Jordan Food & Drug Administration

GUIDANCE FOR REGISTRATION OF BIOSIMILARS

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Policy statement:

While the launch of similar biological products (Biosimilar) would provide patients with potential access to affordable medicines, it is also prudent to ensure that the quality, safety and efficacy of such products are not compromised.

It is therefore important to ensure that control measures are in place to ensure the quality of the manufacturing process and of its products and to safeguard patients against possible adverse events.

So far, the European Union (EU) through the European Medicines Agency (EMA) has the most well developed regulatory framework for biosimilars and which is supported by specific guidelines, the information in this guidance is adopted from the EMA guidelines so in implementing this guideline, all the relevant Guidelines on biological products containing biotechnology-derived proteins as active substance and the Guidelines on similar biological medicinal products (also known as biosimilars), will be used as the basis for defining the registration requirements for biosimilars.

Jordan Food and Drug Administration (JFDA) reserves the right to request information or material, or define conditions not specifically described in this document, in order to ensure the safety, efficacy & quality of a therapeutic biologic product, JFDA is committed to ensure that such requests are justifiable on a science based issues & that decisions are clearly documented, as the experience and knowledge will be generated while applying these guidelines.

Also we recommend Applicants to seek advice before submitting their Biosimilar product application.

And finally, our experience demonstrates that transparent and open dialogue with all relevant stakeholders is key to put in place a robust and adapted regulatory framework in this emerging field.

Purpose:

This guideline is meant to:

- Provide assistance to applicants (industry) on how to comply with the regulations.
- Introduce the concept of biosimilars.
- Require a baseline scientific comparison of the Biosimilar with the Reference drug (the comparability requirements).
- Identify the level of clinical data that will be needed to evaluate and approve the Biosimilar.
- Focus on the Quality assessment, with head-to-head comparison to the Reference drug with full characterization of quality parameters.
- Focus on-marketing safety studies in order to monitor any potential differences in safety and efficacy between the Biosimilar and Reference drug that become apparent once a Biosimilar enters the market.
- Specify details to ensure traceability for biosimilars.
**Concept of biosimilars**

Biosimilars are not generic biologics/biogenerics, thus the classic generic approach is scientifically not applicable (i.e demonstration of bioequivalence of the generic drug with the reference product is usually appropriate to infer therapeutic equivalence) and many characteristics associated with approval process used for generic drugs do not apply to biosimilars because of their large and complex molecular structures and additional non-clinical and clinical data are usually required.

The aim of the biosimilar approach is to demonstrate close similarity of the biosimilar product in terms of quality, safety and efficacy to the reference medicinal product.

**Scope and application**

The demonstration of similarity depends upon detailed and comprehensive product characterization, therefore, information requirements outlined within this document apply to biologic drugs that contain, as the active substances, well characterized proteins derived through modern biotechnological methods such as recombinant DNA, into microbial or cell culture.

Conversely, the biosimilar approach is more difficult to apply to other types of biologics which by their nature are more complex, more difficult to characterize or to those for which little clinical regulatory experience has been gained so far. Therefore, it does not cover complex biologics such as blood-derived products, vaccines, immunologicals, gene and cell therapy products.

The manufacturer must conduct a direct and extensive comparability exercise between its product and the reference product, in order to demonstrate that the two products have a similar profile in terms of quality, safety and efficacy, Only one reference product is allowed throughout this exercise.

Eligibility for a biosimilar pathway hinges on the ability to demonstrate similarity to a reference product. Product employing clearly different approaches to manufacture than the reference product (for example use of transgenic organisms versus cell culture) will not be eligible for the regulatory pathway for biosimilars.

Approval of a product through the biosimilar pathway is not an indication that the biosimilar may be automatically substituted with its reference product. The decision for substitutability with the reference product shall be based on science and clinical data.

A biosimilar product cannot be used as a reference product by another manufacturer because a reference product has to be approved on the basis of a complete/full quality and clinical data package.

Non-clinical and clinical requirements outlined for biosimilar submission in this guidance document are applicable to biosimilars that have demonstrated to be similar to the reference
product, based on results of the comparability exercises from chemistry, manufacturing and control (CMC) perspectives. When similarity of a biosimilar cannot be adequately established, the submission of such a product should be as a stand-alone biotechnological product with complete non-clinical and clinical data.

Non-clinical and clinical issues of specific products are further elaborated in product-class specific guidelines (see Annexes) Based on the comparability approach and when supported by state-of-the-art analytical systems, the comparability exercise at the quality level may allow a reduction of the non-clinical and clinical data requirements compared to a full dossier. This in turn, depends on the clinical experience with the substance class and will be a case by case approach.

**Definitions:**

**Reference product:**
A biological medicinal product either already approved/registered in Jordan or registered in the reference countries in the EU (via the centralized procedure), this product should be registered on the basis of a complete dossier (full quality, safety & efficacy). The reference product is used in demonstrating the comparability of a biosimilar product through quality, non-clinical and clinical studies.

**Biosimilar product (or similar biological medicinal product):**
A biological medicinal product that is similar to the reference product in terms of quality, safety & efficacy through the comparability studies, having the same active substance, dosage form, concentration and route of administration of the reference product.

Biologics are large, highly complex molecular entities manufactured using living cells and are inherently variable. The manufacturing process is highly complex and critical to defining the characteristics of the final product. Maintaining batch-to-batch consistency is a challenge. Subtle variations in the production or even transport or storage conditions may potentially result in an altered safety and efficacy profile of the final product in some cases. Hence, the principle “the process is the product” is often used in reference to biologics.

Based on the current analytical techniques, two biological produced by different manufacturing processes cannot be shown to be identical, but similar at best. Therefore, the term biosimilar is appropriate.

**2.0 IMPLEMENTATION OF GUIDANCE:**

**2.1 Comparability exercise considerations:**

- Biosimilars can be approved based in part on an exercise to demonstrate similarity to an already approved reference product.
- The same reference product should be used throughout the comparability program in order to generate coherent data and conclusions.
- Comparative quality, non-clinical and clinical studies are needed to substantiate the similarity of structure/composition, quality, efficacy and safety; immunogenicity between the
biosimilar and the reference product.

- The pharmaceutical form, strength and route of administration should be the same as that of the reference product. Any differences between the biosimilar and the reference product should be justified by appropriate studies on a case-by-case basis.
- Comparability with the chosen reference product should be addressed for both the active substance and drug product.
- It is not expected that the quality attributes in the biosimilar and the reference product will be identical. For example, minor structural differences in the active substance such as variability in post-translational modifications may be acceptable, however, should be justified, supportive information demonstrating that such differences will not affect the safety and efficacy should be provided.
- Quality differences may impact the amount of non-clinical and clinical data needed, and will be a case by case approach.
- If the reference drug substance used for characterisation is isolated from a formulated reference drug product additional studies should be carried out to demonstrate that the isolation process does not affect the important attributes of the drug substance/moiety.
- Representative batches produced according to a manufacturing process with proven consistency should be used in comparability studies.
- State-of-the-art analytical techniques and validated analytical procedures which are able to detect any differences between a biosimilar product and a reference product are recommended in a comparability study.

2.2 QUALITY GUIDELINES

A biosimilar product is derived from a separate and independent master cell bank, using independent manufacturing and control method, and should meet the same quality standards as required for innovator products. A full quality dossier is always required. In addition the biosimilar manufacturer is required to submit extensive data focused on the similarity, including comprehensive side by-side physicochemical and biological characterisation of the biosimilar and the reference product.

The base requirement for a biosimilar is that it is demonstrated to be highly similar to the reference product. Due to the heterogenous nature of therapeutic proteins, the limitations of analytical techniques and the unpredictable nature of clinical consequences to structure/biophysical differences, it is not possible to define the exact degree of biophysical similarity that would be considered sufficiently similar to be regarded as biosimilar, and this has to be judged for each product independently.
Applicants should note that the comparability exercise for a biosimilar versus the reference product is an additional element to the requirements of the quality dossier and should be dealt with separately when presenting the data.
Information on the development studies conducted to establish the dosage form, the formulation, manufacturing process, stability study and container closure system including integrity to prevent microbial contamination and usage instructions should be documented.
2.2.1 Manufacturing process considerations:

The biosimilar product is defined by its own specific manufacturing process for both active substance and finished product.

The process should be developed and optimized taking into account state-of-the-art science and technology on manufacturing process & consequences on product characteristics.

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

ICH Q5D & ICH Q5A (R1) shall be followed for cell line qualification (MCB and WCB), cells at the limit of in vitro cell age used for production, recommended viral detection and identification assays, virus testing in unprocessed bulk, virus clearance and virus testing on purified bulk, evaluation and characterization of virus clearance procedures, and all other issues in this guidance.

A separate comparability exercise, as described in ICH Q5E, should be conducted whenever change is introduced into the manufacturing process during development (include improvement of process, increasing scale, improving product stability, and complying with changes in regulatory requirements), the manufacturer generally evaluates the relevant quality attributes of the product to demonstrate that modifications did not occur that would adversely impact the safety and efficacy of the drug product, such an evaluation should indicate whether or not confirmatory nonclinical or clinical studies are appropriate, as the 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 quality attributes and safety and efficacy has not been established, and differences between quality attributes of the pre- and post-change product are observed, it might be appropriate to include a combination of quality, nonclinical, and/or clinical studies in the comparability exercise.

The manufacturer(s) for biosimilar should be approved by JFDA starting from the drug substance to the final finished product according to the drug manufacturer accreditation criteria.

2.2.2 Reference product considerations:

- Appropriate comparative tests at the level of the isolated active substance from the formulated reference product are generally needed, except in some cases when quality attributes of the active substance can be tested on the finished product.
- The manufacturer should demonstrate that the active substance used in the comparability studies is representative of the active substance of the reference product.
- Comparisons of the active substance in the biosimilar product made against public domain information e.g. pharmacopoeial monographs are not sufficient to demonstrate similarity, reference standards are not appropriate for use as a reference product.
- The same reference product should be used for all three parts of the dossier (i.e Quality, Safety and Efficacy).
- The brand name, pharmaceutical form, formulation and strength of the reference product
used in the comparability exercise should be clearly identified.
- The shelf life of the reference product and its effect on the quality profile adequately addressed, where appropriate.

2.2.3 Analytical procedure considerations:
- Extensive state-of-the-art analytical methods should be applied to maximize the potential for detecting light differences in all relevant quality attributes.
- Methods used in both the characterisation studies and comparability studies should be appropriately qualified and validated [as in ICH Q2(R1)]
- If available, standards and international reference materials [e.g from European Pharmacopeia (Ph.Eur), WHO etc.] should be used for method qualification and validation.

2.2.4 Characterisations considerations:

Characterisations of a biotechnological/biological product by appropriate techniques, as described in ICH Q6B, includes the following key points:

1- **Physicochemical properties:**
   Determination of composition, physical properties also should consider the concept of the desired product (and its variants) as defined in ICH Q6B. The complexity of the molecular entity with respect to the degree of molecular heterogeneity should also be considered and properly identified.

2- **Biological activity:**
   Include an assessment of the biological properties towards confirmation of product quality attributes that are useful for characterisation and batch analysis, and in some cases, serve as a link to clinical activity. Limitations of biological assays could prevent detection of differences that occur as a result of a manufacturing process change.
   When changes are made to a product with multiple biological activities, manufacturers should consider performing a set of relevant functional assays designed to evaluate the range of activities.

3- **Immunological properties:**
   When immunological properties are part of characterisation, the manufacturer should confirm that the biosimilar product is comparable to the reference product in terms of specific properties.

4- **Purity, impurities and contaminants:**
   Should be assessed both qualitatively and quantitatively using state-of-the-art technologies and firm conclusion on the purity and impurity profiles be made.
   Accelerated stability studies of the reference and of the biosimilar product can be used to further define & compare the degradation pathways/stability profiles.
   Process-related impurities are expected, but their impact should be confirmed by appropriate studies (including non-clinical and/or clinical studies).
   Complete side by-side characterisation is generally warranted to directly compare the biosimilar and the reference product. However, additional characterisation may be indicated in some cases
   Measurement of quality attributes in characterisation studies does not necessarily entail
the use of validated assays, but the assay should be scientifically sound and provide results that are reliable. Those methods used to measure quality attributes for batch release should be validated in accordance with ICH guidelines (ICH Q2A, Q2B, Q5C, Q6B), as appropriate.

2.2.5 Setting specifications:

The analytical procedures chosen to define drug substance or drug product specifications alone are not considered adequate to assess product differences since they are chosen to confirm the routine quality of the product rather than to fully characterise it. The manufacturer should confirm that the specifications chosen are appropriate to ensure product quality. Specification limits should not be wider than the range of variability of the reference product.

2.2.6 Stability considerations:

- Proteins are frequently sensitive to changes, such as those made to buffer composition, processing and holding conditions, and the use of organic solvents.
- Accelerated and stress stability studies are useful tools to establish degradation profiles and can therefore contribute to a direct comparison of biosimilar and the reference product. Appropriate studies should be considered to confirm that storage conditions and controls are selected.
- ICH Q5C and Q 1A(R2) should be consulted to determine the conditions for stability studies.
- For a biosimilar approach, it would be worth comparing a biosimilar with reference product by accelerated stability studies as these studies at elevated temperature may provide complementary supporting evidence for the comparable degradation profile.

