors to be considered when the need for in-vivo non-clinical studies are evaluated, include, but are not restricted to:**

- Qualitative and/or quantitative differences in potentially or known relevant quality attributes between the biosimilar and its reference product (for example, qualitative and/or quantitative differences in the post-translational glycosylation of proteins); and
- Relevant differences in formulation (for example, use of excipients in the biosimilar not widely used in medicinal products).

- In all cases the limitations of an in vivo study (such as sensitivity and variability) should be taken into account when interpreting results comparing the proposed product and the reference product.
- If a relevant and sufficiently sensitive in vivo animal model cannot be identified, the Applicant may choose to proceed directly to clinical studies, taking into account strict principles to mitigate any potential risk.

3.1.2. Step 3 In-vivo studies:

❖ **General aspects to be considered:**

- If Structural and Functional data are limited or there are concerns about the biosimilar product quality, general *Comparative Bridging Toxicology* studies may needed that include full animal pathology, histopathology, PK, PD and immunogenicity assessment.
- Animal studies should be designed to maximize the information obtained, for example, the duration of the study (including observation period) should be justified, taking into consideration the PK behavior of the RP and its clinical and the time to onset of formation of anti-drug antibodies (ADAs) in the test species
- The principles of the **3Rs** (replacement, refinement, reduction) should always be followed to minimize the use of animals in testing (e.g, such a study may be non-sacrificial depending on the endpoints used).

❖ **Specific aspects to be considered:**

- ***PK and/or PD studies:***

- In cases where such studies are considered necessary, the PK and/or PD of the biosimilar and the reference product should be compared quantitatively and also can be incorporated into a single animal toxicity study, where appropriate, using a dose–response assessment that includes the intended exposure in humans.
- The studies may include animal models of disease to evaluate functional effects on disease-related PD markers or efficacy measures, therefore, blood samples should be taken and stored for future evaluations of PK/toxicokinetic data if then needed. However, animal PK and PD assessment will not negate the need for human PK and PD studies.

- ***Safety studies:***

  - Safety data derived from animal toxicity studies generally are **not expected** if clinical data (e.g., from studies or marketing experience outside the Arab Republic of Egypt) using the proposed product are available (with the same proposed route of administration and formulation) that provide sufficient evidence for its safe use, unless animal toxicity studies are otherwise needed to address a specific product quality concern.
  - Where in vivo safety studies are deemed necessary, a flexible approach should be considered. If appropriately justified, repeated dose toxicity with refined design (e.g.: using just one dose level of biosimilar and Reference products and/or just one gender and /or no recovery animals) or In-Life Evaluation of Safety Parameters (clinical signs, body weights, and vital function) may be considered. Moreover, if only one dose to be evaluated, this would be selected at the high end of the dosing range and should be justified on basis of the expected toxicity of the RP.
  - Animal models are often not sensitive enough to detect small differences between the biosimilar and reference product. Moreover, the effects of reference products are often species specific. Therefore, animal toxicity studies are generally not useful if there is no animal species that can provide pharmacologically relevant data for the product. However, there may be some instances when animal data from a pharmacologically nonresponsive species (including rodents) may be useful to support clinical studies with a proposed product that has not been previously tested in human subjects (e.g., comparative PK and systemic tolerability studies).
  - If animal toxicity studies are **not warranted** particularly in situations where there are no animal species available for safety testing and based on an acceptable scientific justification, **additional comparative in vitro testing** (using human cells or tissues when appropriate) is encouraged, as human cells can provide important comparative information that complements the animal and clinical data in assessing the potential clinical effects of minor differences in structure between the biosimilar and the reference product. For example, cell-based bioactivity assays may be used to detect the potential for inducing cytokine release syndrome in vivo.

- ***Immunogenicity studies***

  - Although immunogenicity assessment in animals is generally **not predictive** for immunogenicity in humans, it may be needed for interpretation of in vivo studies. However, difference(s) in qualitative or quantitative product-related variants (e.g, glycosylation patterns, charge, excipient, aggregates, and impurities such as host-cell proteins) between biosimilar and the reference product may have an effect on immunogenic potential and on the potential to cause hypersensitivity. Additionally, determination of antibody formation against the study drugs may be required for the interpretation of PK/toxicokinetic data in cases where in vivo animal studies are needed providing useful information that may reflect

potential structural or functional differences between the two products not captured by other analytical methods

- ***Local tolerance studies:***

  - Studies on local tolerance are usually **not required**. However, if excipients introduced are with no or little experience with the intended clinical route of administration may need to be evaluated and usually evaluated as part of repeated dose toxicity study instead of the performance of separate local tolerance studies.

- ***Other studies:***

  - Reproductive and development toxicity studies – as well as genotoxicity and carcinogenicity studies– are **not warranted** when the proposed product and the reference product have been demonstrated to be highly similar through extensive structural and functional characterization and animal toxicity studies (if such studies were conducted).
  - Furthermore, tissue cross-reactivity studies are not suitable to detect subtle changes in critical quality attributes and are thus not recommended for assessing comparability.

3.2. Clinical Studies:

- Generally, the aim of clinical data is to address slight differences shown at previous steps and to confirm comparable clinical performance of the biosimilar and the reference product. Clinical data cannot be used to justify substantial differences in quality attributes
- The clinical biosimilar comparability exercise is normally stepwise procedure that should begin with comparative human PK and PD studies and a clinical immunogenicity assessment.
- The main clinical data should be generated using the biosimilar product derived from the final manufacturing process (Intended for commercial use).
- Any deviation from this recommendation needs to be justified and additional data may be required.
- Comparative clinical trials are specifically designed to rule out clinically relevant differences in safety or efficacy between the biosimilar and the RP, and to confirm biosimilarity.
- Manufacturers should consult with regulators when proposing a clinical programme solely relying on PK/PD studies.
- The clinical comparability exercise should generally include a **comparative PK study**, if the drug substance can be measured in the blood, and should also include **the measurement of PD markers** if available and also **immunogenicity data.**
- A comparative bioequivalence study involving **PK and/or PD comparability** is generally required for clinical evaluation. An adequately powered comparative efficacy and safety

trial will **not be necessary** if sufficient evidence of biosimilarity can be drawn from other parts of the comparability exercise. The need for a comparative clinical efficacy and safety trial for the proposed biosimilar (and type of trial if required) will be influenced by **factors such as**:

- How well the biosimilar can be characterized;
- The availability of suitable, sensitive and orthogonal assays for adequate analytical and functional characterization;
- The degree of analytical and functional similarity between the biosimilar and RP;
- The existence of a relevant PD parameter;
- The degree of understanding of the mechanism(s) of action of the biological product in different indications and how well these can be investigated in binding and functional in vitro tests – the contribution of each mechanism of action to the observed clinical effect is not relevant as long as it can be measured;
- Knowledge of any (potentially) unwanted immunogenicity – for example, ADA incidence and the magnitude of ADA response including level of neutralizing antibodies, and antibodies targeting endogenous substances (for example, erythropoietin and coagulation factors); and
- Whether the impurity profile or the nature of excipients of the biosimilar gives rise to clinical concerns. This is will be A by case-by-case regulator's point view.

N.B. All clinical studies of the proposed biosimilar product should be performed using materials from the final manufacturing process expected to be used in the market product if approval is granted.

2.3.1. Pharmacokinetic Studies

- The Pk study should be generally a part of the clinical comparability exercise. It should be designed to establish similar pK profiles for the biosimilar and the RP.
- If the biosimilar product and the RP have different routes of administration (most commonly intravenous and subcutaneous), its preferred to carry out the study(ies) based on the non-intravenous route because it's the more immunogenic route .Also the subcutaneous route evaluation will give sufficient data about absorption and elimination and thus it will provide more relevant information about the comparability exercise.

So, the waiver of PK Study of other approved routes of administration must be justified –for example, when the molecule has an absorption constant that is much lower than the elimination constant (flip flop kinetics).

- The design of a PK study depends on various factors, including clinical context, safety, PK characteristics of the reference product (target-mediated disposition, linear or non-linear PK, time dependency, half-life, etc.).

❖ **General considerations when performing Pharmacokinetic studies:**

1) The Sample Size:

The sample size should be appropriate, taking into account PK variability in the study population, and consideration should be given to whether a cross-over or parallel group design would be the most adequate. If appropriate population PK or PK-PD models are available for the RP in the literature, modelling and simulation can be considered for optimizing study design – for example, justification of dose(s) and selection of the most sensitive study population to detect potential PK differences, and choice of sample size.

2) The preferred population:

PK studies should preferably be performed in healthy volunteers (if considered ethical) and care should be taken to standardize the population with regard to factors that may influence variability (for example, ethnic origin, body weight and gender). If the drug substance under investigation is associated with risks or tolerability issues that are considered to be **unacceptable for healthy volunteers**, it will be necessary to perform the PK studies in patients.

3) A multiple-dose study:

- A multiple-dose study in patients is acceptable as a pivotal PK study if a single-dose study cannot be conducted in healthy volunteers due to risks or tolerability reasons or if a single-dose study is not feasible in patients.
- Multiple-dose studies may also be acceptable in **rare situations** where problems with the sensitivity of the analytical method preclude sufficiently precise plasma or serum concentration measurements after a single dose administration. However, given that a multiple-dose study is less sensitive in detecting differences in C_{max} than a single-dose study, **this will only be acceptable with sound justification**.

4) PK comparison of the biosimilar and the RP:

Should not only include the rate and extent of absorption but also a descriptive analysis of elimination characteristics – that is, clearance and/or elimination half-life – which might differ between the biosimilar and the RP. Linear (nonspecific) clearance and nonlinear

(target-mediated) clearance should be evaluated by assessment of partial areas under the curve (pAUCs)**.**

5) Acceptance criteria for the demonstration of PK similarity:

- Acceptance criteria for the demonstration of PK similarity between the biosimilar and the RP must be predefined and appropriately justified.
- It should be noted that the criteria used in standard clinical PK comparability studies (bioequivalence studies) may not necessarily be applicable to all biotherapeutic products. However, the traditional 80–125% equivalence range will in most cases be sufficiently conservative to establish similar PK profiles.

6) Correction for protein content:

May be acceptable on a case-by-case basis if pre-specified and adequately justified, with the assay results for the biosimilar and RP being included in the protocol. If adjustments for covariates are intended for parallel group studies (for example, in the case of adalimumab, stratification for body weight and gender), they should be predefined in the statistical analysis plan rather than being included in post hoc analyses.

7) Other PK studies

Such as interaction studies (with drugs likely to be used concomitantly) or studies in special populations (for example, children, the elderly and patients with renal or hepatic insufficiency), are **not required** for a biosimilar.

8) Particular consideration should be given to the analytical method selected and its ability to detect and follow the time course of the protein in a complex biological matrix that contains many other proteins.

- The method should be optimized to provide satisfactory specificity, sensitivity and a range of quantification of adequate accuracy and precision.
- The same assay should be used to detect the serum concentrations of both the biosimilar and RP.
- A single PK assay (same binding reagents and a single analytical standard, usually a biosimilar) for determining biosimilar and RP concentration in a biological matrix can be adopted based on verification of the bioanalytical comparability of the two products within the method, with supporting data**.**

9) In some cases, the presence of measurable concentrations of endogenous protein

- May substantially affect the measurement of the concentration–time profile of the administered exogenous protein.
- In such cases the manufacturer should describe and justify the approach taken to minimize the influence of the endogenous protein on the results (for example, baseline correction).

10) In some cases, it may not be possible or meaningful to establish PK similarity

Due to the nature of the substance, the route of administration (for example, intraocular administration of aflibercept or ranibizumab) or unacceptably high PK variability (for example, romiplostim). In such cases clinical similarity should be supported by PD, immunogenicity and/or other clinical parameters.