2.3 NON-CLINICAL AND CLINICAL GUIDELINES

2.3.1 General

The information in this section provides only general guidance on non-clinical and clinical data requirements for biosimilars, the non-clinical studies should be conducted prior to the initiation of any clinical studies. These studies should be comparative and aim to detect differences between the biosimilar and the reference product.

The requirements for the drug classes (for example: insulin, growth hormone) may vary, the requirements may also vary depending on various clinical parameters such as therapeutic index, the type and number of indications applied. Efficacy & safety for each indication will either have to be demonstrated or an extrapolation from one indication to another justified.

The final biosimilar product (using the final manufacturing process) should be used for non-clinical and clinical studies. Clinical comparability is done in stages, much like a traditional program.
Proposed indications for biosimilar must be identical or within the scope of indications granted for the reference product. In case the reference product has more than one therapeutic indication, the efficacy and safety of the biosimilar has to be justified or, if necessary, demonstrated separately for each of the claimed indications. In certain cases it may be possible to extrapolate therapeutic similarity shown in one indication to other indications of the reference product, but this is not automatic.

- The non-clinical section addresses the pharmaco-toxicological assessment.
- The clinical section addresses the requirements for PK/PD, efficacy studies.
- The section on clinical safety & pharmacovigilance addresses clinical safety studies as well as the risk management plan with special emphasis on studying immunogenicity of the biosimilar as clinical trials and a robust post marketing Pharmacovigilance are essential to guarantee the products safety and efficacy over time.

2.3.2 Non-clinical requirements

- Biosimilars should undergo appropriate non-clinical testing sufficient to justify the conduct of clinical studies in healthy volunteers or patients. These studies should be comparative and aim to detect differences between the biosimilar and the reference product and not just response per se.
- Ongoing consideration should be given to the use of emerging technologies (For e.g In vitro techniques such as e.g real-time binding assays may prove useful. In vivo, the developing genomic/proteomic microarray sciences may, in the future, present opportunities to detect minor changes in biological response to pharmacologically active substances)

**In vitro studies:**
- Receptor-binding studies or cell-based assay (e.g cell-proliferation assay) should be conducted when appropriate

**In vivo studies:**
- Animal pharmacodynamic study, relevant to clinical use
- At least one repeat-dose toxicity study, including toxicokinetic measurements, should be conducted in relevant species.
- Relevant safety observations (for e.g local tolerance) can be made during the same toxicity study.

The rationale for request of antibody measurements in the context of the repeat dose toxicity study:
- Generally, the predictive value of animal models for immunogenicity in humans is considered low. Nevertheless, antibody measurements (e.g antibody titres, neutralising capacity, cross reactivity) as part of repeated dose toxicity studies is required to aid in the interpretation of the toxicokinetic data and to help assess, as part of the comparability exercise, if structural differences exist between the biosimilar and the reference product.

Other toxicological studies, including safety pharmacology, reproductive toxicology,
mutagenicity and carcinogenicity studies are not required for biosimilar unless warranted by the results from repeated toxicological studies.

2.3.3 Clinical requirements

2.3.3.1 Pharmacokinetic (PK) studies

Comparative pharmacokinetic studies should be conducted to demonstrate the similarities in pharmacokinetic (PK) characteristics between biosimilar and the reference product.

If appropriate from an ethical point of view, healthy volunteers will in most cases represent a sufficiently sensitive and homologous model for such comparative PK studies.

choice of designs must be justified and should consider factors such as clearance and terminal half-life, linearity of PK parameters, where applicable the endogenous level and diurnal variations of the protein under study, production of neutralizing antibody, conditions and diseases to be treated, route of administration.

The acceptance range/ equivalence margin to conclude clinical comparability should be defined prior to the initiation of the study, taking into consideration known PK parameters and their variations, assay methodologies, safety and efficacy of the reference product.

Other PK studies such as interaction studies or other special populations (e.g. children, elderly, and patients with renal or hepatic insufficiency) are usually not required.

2.3.3.2 Pharmacodynamic (PD) studies

PD studies should be comparative in nature, the Parameters should be clinically relevant or a surrogate marker which is clinically validated. The PD study may be combined with a PK study and the PK/PD relationship should be characterised.

2.3.3.3 Confirmatory Pharmacokinetic/ Pharmacodynamic (PK/PD)

Comparative PK/PD studies may be sufficient to demonstrate comparable clinical efficacy, provided all the followings are met: (however, cases when approval on the basis of PK/PD data might be acceptable are highly limited):

- PK & PD properties of the reference product are well characterized.
- Sufficient knowledge of PD parameters is available.
- At least one PD marker is accepted as surrogate marker for efficacy.
- Dose response is sufficiently characterised (ICH E10).
- Equivalence margin is pre-defined and appropriately justified.
2.3.3.4 Clinical efficacy trials.

Comparative clinical trials (head-to-head adequately powered, randomized, parallel group clinical trials, so-called equivalence trials) are required to demonstrate the similarity in efficacy and safety profiles between biosimilar and the reference product. The design of the studies is important. Assay sensitivity must be ensured (ICH E10). Equivalence margins should be pre-specified, adequately justified on clinical grounds. Equivalent rather than non-inferior efficacy should be shown in order for the biosimilar to adopt the posology of the reference product and to open the possibility of extrapolation to other indications, which may include different dosages. Demonstration of similarity may also allow extrapolation of efficacy and safety data to other indications of the reference product.

2.3.3.5 Clinical safety and Immunogenicity

- The safety of biosimilar should be demonstrated to be similar to the reference product in terms of nature, seriousness and frequency of adverse events. Thus data from sufficient number of patients and sufficient study duration with sufficient statistical power to detect major safety differences are needed.
- For products intended for administration for longer than 6 months, the size of the safety database should typically conform with the recommendations of ICH E1 on the extent of population exposure to assess clinical safety.
Data from pre-approval studies are insufficient to identify all differences in safety. Therefore, safety monitoring on an ongoing basis after approval including continued benefit-risk assessment is mandatory.

Immunogenicity which is the ability of a substance to trigger an immune response in a particular organism, also the capability of a specific substance to induce the production of antibodies in the human body. Potential immunogenicity is a key issue for biosimilars and may have serious clinical consequences. In fact, all biopharmaceuticals, in contrast to conventional drugs, demonstrate a greater capacity to induce antibodies and to elicit immune reactions.

Immunogenicity may be influenced by factors related to the biopharmaceutical itself, such as manufacturing process, formulation, aggregates, contaminants and impurities, and also by factors related to the patient, the type of disease, dose and length of treatment, the route of administration or depressed immune response in cancer patients.

A written rationale on the strategy for testing immunogenicity should be provided. State-of-the-art methods should be used, validated to characterize antibody content (concentration or titre), neutralizing antibody & cross-reactivity.

Special attention should be paid to the possibility that the immune response seriously affects the endogenous protein and its unique biological function.
2.3.3.6 Pharmacovigilance Plan/Risk Management Plan (RMP)

Any post-market Risk Management Plan (RMP) should include detailed information of a systematic testing plan for monitoring immunogenicity of the biosimilar post-market. The RMP should include:
- Risk Identification and characterization (e.g. case definitions, antibody assays);
- Risk Monitoring (e.g. specific framework to associate risk with product);
- Risk Minimisation and Mitigation strategies (e.g. plans to restrict to intravenous use where necessary, actions proposed in response to detected risk etc.);
- Risk communication (e.g. minimizing and mitigation messages for patients & physicians);
- Monitoring activities to ensure effectiveness of risk minimisation.
- Detailed information of a systematic evaluation of the immunogenicity potential of the biosimilar product.
- A discussion about methods to distinguish adverse event reports from those for other licensed products, including the reference product, should be included in the RMP.
- The RMP may be maintained and implemented throughout the life-cycle of the product. Risk minimization activities may differ from region to another so it’s very important that the manufacturers should take the region specifications into consideration while setting the Risk Management plan.

The Pharmacovigilance plan must be approved prior to approval of product and the system must be in place to conduct monitoring.

The Pharmacovigilance plan should be designed to monitor and detect both known inherent safety concerns and potentially unknown safety problems that may have resulted from the impurity profiles of a biosimilar.

The Pharmacovigilance, as part of a comprehensive RMP, should include regular testing for consistent manufacturing of the biosimilar.

3.0 POST MARKET REQUIREMENTS

The Pharmacovigilance plan should be able to distinguish between and tracking different products and manufacturers of products in the same class of medicinal products (e.g. epoetins, insulins, interferons). Such capability is essential to help ensure adverse events are properly attributed to the relevant medicinal product (i.e. traceability).

Traceability of the product should involve product identification defined in terms of product name, brand name, pharmaceutical form, formulation, strength, manufacturers name and batch number(s).

Periodic Safety Update Reports (PSURs) of biosimilars should be submitted and evaluation of benefit/risk of the biosimilar post-market should be discussed. Such systems should include provisions for passive Pharmacovigilance & active evaluations such as registries and post-marketing clinical studies.

The compliance of the marketing authorization holder with their commitment and Pharmacovigilance obligations (implementation of RMP) will be closely monitored, reports should be continuously submitted to the authority (where appropriate) and SmPC should be updated whenever new findings.
4.0 ORGANISATION OF DATA / DOSSIER SUBMISSION.

The data for submission are organized according to the Common Technical Dossier (CTD), with full quality data (module 3) plus comparability exercise and abridged studies of the non-clinical (module 4) and clinical components (module 5). Biosimilar should be submitted as new DRUGS and should take NDA number & evaluated by new drugs registration Committee.

Module 1: Regional Requirements, Certificates, Information & Administrative Documents.  
Module 2: CTD file summaries.  
Module 3: Quality part (drug substance & drug product)  
Module 4: Non clinical studies  
Module 5: Clinical studies, RMP & PSUR.  
(Plasma master file should be submitted if product contains any material from plasma)  
(Also to include batch record of three consecutive batches)

5.0 INTERCHANGEABILITY.

Biosimilars are not generic products and cannot be identical, but similar to their reference products, further biosimilar do not necessarily have the same indications or clinical use as the reference product, the decision to treat a patient with a reference product or biosimilar medicine should be taken following the opinion of a qualified health professional.

Automatic substitution (i.e the practice by which a different product to that specified on the prescription is dispensed to the patient without the prior informed consent of the treating physician) and active substance-based prescription cannot apply to biologicals, including biosimilars.

6.0 NAME OF PRODUCTS

In order to facilitate effective Pharmacovigilance monitoring and tracing of adverse safety events and to prevent inappropriate substitution, the specific medicinal product (innovator or biosimilar) prescribed by the treating physician and dispensed to the patient should be clearly identified. Therefore, all biosimilars should be distinguishable by name i.e assign a brand name explicitly, using names that are not suggestive towards the originator nor towards other biosimilars, so every medicine will either have an invented (trade) name, or the name of the active substance (according to the current International Non-proprietary Name (INN) system) together with the company name/trademark.  
The approved name, together with the batch number, the country of origin & manufacturer name are important for clear identification to support adverse drug reaction reporting and monitoring of the safe use of the medicine.
7.0 LABELING / PACKAGE INSERT

The labeling of biosimilars should provide transparent information to healthcare professionals and patients on issues that are relevant to the safe and effective use of the medicinal product.

It is expected that the labeling of biosimilar meet the following criteria:

- The SmPc should be the same as the reference medicinal product, only for approved/extrapolated indications.
- A clear indication that the medicine is a biosimilar of a specific reference product.
- Invented (trade) name, or the name of the active substance together with the company name/trademark.
- Clinical data for the biosimilar describing the clinical similarity (i.e. safety and efficacy) to the reference product and in which indication(s).
8.0 **PRODUCT–SPECIFIC BIOSIMILAR GUIDLINES:**

(Should be read in conjunction with the requirements laid down in the EU Pharmaceutical legislation and with other relevant CHMP guidelines)

- Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant erythropoietins.

- Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues.

- Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human follicle stimulating hormone (r-hFSH) EMA/597110/2012

- Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant Granulocyte Colony-stimulating Factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005)

- Guideline on non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight-heparins. (EMA/134870/2012)

- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues the non-clinical and clinical requirements for Somatropin- (EMEA/CHMP/BMWP/94528/2005 )

- Guidelines on non-clinical and clinical development of of similar biological medicinal products containing recombinant Interferon alfa-containing medicinal products (EMEA/CHMP/BMWP/102046/2006)

- Guidelines on non-clinical and clinical development of of similar biological medicinal products containing recombinant Interferon beta-containing medicinal products (EMA/CHMP/BMWP/652000/2010)

- Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues (EMA/CHMP/BMWP/403543/2010)
8.1 Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant erythropoietins.

(EMEA/CHMP/BMWP/301636/2008)

INTRODUCTION.

Human erythropoietin is a 165 amino acid glycoprotein mainly produced in the kidneys and is responsible for the stimulation of red blood cell production. Erythropoietin for clinical use is produced by recombinant DNA technology using mammalian cells as expression system and termed epoetin.