❖ **PK measures:**

Single dose Pk study		
	IV	**SC**
Primary endpoints	AUC $_{(0\text{-inf})}$	AUC $_{(0\text{-inf})}$ C_{max}
Secondary endpoints	-T_{max} -Vd : Volume of distribution -$T_{1/2}$: Half-life	
Other mandatory endpoints	Anti-drug antibodies should be measured in parallel to PK assessment using appropriate sampling time points.	

Multiple dose Pk study	
Primary endpoints	- $AUC_{0\text{-tau:}}$ Truncated area under the curve after the first administration until the second administration. - AUC I: area under the curve over dosage interval at steady state.
Secondary endpoints	- C_{max} - C_{trough} at steady state
Other mandatory endpoints	- Anti-drug antibodies should be measured in parallel to PK assessment using appropriate sampling time points.

❖ **Pharmacodynamics**

- A human PD study that demonstrates a similar effect on a relevant PD measure(s) related to effectiveness or specific safety concerns (except for immunogenicity, which is evaluated separately) represents even stronger support for a biosimilarity determination.
- It is recommended that pharmacodynamic (PD) markers are added to the pharmacokinetic studies whenever feasible. The PD markers should be selectedon the basis of their relevance to the clinical outcome. In some cases, PK studies cannot reasonably be conducted and PD markers may then play a more important role.
- The PD biomarker(s) used to measure PD response should be a single biomarkeror a composite of biomarkers that effectively demonstrate the characteristics of the product's target effects. Use of scientifically appropriate PD biomarker can reduce residual uncertainty regarding the existence of any clinically meaningful differences between products and can significantly add to the overall demonstration of biosimilarity.

When determining which biomarkers should be used to measure response, it is important to consider the following five characteristics:

1) The time of onset of change in the PD biomarker relative to dosing and its return to baseline with discontinuation of dosing.
2) The dynamic range of the PD biomarker over the exposure range to the biological product.
3) The sensitivity of the PD biomarker to differences between the proposed biosimilar product and the reference product.
4) The relevance of the PD biomarker to the mechanism of action of the drug (to the extent that the mechanism of action is known for the reference product.
5) The analytical validity of the PD biomarker assay.

- In some instances, PD biomarkers with the relevant characteristics listed above arenot identified, but the sponsor is still encouraged to incorporate PD biomarkers that achieve a large dynamic range over the concentration range in the PK evaluation because these PD biomarkers represent potential orthogonal tests that can support similarity.
- When PD biomarkers are not sensitive or specific enough to detect clinically meaningful differences, the derived PK parameters should be used as the primarybasis for evaluating similarity from a clinical pharmacology perspective, and the PD biomarkers can be used to augment the PK data.
- A combination of PK and PD similarity can be an important assessment in demonstrating that there are no clinically meaningful differences between the proposed biosimilar product and the reference product.

- ***PD measures:***

- Assessment of Biosimilarity should be based on similarity in PD using biomarkers that reflect the mechanism of drug action when PD measure has wide dynamic range over the range of the drug concentrations achieved during PK study. In such instances, full evaluation of safety and immunogenicity should still be conducted.
- Selection of time points and durations for the measure of PD biomarkers will depend on the characteristics of PD biomarkers (e.g.: Timing of PD response after administration of product based on half-life of the product and the anticipated duration of the product's effect).
- When PD response lags after initiation of Product administration, a study of multiple dose and steady state conditions can be important, especially if the proposed product is intended for long-term use. The PD biomarkers evaluated for biosimilar product and the reference should be compared by determining area under the effect cure (AUEC).
- If only one PD measurement is available because of the characteristics of the PD biomarker, the measurement should be linked to a simultaneous drug concentration measurement. The relationship of drug concentration and the PD biomarker should then be used as a basis for comparison between products.
- When available and appropriate, clinical endpoints in clinical pharmacology studies can also provide useful information about the presence of clinically meaningful differences between two products.

- ***Developing clinical pharmacology data for supporting a demonstration of biosimilarity:***

A- <u>Study Design:</u>

There are two designs are available: Cross-Over Design and Parallel Design.

Cross-over design	**Parallel design**
- Single dose, randomized study which recommended for product with **<u>short half-life (shorter than 5 days</u>**), rapid PD response (e.g.: Time of onset, maximal effect, and disappearance in conjugation with drug exposure), and low incidence of immunogenicity. - Should include full characterization of PK profile, late elimination phase. - This design is considered the most sensitive to assess PK similarity.	- It is Appropriate for Biological products have long half-life and elicit immunogenic response (especially for products where repeated exposure can lead to increase immunogenicity) may on PK/PD biosimilarity assessment. - This design is also appropriate for diseases that exhibit time-related changes associated with exposure to drug.

- For PD similarity, use multiple dose design may be appropriate when PD effect is delayed or not parallel to single-dose drug PK profile. - The time of appearance and disappearance of immunogenicity and its relation to washout period should be considered using this type of study design.	

- A clinical study or studies designed to establish statistical evidence that the proposed product is neither inferior to the reference product by more than a specified margin nor superior to the reference product by more than a (possibly different) specified margin.
- A well-designed clinical PK and PD study should include information about the Exposure and, when possible, the Exposure-Response to the biological products, which are important for assessing whether there are any potential clinically meaningful differences between two products. Determining the exposure-response to a biological product can be particularly challenging because of the complex nature and heterogeneity of biological products. An evaluation of clinical pharmacology similarity should include assessments of PK similarity, and if applicable, PD similarity.

B-Dose Selection:

The most sensitive dose should be selected to detect and to evaluate differences in the PK and PD profiles between the proposed biosimilar product and the reference product should be one most likely to provide clinically meaningful and interpretable data.