All epoetins in clinical use have a similar amino acid sequence as endogenous erythropoietin but differ in the glycosylation pattern. Glycosylation influences pharmacokinetics and may affect efficacy and safety including immunogenicity. Physico-chemical and biological methods are available for characterisation of the protein.

Epoetin-containing medicinal products are currently indicated for several conditions such as anaemia in patients with chronic renal failure, chemotherapy-induced anaemia in cancer patients, and for increasing the yield of autologous blood from patients in a pre-donation programme. The mechanism of action of epoetin is the same in all currently approved indications but the dosages required to achieve the desired response may vary considerably and are highest in the oncology indications.

Epoetin can principally be administered intravenously (IV) or subcutaneously (SC). Epoetins have a relatively wide therapeutic window and are usually well tolerated provided that the stimulation of bone marrow is controlled by limiting the amount and rate of haemoglobin increase. The rate of haemoglobin increase may vary considerably between patients and is dependent not only on the dose and dosing regimen of epoetin but also other factors, such as iron stores, baseline haemoglobin and endogenous erythropoietin levels, and the presence of concurrent medical conditions such as inflammation.

Exaggerated pharmacodynamic response may result in hypertension and thrombotic complications.

Moreover, pure red cell aplasia (PRCA) due to neutralising anti-epoetin antibodies has been observed, predominantly in renal anaemia patients treated with subcutaneously administered epoetin. Because antibody-induced PRCA usually is a very rare event taking months to years of epoetin treatment to develop, such events are unlikely to be identified in pre-authorisation studies. In addition, possible angiogenic and tumour promoting effects of epoetin might be of importance in selected populations.

NON-CLINICAL STUDIES

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in the pharmaco-toxicological response between the similar biological medicinal product and the reference medicinal product and should not just assess the response per se. The approach taken will need to be fully justified in the non-clinical overview.

Pharmacodynamics studies

In vitro studies:
In order to assess any alterations in reactivity between the similar biological medicinal and
the reference medicinal product, data from a number of comparative bioassays (e.g. receptor-binding studies, cell proliferation assays), many of which may already be available from quality-related bioassays, should be provided.

**In vivo studies:**

The erythrogenic effects of the similar biological medicinal product and the reference medicinal product should be quantitatively compared in an appropriate animal assay. Information on the erythrogenic activity may be obtained from the described repeat dose toxicity study or from a specifically designed assay (e.g. the European Pharmacopoeia normocythaemic mouse assay; data may be already available from quality-related bioassays).

**Toxicological studies**

Data from at least one repeat dose toxicity study in a relevant species (e.g. rat) should be provided.

Study duration should be at least 4 weeks. The study should be performed in accordance with the requirements of the “Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals“ (CPMP/ICH/302/95) and the “Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues“ (CHMP/42832/05). Specific guidance on the design and conduct of this study can also be found in the "Note for guidance on repeated dose toxicity" (CPMP/SWP/1042/99). Appropriate toxicokinetic measurements should be performed ("Note for guidance on toxicokinetics: A guidance for assessing systemic exposure in toxicological studies", CPMP/ICH/384/95) as part of the repeat dose toxicity study and include a determination of antibody formation (“Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins”, EMEA/CHMP/BMWP/14327/2006).

Data on local tolerance in at least one species should be provided in accordance with the "Note for guidance on non-clinical local tolerance testing of medicinal products" (CPMP/SWP/2145/00). According to the “Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals” (CPMP/ICH/286/95), it is preferable to perform local tolerance testing as part of the described repeat dose toxicity study.

Safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing EPO as active substance.

**Clinical studies**

**Pharmacokinetic studies**

The pharmacokinetic properties of the similar biological medicinal product and the reference product should be compared in single dose crossover studies for the routes of administration applied for, usually including both subcutaneous and intravenous administration. Healthy volunteers are considered an appropriate study population. The selected dose should be in the sensitive part of the dose-response curve.
The pharmacokinetic parameters of interest include AUC, Cmax and T1/2 or CL/F. Equivalence margins have to be defined a priori and appropriately justified. Differences in T1/2 for the IV and the SC route of administration and the dose dependence of clearance of epoetin should be taken into account when designing the studies.

**Pharmacodynamic studies**

Pharmacodynamics should preferably be evaluated as part of the comparative pharmacokinetic studies.

The selected dose should be in the linear ascending part of the dose-response curve. In single dose studies, reticulocyte count is the most relevant and therefore recommended pharmacodynamic marker for assessment of the activity of epoetin. On the other hand, reticulocyte count is not an established surrogate marker for efficacy of epoetin and therefore not a suitable endpoint in clinical trials.

**Clinical efficacy studies**

Similar clinical efficacy between the similar and the reference product should be demonstrated in adequately powered, randomised, parallel group clinical trials. Since pharmacokinetics and dose requirements usually differ for IV and SC use, similar efficacy between the test and the reference product should be ensured for both routes of administration. This could be achieved by performing separate clinical trials for both routes or by performing one clinical trial for one route and providing adequate bridging data for the other route (see below). Confirmatory studies should preferably be double-blind to avoid bias. If this is not possible, at minimum the person(s) involved in decision-making (e.g. dose adjustment) should be effectively masked to treatment allocation.

Sensitivity to the effects of epoetin is higher in erythropoietin-deficient than non erythropoietin-deficient conditions and is also dependent on the responsiveness of the bone marrow. Patients with renal anaemia and without major complications (such as severe/chronic infections or bleeding, or aluminium toxicity), expected to relevantly impair the treatment response to epoetin, are therefore recommended as the study population. Other reasons for anaemia should be excluded. Since epoetin doses necessary to achieve or maintain target haemoglobin levels usually differ in pre-dialysis and dialysis patients, these two populations should not be mixed in the same study.

The following sections present different options and recommendations on how to demonstrate similar efficacy of two epoetin-containing medicinal products. A sponsor may choose from these options or modify them but should always provide sound scientific justification for the approach taken.

**Demonstration of efficacy for both routes of administration**

a) **Similar efficacy for both routes of administration may be demonstrated by performing two separate clinical trials.**

The combination of a ‘correction phase’ study using SC epoetin (e.g. in a pre-dialysis population) and a ‘maintenance phase’ study using IV epoetin (e.g. in a haemodialysis population) would be expected to provide a maximum of information on the biosimilar epoetin.
A correction phase study will determine response dynamics and dosing during the anaemia correction phase and is particularly suitable to characterize the safety profile related to the pharmacodynamics of the similar biological medicinal product. It should include treatment naïve patients or previously treated patients after a suitably long epoetin-free and red blood cell transfusion-free period (e.g. 3 months). In case of pre-treatment with long-acting erythropoiesis stimulating agents (such as pegylated epoetin), the treatment-free phase may need to be longer.

A maintenance phase study, on the other hand, may be more sensitive to detect differences in biological activity between the similar and the reference product, although experience suggests that correction phase studies are also likely to be sufficiently discriminatory. The study design for a maintenance phase study should minimise baseline heterogeneity and carry over effects of previous treatments. Patients included in a maintenance phase study should be optimally titrated on the reference product (stable haemoglobin in the target range on stable epoetin dose and regimen without transfusions) for a suitable duration of time (usually at least 3 months). Thereafter, study subjects should be randomised to the similar or the reference product, maintaining their pre-randomisation epoetin dosage, dosing regimen and route of administration.

Alternatively, both the SC and the IV study may be performed in the maintenance setting if appropriately justified.

In the course of both studies, epoetin doses should be closely titrated to achieve (correction phase study) or maintain (maintenance phase study) target haemoglobin concentrations. The titration algorithm should be the same for both treatment groups and be in accordance with current clinical practice.

In the correction phase study ‘haemoglobin responder rate’ (proportion of patients achieving a prespecified haemoglobin target) or ‘change in haemoglobin’ is the preferred primary endpoint. In the maintenance phase study ‘haemoglobin maintenance rate’ (proportion of patients maintaining haemoglobin levels within a pre-specified range) or ‘change in haemoglobin’ is the preferred primary endpoint. However, the fact that epoetin dose is titrated to achieve the desired response reduces the sensitivity of the haemoglobin-related endpoints to detect possible differences in the efficacy of the treatment arms. Therefore, epoetin dosage should be a co-primary endpoint in both study types.

Data for calculation of the primary efficacy endpoints should be collected during an appropriate evaluation period. A 4-week evaluation period from study month 5 to 6 in both the correction phase as well as the maintenance phase study has been found suitable in order to avoid potential carry-over effects from baseline treatment and allow full assessment of potential differences in both endpoints in the presence of stabilised haemoglobin levels and epoetin dosages. If the primary efficacy assessment is performed at an earlier time point the applicant will need to demonstrate that potential differences in efficacy have been fully captured.

Equivalence margins for both co-primary endpoints should be pre-specified and appropriately justified and should serve as the basis for powering the studies. If change from baseline in haemoglobin is used as the primary endpoint, an equivalence margin of ± 0.5 g/dL is recommended. Transfusion requirements should be included as an important secondary endpoint.
b) Another approach to demonstrate similar efficacy for both routes of administration would be to show comparable efficacy for one route of administration in a comparative clinical trial and provide comparative single dose and multiple dose PK/PD bridging data in an epoetin-sensitive population (e.g., healthy volunteers) for the other route of administration. The multiple dose PK/PD study should be at least 4 weeks in duration using a fixed epoetin dosage within the therapeutic range and change in haemoglobin as primary PD endpoint.

Since comparative immunogenicity data will always be required for SC use, if applied for, the most reasonable approach in this alternative scenario would be to perform a clinical trial using SC epoetin and to provide PK/PD bridging data for the IV route.

In this case, patients included in a SC study should be treated with test or reference ideally for a total of 12 months to obtain 12-month comparative immunogenicity data. At this point patients on the reference medicinal product should be switched to the test product and all patients followed, e.g., for another 6 months, to increase the safety and immunogenicity database of the similar medicinal product. Otherwise, regarding the design, enrolled population and endpoints of the clinical trial, the same considerations apply as stated in subsection a) above.

Demonstration of efficacy for one route of administration If only one route of administration is intended to be applied for, a single dose PK/PD study and either a correction phase or a maintenance phase study for the desired route should be performed. Regarding the design, enrolled population and endpoints of the clinical trial, the same considerations apply as stated in subsection a) above.

The lack of data in the other route of administration will be clearly reflected in the SmPC.

**CLINICAL SAFETY**

Comparative safety data from the efficacy trials are usually sufficient to provide an adequate pre-marketing safety database. Adverse events of specific interest include hypertension/aggravation of hypertension and thromboembolic events.

The applicant should submit at least 12-month immunogenicity data pre-authorisation. Principles of immunogenicity assessment are laid down in the “Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins” (EMEA/CHMP/BMWP/14327/2006). In the absence of standardized assays, concomitant immunogenicity data on the reference medicinal product are required for proper interpretation of results. The comparative phase should preferably cover the complete 12 month assessment period. For shorter comparative phases, the applicant will need to provide sound argument that this does not increase the uncertainty about the immunogenic potential of the biosimilar epoetin.

The use of a validated, highly sensitive antibody assay, able to detect both early (low affinity antibodies, especially IgM class) and late (high affinity antibodies) immune responses, is mandatory.

Detected antibodies need to be further characterized including their neutralising potential.
Retention samples for both correction phase and maintenance phase studies are recommended. Due to their rarity, neutralising antibodies or even PRCA are unlikely to be captured pre-marketing and, if occurring, would constitute a major safety concern. Although, the relevance of binding, non-neutralizing antibodies is not clear, a markedly increased frequency of such antibodies for the test product would elicit a safety concern and contradict the assumption of biosimilarity.

Since the SC route of administration is usually more immunogenic than the IV route and patients with renal anaemia constitute the population at risk for developing anti-epoetin antibody induced PRCA, the immunogenicity database should include a sufficient number of SC treated patients with renal anaemia, unless SC use in this population is not applied for.

**PHARMACOVIGILANCE PLAN**

a risk management programme/pharmacovigilance plan should be submitted. The risk management plan should particularly focus on rare serious adverse events such as immune mediated PRCA and tumour-promoting potential.

**EXTENSION OF INDICATION**

Since the mechanism of action of epoetin is the same for all currently approved indications and there is only one known epoetin receptor, demonstration of efficacy and safety in renal anaemia will allow extrapolation to other indications of the reference medicinal product with the same route of administration.
8.2 Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues
(EMA/134217/2012)

Introduction

Human insulin is a non-glycosylated, disulphide-bonded heterodimer of 51 aminoacids. Insulin analogues differ from human insulin by the substitution of aminoacids or other chemical changes such as addition of a fatty acid chain within the molecule. Insulin preparations differ mainly by their kinetic/pharmacodynamic profiles. They are usually classified as rapid-, short-intermediate-, and long-acting preparations, and are used alone or as free mixtures or premixed preparations of rapid/short-acting insulin and intermediate/long-acting insulin in various proportions.

There is extensive experience with the production of insulin for therapeutic use from animal sources, in the form of semisynthetic insulin, and through different recombinant techniques. Physico-chemical and biological methods are available to characterise the primary, secondary and tertiary structures of the recombinant insulin molecule, as well as its receptor affinity and biological activity in vitro and in vivo. Current quality guidelines on comparability provide information on the characterisation and analysis of similar biological medicinal product and its comparator. For recombinant insulins, attention should be given to product related substances/impurities and process related impurities, and in particular to desamido forms and other forms that may derive from the expression vector or arise from the conversion steps removing the C-peptide and regenerating the three-dimensional structure.