Criteria for Dose selection

1- If a study is conducted in a Patient Population, the approved dose for the reference product can be the appropriate choice, because this approved dose can best demonstrate the pharmacological effects in a clinical setting.
2- A lower dose on the steep part of the exposure-response curve is generally appropriate when PD is being measured or when Healthy Subjects are selected for evaluation (Studying doses on the Plateau of the dose response curve is unlikely to detect clinically meaningful difference between two products).
3- In certain cases, a dose selected from a range of doses can be useful for a clinical PK and PD similarity assessment. For example, if the concentration-effect relationship of the reference product is known to be highly variable or nonlinear, a range of doses can be used to assess dose response.
4- If the product can only be administered to patients, an alternative dosing regimen such as a single dose for a chronic indication or a lower dose than the approved dose may be preferable to increase the sensitivity for detecting differences if the approved dose either

results in nonlinear PK or exceeds the dose required for maximal PD effect. The appropriateness of an alternative dosing regimen will depend on certain factors, e.g., whether the lower dose is known to have the same effect as the approved dose and whether it is ethically appropriate to give lower doses not withstanding differences in effect.

An adequate justification for the selection of an alternative dosing regimen should be provided.

C-Routes of administration:

- Clinical PK and PD studies should be conducted using the same route of administration for the proposed biological product and the reference product.
- If the reference product can be administered both intravenously and subcutaneously, the evaluation of subcutaneous administration will usually be sufficient as it covers both absorption and elimination. Thus, it is possible to waive the evaluation of intravenous administration if biosimilar comparability in both absorption and elimination has been demonstrated for the subcutaneous route or other extravascular routes. Omission of the PK study of intravenous administration needs to be justified, e.g., in cases when the molecule has absorption constant 1400 which is much slower than the elimination constant (flip flop kinetics).

D-Study Population:

- The total number of subjects studied should provide adequate statistical power for PK, and, when relevant, PD similarity assessment.
- The choice of study population (Healthy subjects or Patient) should allow for an assessment of clinically meaningful differences between the proposed product and the reference product; often the study population will have characteristics consistent with those of the population studied for the licensure of the reference product for the same indication.
- However, there are cases where a study population could be different from that in the clinical studies that supported the licensure of the reference product.
- For example, if a genetic predictor of response was developed following licensure of the reference product, it may be possible to use patients with the response marker as the study population.

Healthy subjects	Patients
- Clinical PK and PD should be conducted in healthy subjects if the product can be safely administered to them. - Healthy subjects is considered to be more sensitive in evaluating the product similarity because it is likely to produce less PK and/or PD variability compared with study in patients with potential confounding factors such as	- If safety or ethical consideration prevent participation of healthy subjects in such studies for certain products (immunogenicity or known toxicity from Reference) or if PD biomarkers can only be relevant in patients with the relevant condition or disease, the clinical pharmacology studies should be conducted in such patients.

concomitant disease and concomitant medications.	

➔ **Demographic group**

Clinical pharmacology studies should be conducted in subjects or patients with **the same demographic group** (e.g.: Gender, Age, Race, marital state, etc.) most likely to provide a sensitive measure of difference between biosimilar and the Reference product.

- The sponsor should justify why the subject or patient group chosen for studies will provide adequately sensitive measure of difference between two products.

E-Statistical Comparison of PK and PD results:

- The assessment of the clinical pharmacology similarity of a proposed biosimilar product and the reference product in PK and PD studies is based on statistical evaluation. The recommended clinical pharmacology similarity assessment relies on:
  - A criterion to allow the comparison.
  - A confidence interval for the criterion, and an acceptable limit for the biosimilarity assessment.

- Sponsors should use an Average Equivalence Statistical Approach to compare PK and PD parameters for both replicate and non-replicate design studies. This average equivalence approach involves a calculation of a 90% confidence interval for the ratio between the geometric means of the parameters of the proposed biosimilar product and the reference product.
- To establish PK and/or PD similarity, the calculated confidence interval should fall within an acceptable limit. Selection of the confidence interval and the acceptable limits can vary among products. An appropriate starting point for an acceptable limit for the confidence interval of the ratio is 80–125%; if other limits are proposed, the sponsor should justify the limits selected for the proposed biosimilar product.
- There can be situations in which the results of the PK and/or PD study fall outside the pre-defined limits, that can suggest existence of differences between the proposed biosimilar product and the reference product, the sponsors should analyze and explain such findings.

❖ **Confirmatory PK and /or PD studies**

- If an adequately powered comparative efficacy trial is not necessary, comparative PK and/or PD studies may be sufficient for establishing confirmative evidence of the similar clinical performance of a biosimilar and its RP, provided that*:*
  1) The acceptance ranges for confirmatory PK and/or PD end-points are predefined and appropriately justified
  2) The PD biomarker reflects the mechanism of action of the biological product;

3) The PD biomarker is sensitive to potential differences between the proposed biosimilar and the RP
4) The PD biomarker assay is validated.
5) The applicant should consider the option of using additional PD measures **(usually as secondary end-points)** to assess the comparability of the PD properties of the RP and proposed biosimilar.
6) If relevant PD measures are not available, sensitive PD end-points may be assessed if such assessment may help to reduce residual uncertainty about biosimilarity.

- **An example of acceptable confirmatory PK/PD studies** would be the use of euglycaemic clamp studies to compare the efficacy of two insulins. In addition, absolute neutrophil count and CD34+ cell count are the relevant PD markers for assessing the activity of G-CSF and could be used in PK/PD studies in healthy volunteers to demonstrate the similar efficacy of two medicinal products containing G-CSF.
- **The study population and dosage should represent a test system that is known to be sensitive in detecting potential differences between a biosimilar and the RP.** In the case of insulin, for example, the study population should consist of non-obese healthy volunteers or patients with type 1 diabetes rather than insulin-resistant obese patients with type 2 diabetes. Otherwise, **it may be necessary to investigate more than one dose to demonstrate that the test system is discriminatory.**
- **The acceptance ranges for confirmatory PK and/or PD parameters (that is, for primary end-points) should be predefined and appropriately justified.** If PD comparison is not essential for a conclusion of biosimilarity but the results are still expected to reasonably support biosimilarity then a purely descriptive analysis of the PD results may be justified. This may be the case for biological substances that have been extensively characterized and for which biosimilarity can already be concluded from the analytical, functional and PK comparisons. If appropriately designed and performed, such PK/PD studies are usually more sensitive in detecting potential differences in efficacy than trials using hard clinical end-points. However, PD markers may also be used as end-points in clinical efficacy studies in patients.

Examples of appropriate markers include:

- Hemoglobin for measuring the efficacy of an epoetin,
- Lactate dehydrogenase (which is a sensitive biochemical marker of intravascular hemolysis) for evaluating the efficacy of a complex drug such as eculizumab.
- For denosumab, investigation of bone formation and resorption markers as part of the PK study may be useful or possibly sufficient. This would involve measurement of bone mineral density and bone turnover markers such as serum C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propertied (P1NP) after denosumab administration.

- In certain cases (for example, when analytical similarity of the active ingredient in the biosimilar and the RP can be demonstrated to such a degree that clinical differences can be excluded) a comparative PK study may provide sufficient clinical evidence to support biosimilarity. However, a risk assessment (including for example, the impurity profile) should be conducted to determine the need for additional safety/immunogenicity data on the biosimilar.

❖ **Efficacy trials**

- In the absence of surrogate markers for efficacy, it is usually necessary to demonstrate comparable clinical efficacy of the biosimilar and the RP in adequately powered, Randomized, Parallel Comparative clinical trial(s), preferably Double Blind by using efficacy endpoints.
- The Study Population should be generally representative of the approved therapeutic indication(s) of the reference and sensitive for detecting potential differences between biosimilar and the RP.
- In general, equivalence trial designs (requiring lower and upper comparability margins) are preferred for comparing the efficacy and safety of the biosimilar and RP. Non-inferiority designs (requiring only one margin) or trials with asymmetrical margins may be considered if appropriately justified. Regardless of which design is selected in a particular case, the comparability margin(s) must be pre-specified and justified on the basis of clinical relevance – that is, **the selected margin should represent the largest difference in efficacy that would not matter in clinical practice**. Treatment differences within this margin would therefore be acceptable as they would have no clinical relevance

A) Study design

- In general, an equivalence design should be used, The use of a non-inferiority design may be acceptable if justified on the basis of a strong scientific rationale and taking into consideration the characteristics of the reference product, e.g. (safety profile/tolerability, dose range, dose-response relationship).

- Non-Inferiority Trial:

- May only be accepted where the possibility of significantand clinically relevant increase in efficacy can be excluded on scientific and mechanistic grounds. However, as in equivalence trials, assay sensitivity has to be considered. A non-inferiority design could be acceptable, if justified by the applicant, for example:
  - For biological products with high efficacy (for example, a response rate of over 90%), making it difficult to set an upper margin; or
  - In the presence of a wide safety margin.

- It is recommended to discuss the use of a non-inferiority design with regulatory authorities.
- When using asymmetrical margins, the narrower limit should rule out inferior efficacy and the broader limit should rule out superior efficacy. The use of asymmetrical margins should be fully justified by the sponsor of the proposed biosimilar. Factors that would allow for the use of such margins in a clinical trial include:
    - If the dose used in the clinical study is near the plateau of the dose–response curve; and
    - There is little likelihood of dose-related adverse effects (for example, toxicity).

B) Efficacy Endpoints:

- The purpose of the efficacy trials is to confirm comparable clinical performance
- Comparability should be demonstrated in appropriately sensitive clinical models and study conditions.
- The applicant should justify that the chosen model is relevant and sensitive to detect potential differences with regard to efficacy and safety. Nevertheless, deviations from endpoints recommended in disease-specific guidelines need to bescientifically justified.
- The correlation between the “hard” clinical endpoints recommended by the guidelines for new active substances and other clinical/pharmacodynamic endpoints that are more sensitive to detect clinically meaningful differences may have been demonstrated in previous clinical trials with the reference product.
- In this case, it is not necessary to use the same primary efficacy endpoints as those that were used in the marketing authorization application of the reference product. However, it is advisable to include some common endpoints (e.g. as secondary endpoints) to facilitate comparisons to the clinical trials conducted with the reference product. Comparability margins should be pre-specified and justified on both statistical **and** clinical grounds by using the data of the reference product(see ICH topic E9 Statistical principles for clinical trials and CHMP guideline CPMP/EWP/2158/99 on the choice of the non-inferiority margin). As for all comparative clinical trial designs, assay sensitivity (see ICH topic E10) has to be considered.
- The primary or secondary end-points can also be analyzed at different time points compared to those used in clinical trials with the RP if these are considered to be more sensitive in capturing the pharmacological action(s) of the biological product – for example, adalimumab efficacy could be measured by responses at week 12 or 16 in addition to week 24.

C) The sample size and duration of the comparative clinical study

- Should both be adequate to allow for the detection of clinically meaningful differences between the biosimilar and RP.
- When a comparative clinical trial is determined to be necessary then adequate scientific justification for the choice of study design, study population, study end-point(s), estimated effect size for the RP and comparability margin(s) should be provided and may be discussed with regulators in order to obtain agreement at least in principle prior to trial initiation.