Currently available insulins are administered subcutaneously or intravenously. The effects of insulin are mediated predominantly via stimulation of the insulin receptor but insulin is also a weak natural ligand of the insulin-like growth factor-1 (IGF-1) receptor. The same receptors are known to be involved in the mechanism of action relevant for the currently approved therapeutic indications of insulins.

Antibodies to insulin occur frequently, mainly as cross-reacting antibodies. These have been rarely described to have major consequences for efficacy or safety. The potential for development of product/impurity-specific antibodies needs to be evaluated. Possible patient-related risk factors of immune response are unknown.

Non-clinical studies

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in the response to the similar biological medicinal product and the reference medicinal product and should not just assess the response per se. The approach taken will need to be fully justified in the non-clinical overview.
Pharmacodynamic studies

In vitro studies

In order to assess any differences in properties between the similar biological medicinal product and the reference medicinal product, comparative studies such as in vitro bioassays for affinity, insulin- and IGF-1-receptor binding assays, as well as tests for intrinsic activity should be performed. Partly, such data may already be available from bioassays that were used to measure potency in the evaluation of physico-chemical characteristics. It is important that assays used for comparability testing are demonstrated to have appropriate sensitivity to detect minute differences and that experiments are based on a sufficient number of dilutions per curve to characterise the whole concentration-response relationship.

In vivo studies

Comparative study(ies) of pharmacodynamic effects would not be anticipated to be sensitive enough to detect differences not identified by in vitro assays, and are normally not required as part of the comparability exercise.

Toxicological

Generally, separate repeated dose toxicity studies are not required. In specific cases, e.g. when novel or less well studied excipients are introduced, the need for additional toxicology studies should be considered. Studies regarding safety pharmacology and reproduction toxicology are not required for non-clinical testing of a biosimilar containing insulin or insulin analogues. Studies on local tolerance are not required unless excipients are introduced for which there is no or little experience with the intended route of administration. If other in vivo studies are performed, local tolerance may be evaluated as part of these studies.

Clinical studies

Pharmacology studies

Demonstration of similar pharmacokinetic and pharmacodynamic profiles is considered the mainstay of proof of similar efficacy of the biosimilar and the reference insulin. For this purpose, cross-over, preferably double-blind insulin clamp studies using single subcutaneous doses of the test and reference agents and performed at an interval of a few days to a few weeks are considered suitable. The time-concentration and time-action profiles may be studied separately or, preferably, simultaneously (in the same clamp study). Separate pharmacology studies for intravenous use, if applicable, are not required.

Study population

The study population should be homogenous and insulin-sensitive to best detect potential product-related differences and may consist of normal-weight healthy volunteers or patients with type 1 diabetes.
Besides their better availability, healthy volunteers have the advantage of relatively consistent fasting blood glucose levels but the disadvantage of presence of endogenous insulin which cannot be distinguished from exogenously administered insulin by the available assays, except for some insulin analogues. Methods for suppressing endogenous insulin or adjusting measured insulin serum concentrations for estimated endogenous insulin should be considered (see below).

Patients with type 1 diabetes recruited into clamp studies should have their serum C-peptide concentration measured to ensure absence of relevant remaining endogenous insulin secretion. It is important to establish stable and comparable baseline blood glucose and insulin levels for some time (e.g. one hour) prior to the study intervention in order to achieve comparable baseline conditions in all experiments, which is usually more difficult in patients with type 1 diabetes compared to healthy subjects. Insulin sensitivity in women may vary during the menstrual cycle and it is unclear whether this may affect study results. Thus, inclusion of only men in the studies would be justified.

**Insulin clamp studies**

There is general agreement that the euglycaemic or isoglycaemic hyperinsulinaemic clamp technique is the best available method for the measurement of insulin action. In these clamp experiments, the plasma insulin concentration is raised (e.g. by subcutaneous injection of insulin) and the blood-glucose level maintained (“clamped”) at a pre-defined level by means of a variable infusion of glucose.

Measurements of plasma insulin concentrations and glucose infusion rate (GIR) allow an estimation of the time-concentration and time-action profile and, if investigated in the same clamp study, of the dose-response relationship of an insulin preparation. For the purpose of comparing the pharmacokinetic and pharmacodynamic profiles of a biosimilar and its reference insulin, these clamp experiments will need to be conducted by experienced investigators under highly standardised conditions.

Different clamp methods and feedback algorithms for maintaining blood glucose levels exist. Clamp studies can be performed manually or using an automated procedure, e.g. the Biostator. With a Biostator the blood glucose concentration is measured continuously (every minute), and the glucose infusion rates are calculated in a computerised manner by means of a negative feedback algorithm. The major disadvantage of the Biostator appears to be its age (successor models are under development) and difficulties to maintain the system. Manual clamps, on the other hand, are associated with higher blood loss when blood glucose measurements are performed with standard laboratory methods (typical measurement intervals 5 to 10 min) and have a considerable demand for manpower. Manual clamps are also more prone to bias by the examiner compared to automatic clamps. A double-blind design is therefore strongly recommended or, if this is not possible, other means to effectively reduce potential investigator-related bias. Both techniques require substantial experience. However, both methods have been reported to provide similar and reproducible results as long as there are no rapid changes in glucose requirements, which may not be recognised in time depending on the length of intervals between the blood glucose measurements during the manual clamp.
Test conditions for a comparative clamp study need to be strictly standardised. Study subjects should undergo the clamp experiments after an overnight fast (usually 10 to 12 hours, only water allowed) and remain fasting throughout the tests to avoid a confounding effect on study results. In patients with diabetes, carry-over effects from the participants’ last pre-study insulin injection should be prevented and intravenous insulin infusion started at least 4-6 hours prior to study insulin administration to attain steady-state baseline glucose levels. Ideally, the clamp glucose target should be reached at least one hour before study insulin administration without any glucose infusion during this last hour.

Standardisation of clamp technique and factors influencing insulin sensitivity such as time of day, physical activity and food intake/diet, avoidance of alcohol, caffeinated drinks, smoking or medication other than the study medication and absence of intercurrent illness/infection or mental stress are important. Standardisation of habits may be relevant up to several days preceding the day of examination. In the test facility, the subjects should be allowed to adapt to the experimental situation (e.g. for 2 hours prior to the test) to establish a comparable metabolic situation and should stay in bed throughout the experiment in a quiet and pleasant environment. This highlights that even small details are very important. There is, however, evidence that, despite such standardisation, the first of the two clamps may be associated with a somewhat decreased insulin sensitivity, possibly due to an unavoidable increase in the test-related stress level of study subjects with the first clamp.

When healthy volunteers are used for the clamp studies, their endogenous insulin production can be suppressed, although usually not entirely, by a priming dose of rapid- or short-acting insulin, followed by a basal rate (e.g., 0.10 to 0.15 mU/min/kg). Alternatively, somatostatin has been used for maximal suppression of endogenous insulin, glucagon and growth hormone during the test period but it should be noted that somatostatin reduces insulin clearance by about 20%, thus prolonging the duration of insulin action artificially. Setting the target glucose level below the patient’s fasting glucose also helps suppress endogenous insulin production. Serum C-peptide should be measured in parallel to insulin concentrations to estimate the extent and consistency of suppression of endogenous insulin throughout the experiment. In the absence of insulin suppression, C-peptide correction methods have also been proposed but their value is unclear. Regardless which method is used, it should be justified and consistent throughout the clamp studies to ensure comparable test conditions.

The subcutaneously administered dose of the test and reference insulin should reflect commonly used therapeutic doses. For rapid-/short-acting insulins doses of 0.2 to 0.3 U/kg bodyweight and for intermediate-/long-acting insulins doses of 0.3 to 0.4 U/kg bodyweight are frequently used. The mid physiological range of hyperinsulinaemia (60-70 µU/ml), which represents the typical insulin concentration after a standard meal, has been shown to correspond to the steepest part of the dose response curve of insulin and can thus be expected to be most sensitive to detect potential differences in the time-action profiles of two insulins. All injections should preferably be performed by the same experienced investigator in order to ensure a reproducible subcutaneous injection. The site of injection, known to potentially influence the rate of absorption of insulin, should also be the same to decrease variability.

In healthy subjects the blood glucose concentrations are usually clamped 5 mg/dL below the subjects fasting glucose or at 80-100 mg/dL (4.4-5.6 mmol/L). In patients with type 1 diabetes blood glucose concentrations may also be clamped in the euglycaemic range or at typical/target fasting blood glucose levels (isoglycaemic clamp), which may exceed the
normal range for healthy subjects. Glucose levels below approximately 60 mg/dL should be avoided because they result in the stimulation of counter regulatory hormones (epinephrine, glucagon, cortisol, growth hormone) to increase blood glucose concentrations and lead to a rapid and pronounced worsening of insulin sensitivity, thus influencing the estimated time-action profile of the investigated insulin preparation.

The duration of the clamp studies needs to take into account the known duration of action of the investigated insulin preparation and its dose-dependency. The duration of action in glucose clamp studies may be defined as the time from insulin injection to GIR returning to baseline or, in patients with diabetes, of blood glucose values exceeding a predefined threshold, e.g. 150 mg/dL (8.3 mmol/l).

Clamp durations of 8 to 10 hours for rapid- and short-acting insulins and of 24 hours and more for long-acting insulins have been reported for healthy volunteers or patients with type 1 diabetes when using therapeutic doses. A rationale for the selection of the clamp duration should be provided in any case.

### Endpoints/statistical analyses

#### Pharmacokinetics

Comprehensive comparative data should be provided on the time-concentration profiles of biosimilar and the reference insulin with AUC and C\text{max} as the primary and T\text{max}, early and late T 50\% and T\text{1/2}, as secondary pharmacokinetic endpoints. Alternatively to early and late T50\%, other measures (e.g. AUC0-T\text{max}) may be used, as appropriate. For the primary endpoints AUC and C\text{max}, the 90\% confidence interval of the ratio test/reference should lie within 80\% to 125\%, the conventional acceptance range for bioequivalence, unless otherwise justified. For the other parameters descriptive statistics would be appropriate.

#### Pharmacodynamics

The glucose-infusion rate (GIR) over time describes the time-action profile of an insulin preparation. GIR\text{AUC} and GIR\text{max} should be measured as primary and T GIR\text{max}, and early and late T\text{GIR}50\% as secondary pharmacodynamic endpoints. Alternatively to early and late T\text{GIR}50\%, other measures (e.g. GIR-AUC0-T\text{max}) may be used as appropriate. Calculation of 95\% confidence intervals will be required for PD T\text{max} parameters. Equivalence margins should be pre-defined and justified.

It is not easy to control the blood glucose concentrations during the clamp study. Depending on the measurement intervals and feedback algorithm, and due to the inherent measurement delay between sampling and resetting the glucose infusion and the subsequent delay of change in blood glucose levels in response to GIR changes, blood glucose values usually do not correspond to the exact target value but vary around it. In response to that, variations (“noise”) in GIR occur. The Applicant should provide an estimate of the quality of the performance of the clamp study, e.g. by calculating the coefficient of variation of the blood glucose concentrations. The mean intra-individual coefficient of variation of well executed euglycaemic hyperinsulinaemic clamps should usually not exceed 10\% for glucose infusion rate. The noise of the GIR measurements can be reduced by fitting a mathematical model. The algorithm for GIR adjustment should be predefined and the appropriateness of the applied smoothing method demonstrated.
Specifics of long-acting insulin preparations

Long-acting insulin preparations are intended to produce a time-concentration profile which, as far as possible, approximates physiological basal insulin secretion. For long-acting insulins with a very flat pharmacokinetic profile, determination of $C_{\text{max}}$ and $T_{\text{max}}$ (for insulin and GIR) may be difficult to assess and may even become meaningless. For long-acting insulins with a slow decline in insulin action, together with the unavoidable variations of the GIR, it may be difficult to determine the duration of action, particularly in healthy subjects with interfering endogenous insulin. Therefore, patients with type 1 diabetes are more suitable to determine the time-action profile of long-acting insulins. Insulin sensitivity may increase over time in long-term clamp studies, which may affect GIR. However, when strict standardisation of the test conditions (as described above) is implemented, a similar increase in insulin sensitivity over time in the same individual would be expected in both treatment phases of the cross-over study and should thus not impair the comparison of the biosimilar with the reference insulin.

Despite these limitations and the increased intra-subject variability of long-acting compared to short-acting insulins, the hyperinsulinaemic euglycaemic clamp has been successfully used for the comparison of the pharmacokinetic and pharmacodynamic profiles of currently approved long-acting insulin preparations. It should be noted that clamp studies for long-acting insulins may need to be of substantial duration (e.g. for insulin glargine in the clinically relevant dosage range, the duration of action is close to 24 hours in patients with type 1 diabetes). Taken together, hyperinsulinaemic euglycaemic/isoglycaemic insulin clamps, with some limitations, may be appropriate to compare the time-concentration and time-action profiles of long-acting biosimilar and reference insulins but will usually require a relatively large sample size and a long duration for the purpose of demonstrating similarity.

Clinical efficacy
There is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered sensitive enough for the purpose of showing biosimilarity of two insulins.

Clinical safety
Convincing demonstration of similar physicochemical characteristics, pharmacokinetic and pharmacodynamic profiles of the biosimilar and the reference insulin will already provide reasonable reassurance that adverse drug reactions which are related to exaggerated pharmacological effects (hypoglycaemia) can be expected at similar frequencies. Therefore, the main focus of the safety study is the evaluation of immunogenicity, although similarity in the adverse event profile, e.g. with regard to hypoglycaemia and local tolerability, of the biosimilar and the reference product should also be confirmed. Immunogenicity studies should always include a reasonable number of patients with type 1 diabetes. If a mixed population is included, stratification for type of diabetes and pre-existing anti-insulin antibodies is necessary. The study duration should be at least 12 months, including a comparative phase of at least 6 months. The primary outcome measure should be the incidence and titres of antibodies to the test and reference medicinal products but there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity.
The potential impact of antibodies, if detected, on glycaemic control, insulin requirements and safety, especially local and systemic hypersensitivity reactions, should be investigated, and the necessity for further characterisation, e.g. with regard to their neutralising potential, considered.

**Pharmacovigilance plan**
Within the authorisation procedure the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should take into account identified and potential risks associated with the use of the reference product and, if applicable, safety in indications licensed for the reference product that are claimed based on extrapolation. In addition, it should be discussed in detail how these safety concerns will be addressed in post-marketing follow-up.

**Extrapolation of indication**

Demonstration of similar pharmacokinetic and pharmacodynamic profiles of the biosimilar and the reference product and absence of safety issues such as excessive immunogenicity with subcutaneous use will allow extrapolation of efficacy and safety data to intravenous use, if applicable, and to other indications and patient populations licensed for the reference product.
8.3 Guideline on non-similar biological medicinal products containing recombinant human follicle stimulating hormone (r-hFSH) EMA/CHMP/BMWP/671292/2010

Introduction

The marketing authorisation application dossier of a new r-hFSH-containing medicinal product claimed to be similar to a reference medicinal product already authorised in the EU needs to provide the demonstration of comparability of the product applied for to this reference medicinal product. Follicle stimulating hormone (FSH) is a pituitary glycoprotein hormone that plays a key role in regulating reproductive function in both males and females. FSH is a heterodimeric hormone composed of two linked subunits. The alpha subunit (92 amino acids) is common to other glycoprotein hormones whereas the beta subunit (111 amino acids) is specific. Both subunits contain oligosaccharide structures. As a consequence of carbohydrate variability, different isoforms of hFSH with different sialic acid content exist. Isoforms with a high sialic acid content remain longer in circulation. Physico-chemical and biological methods are available for characterisation of the protein.

Recombinant human FSH (r-hFSH) is used in assisted reproductive technologies (ART) for women to stimulate growth and recruitment of ovarian follicles, and for men to induce and maintain spermatogenesis. It is administered by subcutaneous or, in some cases, intramuscular injections.

The most important side effect of FSH treatment in ovarian stimulation is the occurrence of ovarian hyperstimulation syndrome (OHSS). This possibly life-threatening condition is characterised in its most serious forms by ascites, haemoconcentration, coagulation and electrolyte disorders and extreme ovarian enlargement. High number of follicles recruited and high estradiol levels (released from matured follicles) are risk factors for the development of OHSS.

Immunogenicity of r-hFSH appears to be low and so far, neutralising antibodies have not been reported. Generalised hypersensitivity reactions were observed in 0.2% and <1/10,000 patients treated with two different approved r-hFSH products. Local reactions were observed more frequently (3% and >1/10 of patients treated with two different rhFSH products).

Non-clinical studies

Non-clinical studies should be performed before initiating clinical development. These studies should be comparative in nature and should be designed to detect differences in the response between the similar biological medicinal product and the reference medicinal product and should not just assess the response per se. The approach taken will need to be fully justified in the non-clinical overview.

Pharmacodynamic studies

in vitro

In order to evaluate potential differences in pharmacodynamic properties between the biosimilar and the reference medicinal product, comparative in vitro bioassays for receptor affinity and activation
should be performed (such data may already be available from bioassays submitted as part of the quality dossier). Two principal approaches exist for this purpose. First, primary granulosa cells or sertoli cells can be used. Second, permanently cultured cells (e.g. CHO) stably transfected with the human FSH receptor may be constructed. The advantage of the first approach is that the FSH receptor is investigated in its natural context. A drawback is that the number of cells is limited which in turn limits the number of replicates and the number of different r-hFSH concentrations that can be tested to obtain reliable concentration-response relationships. The second approach, although providing enough material, relies on an artificial construct (transfected cells). Appropriate sensitivity of the assay used for comparability testing to detect potential differences should be demonstrated and experiments should be based on a sufficient number of dilutions per curve to characterise the whole concentration-response relationship. Binding studies including on-off-kinetics should be provided as well as measures of receptor activation i.e. plasminogen activator production (only in the classical granulosa cell assay) or intracellular cAMP accumulation. Other endpoints are conceivable (e.g. reporter gene activation).

The Applicant should justify the approach taken.

**in vivo**

FSH is a highly glycosylated protein and in vitro studies may not fully reflect the more complex situation in vivo. Hence, to qualify any potential differences between the biosimilar FSH and the reference product, the need for additional comparative in vivo studies should be considered.

Currently, the potency of r-hFSH-containing products is evaluated by calibration against an international standard (or an internal reference standard calibrated against the international standard; Steelman-Pohley assay). As the in vivo potency of both the biosimilar and the reference product may be evaluated in such a way, the number of different assays performed may be reduced by a study design in which the biosimilar and the reference medicinal product are compared and simultaneously calibrated against the reference standard. This reduces inter-assay variation and is more economical with regard to reagents and animals used. The Steelman-Pohley assay is only expected to establish biological activity but not to reveal small differences in potency between reference product and biosimilar. If feasible, an evaluation of safety endpoints, e.g. body weight and local tolerance, could be included within the framework of the in vivo pharmacodynamic studies. If a different bioassay – for example an ex vivo assay such as whole follicle culture or primary granulosa cell culture - is used to compare pleiotropic effects of FSH in a natural tissue environment, this should be justified. Such an approach would further reduce the number of animals needed, circumvent inter-animal variability and would give the possibility for multiple pharmacodynamic readouts.

**Toxicological studies**

Generally, separate repeated dose toxicity studies are not required. In specific cases, e.g. when novel or less well studied excipients are introduced, the need for additional toxicology studies should be considered.
Safety pharmacology and reproduction toxicology studies are not required for non-clinical testing of similar biological medicinal products containing r-hFSH as active substance. Studies on local tolerance are not required, unless excipients are introduced for which there is no or only little experience with the intended route of administration. If other in vivo studies are performed, evaluation of local tolerance may be evaluated as part of these studies.

**Clinical studies**

**Pharmacokinetic studies**

The relative pharmacokinetic properties of the similar biological medicinal product and the reference medicinal product should be determined in a single dose cross-over study using subcutaneous injections. With respect to the general study design, the Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98) should be taken into account. Healthy female volunteers are considered appropriate. Suppression of endogenous FSH production with a GnRH agonist or a combined oral contraceptive is recommended. The dose of r-hFSH should be justified, taking into account that a dose in the linear part of the dose response curve is suitable to detect potential differences in the pharmacokinetic profiles of the biosimilar and the reference medicinal product. The pharmacokinetic parameters of interest are AUC, Cmax, tmax, t1/2 and clearance. For the primary endpoints AUC and Cmax, the 90% confidence interval of the ratio test/reference should lie within 80% to 125%, the conventional acceptance range for bioequivalence, unless otherwise justified. For the other parameters descriptive statistics would be appropriate. Separate pharmacology studies for intramuscular use, if applicable, are not required.

**Pharmacodynamic studies**

PD parameters should be investigated as part of the phase III trial.

**Clinical efficacy**

Clinical comparability regarding efficacy between the similar and the reference biological medicinal product should be demonstrated in an adequately powered, randomised, parallel group clinical trial. The recommended model for the demonstration of comparability of the test and the reference product is the stimulation of multifollicular development in patients undergoing superovulation for ART such as in vitro fertilisation (IVF), gamete intrafallopian transfer (GIFT) or zygote intrafallopian transfer (ZIFT). The first treatment cycle should be used for comparison of efficacy. Double-blind trials are recommended. If the performance of a double-blind trial is not feasible, blinded assessment of study outcomes that might be particularly affected by subjective factors, such as ultrasound examinations and parameters of oocyte/embryo quality, should be carried out. The r-hFSH dose should be fixed for the first 5 days of stimulation. A GnRH agonist or GnRH antagonist protocol can be used. “Number of oocytes retrieved” is the recommended primary endpoint. Equivalent efficacy between the test product and the reference product should be demonstrated and equivalence margins prospectively defined and justified. It should be taken into account that over-stimulation as well as understimulation
can result in cycle cancellation and a number of zero oocytes retrieved (primary endpoint). Thus, the data should be presented in such a way that a detailed comparison of the reasons for cancellation of ART cycles is possible.

As an alternative possibility, demonstration of non-inferiority for “ongoing pregnancy rate at least 10 weeks after embryo transfer” is also an acceptable primary endpoint. In the latter case, “number of oocytes retrieved” should be included as co-primary endpoint with an appropriate equivalence margin, or as the most important secondary endpoint.

With regard to secondary endpoints, the following issues should be taken into account:

• If number of oocytes is chosen as the primary endpoint, ongoing pregnancy rate after at least 10 weeks after embryo transfer should be evaluated as secondary endpoint.

• In ART cycles, the dose of FSH has to be adjusted based on ovarian response which might obscure product-specific differences. Thus, dose adjustments and possible differences between the dosages of the similar biological product and the reference product should be carefully considered. Secondary endpoints covering this issue, such as total dose of r-hFSH required, number of days of r-hFSH stimulation and percentage of patients with need to increase or lower the dose of r-hFSH, should be investigated. Major differences with regard to dose requirements between the similar biological product and the reference product would not be in accordance with the concept of biosimilarity.

• Parameters supporting comparable pharmacodynamic properties of the similar biological product and the reference product should be investigated. The respective endpoints should include number and size distribution of follicles during treatment and at the day of ovulation induction. A further endpoint covering the initial PD effect of r-hFSH on the ovary could be the number of follicles after 5 days of FSH stimulation (before dose adjustments). In addition, serum levels of inhibin-B, estradiol, luteinizing hormone and progesterone should be measured.

• Markers of oocyte/embryo quality should be included. Number of good quality oocytes/embryos should be documented.

**Clinical safety**

Data from the efficacy trial will usually be sufficient to characterise the adverse event profile of the biosimilar product.

An adverse reaction of special interest is ovarian hyperstimulation syndrome (OHSS). All events of OHSS should be carefully recorded, using a grading system (mild, moderate, severe) and also distinguishing between early and late onset OHSS.

Immunogenicity of a therapeutic protein is more likely when given intermittently than continuously and the subcutaneous route of administration is more immunogenic than the intravenous one. Both of these factors may apply to r-hFSH as women may receive more than one ART cycle. Therefore, immunogenicity data should be provided on all women included in
the efficacy trial and also on women exposed for more than one ART cycle. Immunogenicity testing should continue up to three months after r-hFSH treatment using validated antibody assays of adequate sensitivity and specificity. The potential impact of FSH-antibodies, if detected, on efficacy and/or safety should be assessed and the necessity for further characterisation, e.g. with regard to their neutralising potential, considered.

**Pharmacovigilance**

Within the authorisation procedure the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should take into account identified and potential risks associated with the use of the reference product and, if applicable, safety in indications licensed for the reference product that are claimed based on extrapolation. In addition, it should be discussed in detail how these safety concerns will be addressed in post-marketing follow-up.

**Extrapolation of indication**

Demonstration of similar efficacy and safety of the test compared to the reference product for stimulation of multifollicular development in patients undergoing superovulation for ART will allow extrapolation to other therapeutic indications approved for the reference product.
8.4 Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant Granulocyte Colony-stimulating Factor (rG-CSF) (EMEA/CHMP/BMWP/31329/2005)

INTRODUCTION

Human G-CSF is a single polypeptide chain protein of 174 amino acids with O-glycosylation at one threonine residue. Recombinant G-CSFs produced in E. coli (filgrastim) and in CHO (lenograstim) are in clinical use. Compared to the human and to the mammalian cell culture derived G-CSF, the E. coli protein has an additional amino-terminal methionine and no glycosylation. The rG-CSF protein contains one free cysteinyl residue and two disulphide bonds. Physico-chemical and biological methods are available for characterisation of the protein.

Effects of G-CSF on the target cells are mediated through its transmembrane receptor that forms homo-oligomeric complexes upon ligand binding. Several isoforms of the G-CSF receptor arising from alternative RNA splicing leading to differences in the intracytoplasmic sequences have been isolated. One soluble isoform is known. However, the extracellular, ligand-binding domains of the known isoforms are identical. Consequently, the effects of rG-CSF are mediated via a single affinity class of receptors.