2.3.2. Clinical safety

- Clinical safety is important throughout the clinical development program and is captured during initial PK and/or PD evaluations and also as part of the pivotal clinical efficacy study. Comparative safety data should normally be collected pre- authorization to characterize the safety profile of Biosimilar product.
- Knowledge of: (a) the type, frequency and severity of adverse events/reactions when compared with the RP; (b) whether these are due to exaggerated pharmacological actions; (c) the degree of analytical and functional similarity of the biosimilar and RP; and (d) the presence of novel impurities and novel excipients in the biosimilar will all inform the type and extent of data required to characterize the safety profile of the biosimilar. Care should be given particularly to adverse events those described in SmPC of the Reference product.
- If the clinical programme for the biosimilar is limited to confirmatory PK/PD studies, this will need to be adequately justified and a risk assessment should be conducted to determine the need to obtain additional safety data for the biosimilar. Highly similar physicochemical characteristics and PK/PD profiles of the biosimilar and RP could provide sufficient reassurance that the risk of safety issues is also similar, obviating the need for further safety data.
- If the biosimilar contains impurities that are not present in the RP (for example, because of the use of a novel expression system) then the generation of further safety data may be necessary, or scientific justification should be provided as to why such data are not needed. As for all medicinal products, further monitoring of the safety of the biosimilar will be necessary in the post-marketing phase.

2.3.3. Immunogenicity:

- Immunogenicity should be investigated as part of the clinical evaluation package of the biosimilar relative to the RP unless the manufacturer can provide a scientific justification that human immunogenicity data are not needed. Such justification should be based on the degree of physicochemical similarity of the biosimilar and RP, and on a thorough risk assessment of any unwanted immunogenicity and clinical consequences known for the RP.
- The goal of the immunogenicity programme is to exclude an unacceptable/marked increase in the immunogenicity of the biosimilar that affect both the safety and the effectiveness for

example, altering PK, inducing anaphylaxis, or promoting development of neutralizing antibodies that neutralizethe product. When compared with the immunogenicity of the RP and to generate descriptive data in support of biosimilar approval and its clinical use.

- Structural, functional, and animal data are generally not adequate to predict immunogenicity in humans. Therefore, at least one clinical study that includes acomparison of the immunogenicity of the proposed product to that of the reference product is recommended. The immunogenicity study report should include data on antibody incidence, magnitude of ADA response and neutralization ability, whether antibodies are transient or persistent, and their impact on PK and clinical correlates.
- The marketing authorization application should include an integrated immunogenicity summary comprising a risk assessment and, if appropriate, the results of testing using appropriately validated and characterized assays, along with details on the clinical study duration, sampling schedules and regimen, and the clinical immunogenicity assessment.
- The immunogenicity studies should be tailored to each product and require a multidisciplinary approach taking into account both quality and clinical considerations. The risk assessment should include:
  - Accumulated information on the immunogenicity of the RP (that is, on the nature, frequency and clinical relevance of the immune response);
  - Consideration of the quality aspects (including the nature and complexity of the drug substance, non-glycosylated/glycosylated, expression system, product- and process-related impurities, and aggregates);
  - Consideration of excipients and container closure system, and stability of the product, route of administration, dosing regimen.
  - Consideration of patient- and disease-related factors (for example, immune competent/compromised and any concomitant immunomodulatory therapy).
  - Consideration of the type of product is also a critical element of the risk assessment, with the risk being higher for a product that has an endogenous non-redundant counterpart (for example, epoetin). In such cases, special attention should be paid to the possibility of the immune response seriously affecting the endogenous protein and its unique biological function, with serious adverse effects. Real-time testing for neutralizing ADAs is recommended for epoetins and other high-risk products (for example, enzyme replacement therapies and coagulation factors). Conversely, for well-characterized biological substances (for example, insulin, somatropin, filgrastim, teriparatide), where an extensive literature and clinical experience indicate that immunogenicity does not impact upon product safety and efficacy, immunogenicity studies may not be necessary, provided that the biosimilar is highly similar to the RP and the risk-based evaluation indicates a low risk. This may also

be applicable to other products, including mAbs. In such cases, manufacturers should consult with the regulatory authorities.

- If a sponsor is seeking to extrapolate immunogenicity findings for one condition of use to other conditions of use, the sponsor should consider using a study population and treatment regimen that are adequately sensitive for predicting a difference in immune responses between the proposed product and the RP across the conditions of use. Usually, this will be the population and regimen for the RP for which development of immune responses with adverse outcomes is most likely to occur.

2.3.4. Clinical evaluation:

- ADAs can affect the PK, PD, safety and/or efficacy of the administered product. The immunogenic risk of a biological is determined by the ADA incidence in the treated population and the magnitude of the unwanted clinical effect, and influences the benefit–risk balance of the therapeutic.
- If human immunogenicity data are needed, they should be generated in a comparative manner throughout the clinical programme. The sensitive patient population (that is, the population with the highest likelihood of mounting an immune response) is preferred for investigating immunogenicity. For example, if an epoetin is licensed for the treatment of renal anaemia and for patients with chemotherapy-induced anaemia, the selection of patients with renal anaemia is advised. Comparative PK and/or PD studies should be designed to also collect immunogenicity data regardless of the population to be included (for example, healthy volunteers and patients).
- A PK/PD cross-over design is possible for immunogenicity testing but if the exposure time until the switch does not provide sufficient immunogenicity data, the sponsor must ensure that a sufficient number of patients are treated without cross-over – for example, by extending the cross-over study with two parallel treatment arms, or by proposing a separate immunogenicity study.
- If ADAs are known to affect the PK of the RP then ADA rate and kinetics assessments could be performed along with assessment of their impact on PK through pre-specified subgroup analysis of ADA-negative and -positive subjects.
- Sampling during immunogenicity testing should include baseline sampling (prior to treatment) for pre-existing antibodies, sampling during treatment and in some cases post-treatment, particularly if ADAs persist or are undetectable at earlier time points (due to immunosuppressive properties of the product or technical problems such as drug interference). The sampling schedule should be synchronized for evaluation of PK as well as for assessment of safety and efficacy to provide an understanding of the impact of antibodies on clinical outcome. Generally, for chronic administration, 6-month data are acceptable to exclude excessive immunogenicity, but in some cases a longer evaluation

period may be appropriate pre-licensing to assess antibody incidence and possible clinical effects.