Antibodies to the currently marketed E. coli derived rG-CSF occur infrequently. These have not been described to have major consequences for efficacy or safety. RG-CSF is administered subcutaneously or intravenously. Possible patient-related risk factors of immune response are unknown.

NON-CLINICAL STUDIES

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in pharmaco-toxicological response between the similar biological medicinal product and the reference medicinal product - not just the response per se. The approach taken will need to be fully justified in the non-clinical overview.

Pharmacodynamic studies

In vitro studies:

At the receptor level, comparability of test and reference medicinal product should be demonstrated in appropriate in vitro cell based bioassays or receptor-binding assays. Such data may already be available from bioassays that were used to measure potency in the
evaluation of biological characteristics in module 3. It is important that assays used for comparability will have appropriate sensitivity to detect differences and that experiments are based on a sufficient number of dilutions per curve to fully characterise the concentration-response relationship.

**In vivo studies:**
In vivo rodent models, neutropenic and non-neutropenic, should be used to compare the pharmacodynamic effects of the test and the reference medicinal product.

**Toxicological studies**

Data from at least one repeat dose toxicity study in a relevant species should be provided. Study duration should be at least 28 days. The study should be performed in accordance with the requirements of the "Note for Guidance on Repeated Dose Toxicity" (CPMP/SWP/1042/99) and include (i) pharmacodynamic measurements and (ii) appropriate toxicokinetic measurements in accordance with the "Note for Guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicological studies" (CPMP/ICH/384/95).

In this context, special emphasis should be laid on the investigation of immune responses to the products.

Data on local tolerance in at least one species should be provided in accordance with the "Note for Guidance for Non-clinical Local Tolerance Testing of Medicinal Products" (CPMP/SWP/2145/00). If feasible, local tolerance testing can be performed as part of the described repeat dose toxicity study.

Safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing recombinant G-CSF as active substance.

**CLINICAL STUDIES**

**Pharmacokinetic studies**

The pharmacokinetic properties of the similar biological medicinal product and the reference medicinal product should be compared in single dose crossover studies using subcutaneous and intravenous administration. The primary PK parameter is AUC and the secondary PK parameters are Cmax and T1/2. The general principles for demonstration of bioequivalence are applicable.

**Pharmacodynamic studies**

The absolute neutrophil count (ANC) is the relevant pharmacodynamic marker for the
activity of r-G-CSF. The pharmacodynamic effect of the test and the reference medicinal products should be compared in healthy volunteers. The selected dose should be in the linear ascending part of the dose-response curve. Studies at more than one dose level may be useful. The CD34+ cell count should be reported as a secondary PD endpoint. The comparability range should be justified.

**Clinical efficacy studies**

rG-CSF can be used for several purposes such as:

- Reduction in the duration of neutropenia after cancer chemotherapy or myeloablative therapy
  followed by bone marrow transplantation.

- Mobilisation of peripheral blood progenitor cells (PBPCs);

- For treatment of severe congenital, cyclic, or idiopathic neutropenia

- Treatment of persistent neutropenia in patients with advanced human immunodeficiency virus (HIV) infection

The posology varies between these conditions.

The recommended clinical model for the demonstration of comparability of the test and the reference medicinal product is the prophylaxis of severe neutropenia after cytotoxic chemotherapy in a homogenous patient group (e.g. tumour type, previous and planned chemotherapy as well as disease stage). This model requires a chemotherapy regimen that is known to induce a severe neutropenia in patients. A two-arm comparability study is sufficient in chemotherapy models with known frequency and duration of severe neutropenia. If other chemotherapy regimens are used, a three arms trial, including placebo, may be needed. The sponsor must justify the comparability delta for the primary efficacy variable, the duration of severe neutropenia (ANC below 0.5 x 10⁹/l). The incidence of febrile neutropenia, infections and the cumulative r-G-CSF dose are secondary variables. The main emphasis is on the first chemotherapy cycle.

Demonstration of the clinical comparability in the chemotherapy-induced neutropenia model will allow the extrapolation of the results to the other indications of the reference medicinal product if the mechanism of action is the same. Alternative models, including pharmacodynamic studies in healthy volunteers, may be pursued for the demonstration of comparability if justified. In such cases, the sponsor should seek for scientific advice for study design and duration, choice of doses, efficacy / pharmacodynamic endpoints, and comparability margins.
Clinical Safety

Safety data should be collected from a cohort of patients after repeated dosing preferably in a comparative clinical trial. The total exposure should correspond to the exposure of a conventional chemotherapeutic treatment course with several cycles. The total follow up of patients should be at least 6 months. The number of patients should be sufficient for the evaluation of the adverse effect profile, including bone pain and laboratory abnormalities. Immunogenicity data should be collected according to the principles described in the “Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues” (EMEA/CPMP/42832/05/).

PHARMACOVIGILANCE PLAN
Within the authorisation procedure the applicant should present a risk management programme pharmacovigilance plan in accordance with current EU legislation and pharmacovigilance guidelines. Attention should be paid to immunogenicity and potential rare serious adverse events, especially in patients undergoing chronic administration. Lack of efficacy should also be monitored, especially in individuals undergoing haematopoietic progenitor cell mobilisation.
8.5 Guideline on non-clinical and clinical development of similar biological medicinal products containing low-molecular-weight-heparins. (EMA/134870/2012)

1. Introduction

Heparin is a highly sulphated and heterogeneous member of the glycosaminoglycan family of carbohydrates consisting of various disaccharide units. The most common disaccharide unit is composed of a 2-O-sulfated ρ-L-iduronic acid and 6-O-sulfated, N-sulfated ρ-D-glucosamine, IdoA(2S)GlcNS(6S). Endogenous heparin is synthesised in the granules of mast cells and possesses the highest negative charge density of all known biological molecules.

Heparin used for therapeutic purposes is sourced from domestic animals, mainly from porcine intestinal mucosa.

Heparin catalyzes the inhibition of several serine proteases of the plasmatic coagulation system by antithrombin (AT). For the binding of heparin to AT, a pentasaccharide sequence, which contains a 3-O-sulphated glucosamine residue, is important. Upon binding to the enzyme inhibitor antithrombin, heparin causes a conformational change in the antithrombin molecule which results in its active site being exposed for inhibition of activated coagulation factors. Furthermore, heparin acts as a catalytic template to which the inhibitor and activated serine proteases such as thrombin and factors (F) IXa and XIa bind. This effect depends essentially on the number of monosaccharides in the heparin molecule. Heparin molecules containing fewer than 18 monosaccharides do not catalyze inhibition of thrombin but still inactivate factor Xa(FXa). Heparin enhances the rate of thrombin–antithrombin reaction at least a thousand-fold resulting in a stable 1:1 complex after the serine-protease attacks a specific Arg-Ser peptide bond in the reactive site of antithrombin.

In addition, heparin has numerous other plasmatic and cellular interactions, but overall, in comparison with the anticoagulatory effect, the clinical relevance of these interactions is uncertain and insufficiently investigated.

Heparin is administered parenterally, as it is degraded when taken orally. It can be injected intravenously, intra-arterially or subcutaneously, whereas intramuscular injections should be avoided because of the risk of inducing hematomas. Low molecular weight heparins (LMWHs) are prepared from unfractionated heparin by various chemical or enzymatic depolymerisation processes. Thus, the starting material of LMWHs is of biological origin and the manufacturing process defines the characteristics of the drug substance. The complexity of LMWH results largely from the nature of the starting material (unfractionated heparin extracted from porcine mucosa or other animal tissues), the extraction, the fractionation and the production processes. Several state of the art methods for physico-chemical characterisation of LMWH products are available. However, although the inhibition of activated FXa activity and the inhibition of thrombin activation reflect the main anticoagulant activities of LMWH, it is presently not clear to which extent the multiple
different polysaccharides contribute to the clinical effects relevant for efficacy and safety of LMWH.

A specific LMWH differs from unfractionated heparin and may differ from other LMWHs in its pharmacokinetic and pharmacodynamic properties. As a result of the depolymerisation process LMWHs are mainly enriched in molecules with less than 18 monosaccharide units. This reduction of molecule size is associated with a loss of thrombin inhibition activity in comparison to standard heparin and an increased inhibition of FXa. Due to difficulties in the physical detection of LMWH, conventional pharmacokinetic studies cannot be performed. Instead, the absorption and elimination of LMWHs are studied by using pharmacodynamic tests, including the measurement of anti-FXa and anti-FIIa activity.

there are several authorised LMWHs that differ in their source material, manufacturing process, pharmacokinetic/pharmacodynamic properties and therapeutic indications, which include treatment and prophylaxis of deep venous thrombosis and prevention of complications of acute coronary syndromes (unstable angina, non-ST elevation myocardial infarction (non-STEMI) and myocardial infarction with ST elevation (STEMI)).

The most common adverse reactions induced by heparins are bleedings, whilst the most serious one is the rarely observed Heparin-induced thrombocytopenia type II (HIT II). This antibody-mediated process is triggered by the induction of antibodies directed against neoantigens of platelet-factor 4 (PF4)-heparin complexes. Binding of those antibody-PF4-heparin complexes may activate platelets and generate thrombogenic platelet microaggregates. Patients developing thrombocytopenia are in danger of arterial and venous thromboembolic complications (heparin-induced thrombocytopenia and thrombosis, HITT). Although the risk of these adverse reactions appears to be reduced in comparison to unfractionated heparin, it is obligatory to monitor the platelet count regularly in all patients using LMWH and to test for PF4-heparin complex-antibodies in those who develop thrombocytopenia or thromboembolic complications during heparin treatment.

In conclusion, the heterogeneity of LMWH is high, the structure-effect relationship is presently not fully elucidated and the PD markers anti-FXa and anti-FIIa activity may not fully reflect/predict efficacy.

Thus, clinical trials will usually be necessary to address remaining uncertainties resulting from the physicochemical and biological comparison.

**Non-clinical studies**

Non-clinical studies should be performed before initiating clinical trials. The studies should be comparative in nature and should be designed to detect differences in the response between the biosimilar and the reference LMWH and not just assess the response *per se*. The approach taken will need to be fully justified in the non-clinical overview.
Pharmacodynamic studies

**In vitro studies:**
In order to compare pharmacodynamic activity of the biosimilar and the reference LMWH, data from a number of comparative bioassays (based on state of the art knowledge about clinically relevant pharmacodynamic effects of LMWH and including, at least, evaluations of anti-FXa and anti-FIIa activity) should be provided. If available, standardised assays (e.g. in accordance with the European Pharmacopoeia) should be used to measure activity. Such data may already be available from bioassays submitted as part of the quality dossier.

**In vivo studies:**
If physicochemical and biological characterisation of the biosimilar and the reference LMWH has been performed with a high level of resolution and convincingly demonstrated close similarity, in vivo studies are not required as part of the comparability exercise. otherwise, the in vivo pharmacodynamic activity of the biosimilar and the reference LMWH should be quantitatively compared in:

- An appropriate in vivo pharmacodynamic model, which takes into account state of the art knowledge about clinically relevant pharmacodynamic effects of LMWH and includes, at least, an evaluation of anti-FXa, and anti-FIIa activity and of release of tissue factor pathway inhibitor. and/or
- In accordance with the intended clinical indication(s), either a suitable animal venous or an arterial thrombosis model.

Toxicological studies

Generally, separate repeated dose toxicity studies are not required.

In specific cases, e.g. when novel or less well studied excipients are introduced, the need for additional toxicology studies should be considered. The conduct of toxicity studies to assess unspecific toxicity only, based on impurities is not recommended. A priori biosimilar and reference product are expected to be highly similar, which should be demonstrated with physicochemical methods. Impurities, such as proteins should be kept at a minimum in accordance with pharmacopoeial monographs, which is the best strategy to minimize any associated risk.

Studies regarding safety pharmacology, and reproduction toxicology, are not required for non-clinical testing of a biosimilar containing LMWH. Studies on local tolerance are not required unless excipients are introduced for which there is no or little experience with the
intended route of administration. If other in vivo studies are performed, local tolerance may be evaluated as part of these studies.

Clinical studies
Pharmacokinetic/Pharmacodynamic studies

Due to the heterogeneity of LMWHs conventional pharmacokinetic studies cannot be performed. Instead, the absorption and elimination characteristics of LMWHs should be compared by determining pharmacodynamic activities (including anti FXa and anti-FIIa), as surrogate markers for their circulating concentrations. In addition other pharmacodynamic tests such as Tissue Factor Pathway Inhibitor (TFPI) activity, as well as the ratio of anti-FXa and anti-FIIa activity should be compared. Assessment of these PD parameters will provide an important fingerprint of the polysaccharidic profile. These pharmacokinetic/pharmacodynamic properties of the similar biological medicinal product and the reference product should be compared in a randomized, single dose two way crossover study in healthy volunteers using subcutaneous administration. In case the originator product is also licensed for the intravenous or intra-arterial route, an additional comparative study should be performed via the intravenous route. The selected doses should be in the sensitive part of the dose-response curve and within the recommended dose ranges for the different indications. Equivalence margins should be pre-specified and appropriately justified.

Clinical efficacy

A comparative clinical efficacy trial will usually be required as part of the comparability exercise. Only if similar efficacy of the biosimilar and the reference product can be convincingly deduced from the comparison of their physicochemical characteristics, biological activity/potency and PD fingerprint profiles, based on the use of highly sensitive and specific methods, then a dedicated efficacy trial may be waived. It is expected that this is an exceptional scenario since the required amount of reassurance from analytical data and bioassays would be considerable. Therapeutic equivalence should be demonstrated in an adequately powered, randomized, double-blind, parallel group clinical trial. In theory, this could be done either in the setting of prevention of venous or arterial thromboembolism, or in the setting of treatment of venous thromboembolism. However, the most sensitive model to detect potential differences in efficacy between the biosimilar LMWH and the reference product should be selected. Surgical patients have the highest prevalence of venous thromboembolism (VTE). Furthermore, the vast majority of published trials have been performed in surgical patients with high VTE risk, especially in patients with hip and knee surgery, and thus the
knowledge about influence of types of surgery, duration of trials and risks for bleeding is the most accurate for this patient population.

Therefore, it is recommended to demonstrate efficacy in the prevention of VTE in patients undergoing surgery with high VTE risk. Preferably, the trial should be conducted in major orthopaedic surgery such as hip surgery. In this clinical setting, patients with hip fracture should be well represented in the study as they have both high thrombotic risk and high perioperative bleeding risk. The posology and administration should follow European recommendations for prophylaxis with the reference product in patients requiring prolonged VTE prophylaxis. The Guideline on clinical investigation of medicinal products for prophylaxis of high intra and post-operative venous thromboembolic risk (CPMP/EWP/707/98), although intended for novel medicinal products, may contain useful information for the conduct of such a trial. However, for the purpose of investigating potential product-related differences in efficacy between the biosimilar and the reference product, the patient population should ideally be as homogenous as possible.

In the VTE-prevention setting, the clinically most relevant composite endpoint consists of proximal deep vein thrombosis (DVT), pulmonary embolism (PE) and VTE-related death to demonstrate patient benefit. However, for the purpose of biosimilarity testing, a composite endpoint consisting of total number of thromboembolic events (total DVTs, including asymptomatic distal DVT, PE and VTE-related 213 death) may be used. Adjudication of VTE events should be performed by a central independent and blinded committee of experts.

Equivalence margins have to be defined a priori and appropriately justified, both on statistical and clinical grounds. The study should be powered to show therapeutic equivalence on one of the two composite endpoints mentioned above.

State of the art imaging technique should be used for the endpoint assessment. While proximal DVTs could be diagnosed with high specificity and sensitivity using ultrasonography, a clear assessment of distal DVT is only possible by using bilateral venography. Thus, this invasive diagnostic procedure would be mandatory in trials including total DVT in the endpoint.

The most relevant components of the primary endpoint (in particular proximal DVTs, PE and VTE-related deaths) should favorably support the biosimilarity of the two products.

Assessment of the primary endpoint should be performed at the time of occurrence of symptoms suggestive of VTE or, in asymptomatic patients, at end of treatment. The overall follow-up should be at least 60 days to detect late thrombotic events.

**Clinical safety**

Human safety data on the biosimilar will usually be needed pre-authorisation, even if similar
efficacy can be concluded from the comparative data on physicochemical characteristics, biological activity/potency and PD fingerprint. Comparative safety data from the efficacy trial will be sufficient to provide an adequate pre-marketing safety database. Care should be taken to compare the type, frequency and severity of the adverse reactions between the similar biological medicinal product and the reference product. Major bleeding events and clinically relevant non-major bleeding events should be carefully assessed and documented. A consistent and clinically relevant classification of bleedings should be used. Similar to the efficacy evaluation, the adjudication of bleeding events by a central independent and blinded committee of experts, using pre-specified limits should be performed. Liver function testing is recommended. Sufficient reassurance will be needed that the biosimilar LMWH is not associated with excessive immunogenicity compared to the reference product. For the detection of the immune-mediated type of Heparin-induced Thrombocytopenia (HIT Type II) monitoring of platelet count and an adequate diagnostic procedure (including determination of PF4-Heparin complex antibodies) in patients developing thrombocytopenia and/or thromboembolism (HITT) during the trial has to be performed. Monitoring of antibodies in all patients participating in the trials is not necessary. Since the frequency of immune-mediated HIT II is usually very low (< 0.1%) such events are not usually expected to occur in pre-authorisation clinical trials.

Pharmacovigilance plan

Within the authorisation procedure the applicant should present a risk management plan in accordance with current EU legislation and pharmacovigilance guidelines. The RMP of the biosimilar should take into account identified and potential risks associated with the use of the reference product and, if applicable, safety in indications authorised for the reference product that are claimed based on extrapolation. Rare serious adverse events known to be associated with LMWHs such as Heparin-induced Thrombocytopenia Type II (HIT II, HITT) as well as anaphylactoid and anaphylactic reactions should specifically be discussed in the risk management plan.

Extrapolation of indication

Demonstration of comparable efficacy and safety in surgical patients at high risk for VTE as recommended or by other means as described above may allow extrapolation to other indications of the reference medicinal product if appropriately justified by the applicant.
8.6 Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues the non-clinical and clinical requirements for Somatropin- (EMEA/CHMP/BMWP/94528/2005 )

INTRODUCTION

The Marketing Authorisation (MA) application dossier of a new recombinant human growth hormone (rhGH, somatropin) claimed to be similar to a reference medicinal product already authorised shall provide the demonstration of comparability of the product applied for to a reference medicinal product authorised in the EU.

The principal bioactive human growth hormone (hGH) is a single chain non-glycosylated 191 amino acid, 22 kDa polypeptide produced in the anterior pituitary gland. Growth hormone for clinical use has an identical amino acid sequence and is produced by recombinant technology using E. coli, mammalian cells or yeast cells as expression system. The structure and biological activity of somatropin can be characterised by appropriate physico-chemical and biological methods. Several techniques and bioassays are available to characterise both the active substance and product-related substances/impurities such as deamidated and oxidized forms and aggregates.

Growth hormone has potent anabolic, lipolytic and anti-insulin effects (acute insulin-like effect). The effects of GH are mediated both directly (e.g. on adipocytes and hepatocytes) and indirectly via stimulation of insulin-like growth factors (principally IGF-1). Somatropin-containing medicinal products are currently licensed for normalising or improving linear growth and/or body composition in GH-deficient and certain non GH-deficient states. The same receptors are thought to be involved in all currently approved therapeutic indications of rhGHs.

Somatropin has a wide therapeutic window in children during the growth phase whereas adults may be more sensitive for certain adverse effects. Antibodies to somatropin have been described, including, very rarely, neutralising antibodies. Problems have been associated with the purity and stability of the formulations. Somatropin is administered subcutaneously; possible patient-related risk factors of immune response are unknown.

NON-CLINICAL STUDIES

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in the pharmaco-toxicological response between the similar biological medicinal product and the reference medicinal product and should not just assess the response per se. The approach taken will need to be fully justified in the non-clinical overview.
**Pharmacodynamics studies**

*In vitro* studies:

In order to assess any alterations in reactivity between the similar biological medicinal and the reference medicinal product, data from a number of comparative bioassays (e.g. receptor-binding studies, cell proliferation assays), many of which may already be available from quality-related bioassays, should be provided.

*In vivo* studies:

An appropriate *in vivo* rodent model (e.g. the weight-gain assay and/or the tibia growth assay in immature hypophysectomized rats; data may already be available from quality-related bioassays) should be used to quantitatively compare the pharmacodynamic activity of the similar biological medicinal and the reference medicinal product.

**Toxicological studies**

Data from at least one repeat dose toxicity study in a relevant species (e.g. rat) should be provided.

Study duration should be at least 4 weeks. The study should be performed in accordance with the requirements of the "Note for guidance on repeated dose toxicity" (CPMP/SWP/1042/99) and include appropriate toxicokinetic measurements in accordance with the "Note for guidance on toxicokinetics: A Guidance for assessing systemic exposure in toxicological studies" (CPMP/ICH/384/95). In this context, special emphasis should be laid on the determination of immune responses.

A Guidance for assessing systemic exposure in toxicological studies" (CPMP/ICH/384/95). In this context, special emphasis should be laid on the determination of immune responses.

Data on local tolerance in at least one species should be provided in accordance with the "Note for guidance on non-clinical local tolerance testing of medicinal products" (CPMP/SWP/2145/00). If feasible, local tolerance testing can be performed as part of the described repeat dose toxicity study.

Safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing rhGH as active substance.

**CLINICAL STUDIES**

**Pharmacokinetic studies**

The relative pharmacokinetic properties of the similar biological medicinal product and the reference medicinal product should be determined in a single dose crossover study using subcutaneous administration. Healthy volunteers are considered appropriate but suppression
of endogenous GH production e.g. with a somatostatin analogue should be considered. The primary pharmacokinetic parameter is AUC and the secondary parameters are Cmax and T1/2. Comparability margins have to be defined a priori and appropriately justified.

**Pharmacodynamic studies**

Pharmacodynamics should preferably be evaluated as part of the comparative pharmacokinetic study. The selected dose should be in the linear ascending part of the dose-response curve. IGF-1 is the preferred pharmacodynamic marker for the activity of somatropin and is recommended to be used in comparative pharmacodynamic studies. In addition, other markers such as IGFBP-3 may be used. On the other hand, due to the lack of a clear relationship between serum IGF-1 levels and growth response, IGF-1 is not a suitable surrogate marker for the efficacy of a somatropin in clinical trials.

**Clinical efficacy studies**

Clinical comparability efficacy between the similar biological medicinal product and the reference medicinal product should be demonstrated in at least one adequately powered, randomised, parallel group clinical trial. Clinical studies should be double-blind to avoid bias. If this is not possible, at minimum the person performing height measurements should be effectively masked to treatment allocation. Sensitivity to the effects of somatropin is higher in GH-deficient than non-GH-deficient conditions. Treatment-naïve children with GH deficiency are recommended as the target study population as this provides a sensitive and well-known model. Study subjects should be pre-pubertal before and during the comparative phase of the trial to avoid interference of the pubertal growth spurt with the treatment effect. This may be achieved e.g. by limiting the age/bone age at study entry. It is important that the study groups are thoroughly balanced for baseline characteristics, as this will affect the sensitivity of the trial and the accuracy of the endpoints. (Change in) height velocity or (change in) height velocity standard deviation score from baseline to the pre-specified end of the comparative phase of the trial is the recommended primary efficacy endpoint. Height standard deviation score is a recommended secondary endpoint. Adjustment for factors known to affect the growth response to somatropin should be considered.

During the comparative phase of the study, standing height should be measured at least 3 times per subject at each time point and the results averaged for analyses. The use of a validated measuring device is mandatory. Consecutive height measurements should be standardised and performed approximately at the same time of the day, by the same measuring device and preferably by the same trained observer. These recommendations aim
to reduce measurement errors and variability. For the determination of reliable baseline growth rates, it is important that also height measurements during the pre-treatment phase are obtained in a standardised manner using a validated measuring device.

Due to significant variability in short-term growth, seasonal variability in growth and measurement errors inherent in short-term growth measurements, the recommended duration of the comparative phase is at least 6 months and may have to be up to 12 months.

Calculation of pre-treatment growth rates should be based on observation periods of no less than 6 and no more than 18 months. Comparability margins have to be pre-specified and appropriately justified, primarily on clinical grounds, and serve as the basis for powering the study.

4.3 CLINICAL SAFETY

Data from patients in the efficacy trial(s) are usually sufficient to provide an adequate pre-marketing safety database.

The applicant should provide comparative 12-month immunogenicity data of patients who participated in the efficacy trial(s) with sampling at 3-month intervals and testing using validated assays of adequate specificity and sensitivity. In addition, adequate blood tests including IGF-1, IGFBP-3, fasting insulin and blood glucose should be performed.

4.4 PHARMACOVIGILANCE PLAN

Within the authorisation procedure the applicant should present a risk management programme / pharmacovigilance plan in accordance with current EU legislation and pharmacovigilance guidelines. This should take into account risks identified during product development and potential risks, especially as regards immunogenicity, and should detail how these issues will be addressed in post-marketing follow-up.

4.5 EXTENSION OF INDICATION

Demonstration of efficacy and safety in GH-deficient children may allow extrapolation to other indications of the reference medicinal product if appropriately justified by the applicant.
8. 7 Guidelines on non-clinical and clinical development of recombinant Interferon alfa-containing medicinal products (EMEA/CHMP/BMWP/102046/2006)

1. INTRODUCTION

Human interferon-alfa 2a or 2b are well-known and characterized proteins consisting of 165 amino acids. The non-glycosylated protein has a molecular weight of approx. 19,240 D. It contains two disulfide bonds, one between the cysteine residues 1 and 98, and the other between the cysteine residues 29 and 138. The sequence contains potential O-glycosylation sites. Physico-chemical and biological methods are available for characterisation of the proteins.

Recombinant Interferon alfa 2a or 2b is approved in a wide variety of conditions such as viral hepatitis B and C, leukaemia, lymphoma, renal cell carcinoma and multiple myeloma. The sub-types Interferons alfa 2a and 2b have different clinical uses. IFN-alfa is used alone or in combination.