- Furthermore, notable differences in immunogenicity between the biosimilar and RP would require further investigation of the underlying cause, and data and justification provided to support any claim that the difference noted was not clinically relevant. An analysis of the clinical impact of ADAs in both arms on PK, efficacy and/or safety should be performed through stratified analysis of ADA-negative and -positive subjects. Any potential for the production of neutralizing antibodies against critical endogenous factors (for example, following epoetin administration) will necessitate clinical studies in patients.

N.B.: If lower immunogenicity for Biosimilar is possible, this would not preclude approval as a biosimilar. In case of reduced development of neutralizing antibodies with the biosimilar that suggest that the biosimilar is more officious that the Reference product. It is recommended to pre-specify an additional subgroup analysis of efficacy and safety in those patients that did not mount an anti-drug antibody response during the clinical trial. This will be helpful to establish efficacy of the biosimilar and the Reference product in principle similar if not impacted by immune response.

Duration of the immunogenicity study should be justified on case-by-case basis depending on the duration of treatment course, disappearance of the product from circulation and the time for emergence of humoral immune response (at least four weeks when an immunosuppressive agent is used).

The Duration of follow-up evaluation should be determined based on:

(1) The time course for the generation of immune responses (such as the development of neutralizing antibodies, cell-mediated immune responses).
(2) Expected clinical sequelae (informed by experience with the reference product).
(3) The time course of disappearance of the immune responses and clinical sequelae following cessation of therapy.
(4) The length of administration of the product

The extent and timing of clinical immunogenicity assessment will vary depending on range of factors:

- Extent of analytical similarity.
- Incidence and clinical consequences of immune Reponses for reference (If Consequences is Severe, more extensive immunogenicity assessment will needed/ If immune response to reference is rare, pre-marketing evaluation may be adequate to support similarity / In

addition, in some cases, crtain safety risks may need to be evaluated through post-marketing surveillance).

- The differences in immune responses between both products in absence of observed clinical sequelae may be of concern and may need further evaluation(extended period of Follow-up evaluation).

- **Immunogenicity testing:**

- A multi-tiered approach comprising screening and confirmatory immunoassays that detect binding ADAs followed by assays which determine ADA magnitude and neutralization potential is generally necessary and deviation from this requires justification.
- The manufacturer will need to justify the antibody-testing strategy and the choice of assays to be used. Attention should be given to the selection of suitable controls for assay validation and to the determination of cut-off points for distinguishing antibody-positive from antibody-negative samples.
- Aspects relating to potential interference by matrix components, including the pharmacological target and the residual drug in the sample, are also important. To mitigate such interference, corrective measures should be implemented. For example, for drug interference (which commonly occurs with samples taken from patients given mAbs) measures such as allowing time for clearance of the drug from the circulation prior to sampling, or incorporating steps for dissociating immune complexes and/or removal of the drug can be used. Care should be taken to ensure that the use of such measures does not compromise ADA detection or patient treatment.
- Where required, comparative immunogenicity testing should be performed using the same assay format and sampling schedule by using comparative blinded, parallel design (i.e., a head-to-head study) in treatment-naïve patients as the most sensitive design for pre-marketing study to assess potential differences in the risk of immunogenicity is recommended. -For immunogenicity assessment in new drug development, antibody testing is performed using the therapeutic given to the patient. In applying this concept to biosimilar, the development of screening assays with a similar sensitivity for the two patient groups (biosimilar and RP) within the same study is very challenging. Therefore, in the biosimilar scenario, relative immunogenicity is often assessed by using a single assay which employs the drug substance of the biosimilar as the antigen for sample testing for both groups. This approach allows for the detection of all antibodies developed against the biosimilar. The manufacturer should demonstrate the suitability of the method(s) used and provide data assuring that the method(s) measure ADA to the RP and to the biosimilar to a similar extent.
- Neutralization assays reflecting the mechanism of action are usually based on the potency assay of the product. Non-cell ligand-based assays are relevant in cases where the

therapeutic binds to a soluble ligand and inhibits its biological action. For products associated with high risk (for example, those with non-redundant endogenous homologs) and those for which effector functions are important, the use of functional cell-based bioassays is recommended. Where necessary, advice on the need for a neutralization assay and on the appropriate format to use (cell-based, ligand-based or based on enzyme activity) may be sought from regulatory authorities.

- Further characterization of antibodies (for example, isotype) should be conducted if considered clinically relevant, or in special situations (for example, the occurrence of anaphylaxis or use of certain assay formats), taking into account the immunogenicity profile of the RP. For example, if the RP does not elicit an IgE response it is unlikely that the biosimilar would elicit one if the same expression system is used. The retention of patient samples under appropriate storage conditions will be necessary for retesting in cases where technical problems occurred with the original assay.

❖ **Extrapolation of Efficacy and Safety from one therapeutic indication to another:**

- The RP may have more than one therapeutic indication. When biosimilar comparability has been demonstrated in one indication, extrapolation of clinical data to other indications of the reference product could be acceptable, but needs to be scientifically justified.
- In case it is unclear whether the safety and efficacy confirmed in one indication would be relevant for another indication, additional data will be required.
- Extrapolation should be considered in the light of the totality of data, i.e. quality, non-clinical and clinical data. The extension of indications from the RP to the biosimilar is only possible if the following requirements are fulfilled:
  - similarity in analytical characteristics and functional properties has been confirmed in sensitive orthogonal assays which provide information on the clinically relevant mechanism of action and/or involved receptor(s) as part of the comparability exercise; and
  - This is supported by clinical data (comparative PK and/or PD study) plus a comparative clinical trial performed in a patient population that allows sensitive measurement of the intended clinical parameters, if necessary.

Additional data are required in certain situations, such as:

1. The active substance of the reference product interacts with several receptors that may have a different impact in the tested and non-tested therapeutic indications.
2. The active substance itself has more than one active site and the sites may have a different impact in different therapeutic indications.
3. The studied therapeutic indication is not relevant for the others in terms of efficacy or safety, i.e., is not sensitive for differences in all relevant aspects ofefficacy and safety.

- Immunogenicity is related to multiple factors including the route of administration, dosing

regimen, patient-related factors and disease-related factors (e.g., co-medication, type of disease, immune status). Thus, immunogenicity could differ among indications. Extrapolation of immunogenicity from the studied indication/route of administration to other uses of the reference product should bejustified.

4.5. Pharmacovigilance:

The guidance provided in this part is addressing only specific Pharmacovigilance requirements in the context of registration of biosimilar products. For more details, regarding these Pharmacovigilance requirements and the other Pharmacovigilance requirements throughout product life cycle refer to the "Egyptian Good Pharmacovigilance Practice" (Egyptian GVP) which should be read **in parallel** with this guideline.

Manufacturing Variabilities:

As the Marketing authorization holders of medicinal products make frequent changes to the manufacturing process of their products post-authorization. This happens for many reasons including for example changes in source materials, facilities or regulatory requirements. In the long-term post-authorization period, the reference product, biosimilars and related products may potentially exhibit different safety profiles as these products evolve through their life-cycle.

Within the authorization procedure the applicant should demonstrate adequate pharmacovigilance system and adequate risk management plan in place in accordance with current Pharmacovigilance guideline.

Regarding the Risk management system:

As a general principle, any post-authorization update to the RMP for a reference product should be similarly applied to the relevant biosimilars and related products, and vice-versa, unless justified, all parts of a RMP – Integrated RMP- are required for a biosimilar, with the exception of RMP part II, module SI "Epidemiology of the target population".

Regarding the Risk management plan (RMP):

- **"Safety specification":**

For biosimilars and related products, the summary of safety concerns should, as a minimum, be the same as the reference product unless otherwise justified.

Immunogenicity should specifically be addressed in this context and reflected in the RMP.

- **Pharmacovigilance Plan:**
- Any specific safety monitoring imposed on the reference product should be adequately addressed in the pharmacovigilance plan of the biosimilar.
- **Regarding "Additional pharmacovigilance activities":**

Post-authorization safety studies:

Biosimilars and related products Any specific safety monitoring imposed on the reference product should be adequately addressed in the pharmacovigilance plan, unless otherwise justified (e.g., if the safety concern was specific to the reference product and not included in the safety specification of the biosimilar or related product).

- **"Risk minimization measures":**

- Risk minimization activities in place for the reference product should, in principle, be included in the RMP of the biosimilars and related products, and vice-versa. Any deviation from this (e.g., when the risk minimization is linked specifically to the reference product) should be justified.
- Evaluating the effectiveness of additional risk minimization measures is necessary to establish whether an intervention has been effective or not, and if not why and which corrective actions are necessary. The evaluation should be performed for the additional risk minimization tools individually and for the risk minimization program as a whole.
- Effectiveness evaluation should be conducted at the most appropriate time, accounting for time required for launch of the risk minimization measures, the estimated use of the product by the healthcare system and other relevant circumstances.
- To evaluate the effectiveness of additional risk minimization measures two categories of indicators should be considered, process indicators and outcome indicators.
- **Regarding post marketing pharmacovigilance activities:**

Marketing authorization holders of the biosimilar products are obligated to:

✓ Individual Case Safety Reports (ICSRs) management
✓ Periodic Benefit Risk Evaluation Report (PBRER) submission
✓ Full signal management processes
✓ Emergency Safety Issues (ESIs) management
✓ Any other pharmacovigilance activities required by EDA

V. Glossary:

ADME:	Absorption, Distribution, Metabolism, Elimination
ASMF:	Active substance master file
CMC:	Chemistry, Manufacturing and Control
EPVC:	Egyptian Pharmacovigilance Center
ICH:	International Conference on Harmonization
PD:	Pharmacodynamic
PK:	Pharmacokinetic
PSUR:	Periodic Safety Update Report
PBRER:	Periodic Benefit-Risk Evaluation Report
RMP:	Risk management plan
SMF:	Site Master File
MA:	Market Authorization
QTPP:	Quality Target Product Profile
W.D:	Working days

VI. Reference

- WHO-guidelines on evaluation of similar biotherapeutic products Annex 2, TRS 977.
- ICH S6: Pre-clinical safety Evaluation of Biotechnology-derived pharmaceutical
- ICH E8: General consideration for clinical trials
- ICH E9: Statistical principles for clinical trials
- ICH Q5C: Quality of Biotechnological products: Stability testing of Biotechnological/Biological products
- ICH Q5D: Derivation and characterization of cell substrates used for production of Biotechnological/Biological products
- ICH Q5A: Viral safety evaluation of Biotechnology products derived from cell lines of human and Animal origin
- ICH Q5B: Quality of biotechnological products: analysis of the expression construct in cells used for production of r-DNA derived protein products
- ICH Q5E: Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process
- ICH guidelines: Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
- ICHQ8(R2) Pharmaceutical Development
- ICH Q9 Quality Risk Management
- ICH Q10 Pharmaceutical Quality System
- ICH Q11- Development and manufacture of drug substances (chemical entities and biotechnological/biological entities
- EMA-Overarching biosimilar guidelines
- EMA- Product-specific biosimilar guidelines
- EMA- GUIDELINE ON SIMILAR BIOLOGICAL MEDICINAL PRODUCTS
- EMA- Other guidelines relevant for biosimilars
- EMA- Scientific Guidelines on Biological Drug substances
- EMA- Scientific Guidelines on Biological Dug Products
- FDA- Scientific Considerations in Demonstrating Biosimilarity to a Reference Product
- FDA- Comparative Analytical Assessment and Other Quality-Related Considerations
- FDA- Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product
- League of Arab States. Guideline on good pharmacovigilance practices (GVP) for Arab countries.
- The Egyptian Pharmaceutical Vigilance Centre. Guidelines. Ministry of Health and Population. http://www.epvc.gov.eg/guidelinesmd
- WHO Guidelines on procedures and data requirements for changes to approved biotherapeutic products Annex 3. TRS No. 1011