Interferon alfa may have several pharmacodynamic effects. The relative importance of these effects in the different therapeutic indications is unknown. In general, interferon-alfa 2a or 2b use in oncology indications has reduced considerably and been superseded by other treatments.

The dose and treatment regimen required to achieve the desired response vary considerably between different therapeutic indications.

Interferon alfa is commonly used subcutaneously although it can also be used through intramuscular or intravenous route. Treatment with Interferon alfa 2a or 2b is associated with a variety of adverse reactions such as flu-like illness, fatigue, and myalgia. In addition Interferon alfa is associated with psychiatric, haematological and renal adverse effects.

Therapy with IFN-alfa 2a or 2b may induce development of auto-antibodies. A variety of immune-mediated disorders such as thyroid disease, rheumatoid arthritis, systemic lupus erythematosus, neuropathies and vasculitis have been observed with the therapeutic use of Interferon alfa.

Both non-neutralising and neutralising antibodies against the administered Interferon alfa have been observed.

NON-CLINICAL STUDIES

Before initiating clinical development, non-clinical studies should be performed. These studies would be comparative in nature and designed to detect differences in the pharmacotoxicological response between the similar Interferon alfa and the reference Interferon alfa and not just assess the response per se. The approach taken will need to be fully justified in the non-clinical overview.
Pharmacodynamics studies

In vitro studies:

In order to compare differences in biological activity between the similar and the reference medicinal product, data from a number of comparative bioassays could be provided (e.g. receptor-binding studies, antiviral effects in cell culture, antiproliferative effects on human tumour cell lines), many of which may already be available from bioassays submitted as part of the quality dossier. Wherever possible, analytical methods should be standardised and validated according to relevant guidelines.

The limitations of studying anti-viral effects in cell culture systems expressing HCV, however, should be recognised, as the results do not correlate well with clinical response. Wherever possible, standardised and validated assays should be used to measure activity and potency.

In vivo studies:

To support the comparability exercise for the sought clinical indications, the pharmacodynamic activity of the similar and the reference medicinal product could be quantitatively compared in:
- an appropriate pharmacodynamic animal model (e.g. evaluating effects on pharmacodynamic markers as for example serum 2',5'-oligoadenylate synthetase activity).
If feasible, these measurements may be performed as part of the toxicological studies described below or
- a suitable animal tumour model (e.g. nude mice bearing human tumour xenografts) or
- a suitable animal antiviral model.

Toxicological studies

Data from at least one repeat dose toxicity study in a relevant species should be considered (for example, human Interferon alfa may show activity in the Syrian golden hamster). The study duration should be at least 4 weeks.

The study should be performed in accordance with the requirements of the “Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals” (CPMP/ICH/302/95) and the “Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (CHMP/42832/05)“.

Specific guidance on the design and conduct of this study can also be found in the "Note for guidance on repeated dose toxicity" (CPMP/SWP/1042/99). Appropriate toxicokinetic measurements should be performed ("Note for guidance on toxicokinetics: A guidance for assessing systemic exposure in toxicological studies", CPMP/ICH/384/95) as part of the repeat dose toxicity study and include a determination of antibody formation (“Guideline on

Data on local tolerance in at least one species should be provided in accordance with the "Note for guidance on non-clinical local tolerance testing of medicinal products" (CPMP/SWP/2145/00). If feasible, local tolerance testing can be performed as part of the described repeat dose toxicity study.

Safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing recombinant human Interferon alfa as active substance.

CLINICAL STUDIES

Pharmacokinetic studies

The pharmacokinetic properties of the similar and the reference medicinal product could be compared in single dose crossover studies using subcutaneous and intravenous administration in healthy volunteers. The recommended primary pharmacokinetic parameter is AUC and the secondary parameters are C and T1/2 or CL/F.

Equivalence margins have to be defined a priori and appropriately justified.

Pharmacodynamic studies

There are a number of PD markers, such as β2 microglobulin, neopterin and serum 2´, 5´-oligoadenylate synthetase activity, which are relevant to the interaction between Interferon alfa and the immune system. The selected doses should be in the linear ascending part of the dose-response curve. Whereas the relative importance of these effects in the different therapeutic indications is unknown a comprehensive comparative evaluation of such markers following administration of test and reference products could provide useful supporting data.

EFFICACY

Patient population

The mechanism of action of interferon comprises of several different unrelated effects. Demonstration of similar efficacy between test and reference products is required. This could be performed in treatment-naïve patients with chronic hepatitis C (HCV) as delineated by the indication for the reference product. Other patient population(s) might be studied depending on the indications desired (see under Extrapolation of evidence).

Study design and duration:

A randomised, parallel group comparison against the reference product over at least 48
weeks is recommended. If possible, the study should be double-blind at least until data to complete the primary analysis have been generated. If this is not feasible, justification should be provided and efforts to reduce/eliminate bias should be clearly identified in the protocol.

The posology (i.e., dose, route and method of administration) should be the same as for the reference product. IFN-alfa should be given in line with the current standard treatment for chronic HCV infection and in accordance with the SmPC of the reference product.

The study could be designed so that the primary efficacy analysis is performed at week 12 for all enrolled patients. Preferably, a homogeneous population is recommended (e.g. one single HCV genotype). However, if a mixed population is chosen, it should be stratified based on the HCV genotype.

**Endpoints**

Primary: Virologic response as measured by the proportion of patients with undetectable levels of HCV RNA by quantitative PCR at week 12. The assay used to measure HCV RNA and the cut-off applied should be justified. A 2-log decrease in viral load may be a co-primary endpoint.

Secondary: virologic response at week 4 and end-of-treatment; sustained virologic response (24 weeks after completion of treatment); change in liver biochemistry including transaminase levels and morbidity.

**SAFETY**

Safety data should be collected from patients after repeated dosing in a comparative clinical trial over the treatment period plus 24 weeks of follow-up. The number of patients should be sufficient for the comparative evaluation of the adverse effect profile. Laboratory abnormalities for immune mediated disorders should be included. The safety profile should be similar to the reference products for the common adverse events (such as flu-like illness, alopecia, myalgia, leucopenia, anaemia and thrombocytopenia).

**Immunogenicity**

Comparative immunogenicity data (antibody levels) should be presented during the treatment period plus 24 weeks of follow-up according to the principles described in the “Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues” (EMEA/CPMP/42832/05/) and the “Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins” (EMEA/CHMP/BMWP/14327/2006).

Antibodies, if present, should be further evaluated e.g., for neutralising capacity and the
resulting potential for impact on efficacy of r-IFN-alfa. In addition, any potential for neutralisation of the effect of endogenous interferon(s) (i.e., development of autoimmunity) should be addressed. Any impact of immunogenicity should be thoroughly evaluated in those individuals:

- not responding to treatment
- losing response during primary treatment
- exhibiting unexpected adverse reactions or known immune-mediated events.

6. EXTRAPOLATION OF EVIDENCE

In principle extrapolation from one therapeutic indication to another is appropriate where the mechanism of action and/or the receptor are known to be the same as the condition(s) for which similarity in efficacy has been established. If indication(s) are sought, where the mechanism of action is not known to be the same, such extrapolation should be adequately justified.

7. PHARMACOVIGILANCE PLAN

Within the authorisation procedure the applicant should present a risk management programme/pharmacovigilance plan in accordance with current EU legislation and pharmacovigilance guidelines.

Attention should be paid to immunogenicity and potentially rare and/or delayed serious adverse events, especially in patients undergoing chronic administration. Safety should be collected from patients representing all approved indications.
8.8 - Guidelines on non-clinical and clinical development of similar biological medicinal products containing recombinant Interferon beta-containing medicinal products (EMA/CHMP/BMWP/652000/2010)

Introduction (background)

Medicinal products containing recombinant IFN-β are currently approved for the first-line treatment of multiple sclerosis (MS); they differ with respect to their molecular structure, injection route, recommended posology, and MS indications.

Recombinant IFN-β-1a is a single glycosylated polypeptide chain containing 166 amino acids. Two products are available, one is administered subcutaneously and the other intramuscularly.

Recombinant IFN-β-1b is produced as a single non-glycosylated polypeptide chain of 165 amino acids with no methionine at the N-terminus and an amino acid substitution at position 17 and is administered subcutaneously.

Medicinal products containing recombinant IFN-β are currently indicated for patients with relapsing MS including those at high risk of developing MS after a single demyelinating event. The mechanism of action of IFN-β in MS is not well established but it has been hypothesized that it acts as an immunomodulator by 1) interfering with T-cell activation in several ways, including downregulating the expression of Type II MHC molecules, inhibiting the production of pro-inflammatory cytokines by Th1 cells, promoting the production of anti-inflammatory cytokines by Th2 cells, activating suppressor T-cells and 2) inhibiting permeability changes of the blood brain barrier and the infiltration of T-cells into the central nervous system (CNS).

The clinical effects of recombinant IFN-β in relapsing MS (RMS) are modest with decreases in the frequency of exacerbations by approximately 30% as compared with placebo and inconsistent results on the progression of disability.

All products are associated with similar adverse reactions which may affect patient adherence to therapy; the most frequent are influenza-like symptoms (fever, chills, arthralgia, malaise, sweating, headache, and myalgia). Injection site reactions and asymptomatic liver and white blood cell abnormalities occur more frequently with the subcutaneous products at the recommended dose regimens. Less common adverse reactions include depression and autoimmune disorders manifested as thyroid or liver dysfunction. All products induce the development of antibodies, and in particular neutralising antibodies (NAb); in clinical trials, the incidence of NAb has been shown to range widely, from 5% for intramuscular IFN-β-1a given weekly to 45% for subcutaneous IFN-β-1b given every other day. Most Nabs develop in the first year of therapy and they have the potential to impact clinical outcomes after 18-24 months of treatment.
Non-clinical studies

As regards non-clinical development, a stepwise approach should be applied to evaluate the similarity of the biosimilar and reference medicinal product. Non-clinical studies should be performed before initiating clinical trials. In vitro studies should be conducted first and a decision then made as to the extent of what, if any, in vivo work will be required. The approach taken will need to be fully justified in the non-clinical overview.

In vitro studies

In order to assess any differences in biological activity between the biosimilar and the reference medicinal product, data from a number of bioassays/pharmacological studies should be provided (e.g. receptor-binding studies; assays for characterisation of antiviral, anti-proliferative and immunomodulatory effects), some of which may already be available from assays submitted as part of the quality dossier (for IFN-β-1a, the requirements of the European Pharmacopoeia monograph Interferon beta-1a concentrated solution apply). These studies should be comparative in nature and should be designed to be sensitive enough to detect differences in the response between the biosimilar and the reference medicinal product and should not just assess the response per se. They should be performed with an appropriate number of batches of product representative of that intended to be used in the clinical trial.

Wherever possible, analytical methods should be standardised and validated according to relevant guidelines (e.g. evaluation of antiviral effects in cell culture in accordance with the provisions of the European Pharmacopoeia, general chapter 5.6 Assay of interferons).

In vivo studies

Generally, in vivo studies in animals are not recommended.

Only when the outcome of the quality evaluation and/or the in vitro bioassays/pharmacological studies leave uncertainties about the comparability of the biosimilar and reference medicinal product, the need for additional studies should be considered.

In vivo studies should be designed to specifically address the remaining uncertainties identified. These could include an in vivo pharmacological study and/or a general repeated dose toxicity study in a relevant species.

Further studies in a pharmacologically responsive animal species should only be considered when it is expected that such studies would provide relevant additional information.
Clinical studies

The clinical comparability exercise should follow a stepwise approach starting with pharmacokinetic and pharmacodynamic studies (PK & PD) and continuing with efficacy and safety studies.

Pharmacokinetics

The pharmacokinetic properties of the biosimilar and reference products should be compared in a crossover study using the route of administration applied for. Healthy volunteers are considered an appropriate study population. The selected dose should be in the linear part of the dose-concentration curve; if available information on the reference product is too scarce, more than one dose should preferably be tested. The choice of a single or repeated dose (e.g. three doses over a week) regimen should be justified; a single dose is preferred as long as the bioanalytical method is sufficiently sensitive to characterise the full PK profile. Although antibody development is not expected after a few doses of IFN-β, their determination should be carried out before/after each treatment course in order to exclude any potential interference with the PK profile.

Serum concentrations of IFN-β are very low after the administration of therapeutic dosages and their measurement is technically difficult. Possible methods of detection include a cell-based myxovirus resistance protein A (MxA) induction assay, which measures the biological activity of IFN-β in serum samples, and ELISA assays, which determine the IFN-β protein mass. The Applicant should justify the rationale for the choice of assay.

The design of the study should take into account the recommendations as outlined in the Guideline on the pharmacokinetics of therapeutic proteins (CHMP/EWP/89249/2004). In particular, the pharmacokinetic parameters of interest should include AUC, Cmax and also T1/2 or clearance. The equivalence margin has to be defined a priori and appropriately justified, especially given the high variability of the relevant PK parameters. A two-stage design may be planned in the protocol provided adjusted significance levels are used for each of the analyses in accordance with the Guideline on bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1 Corr).

Pharmacodynamics

Pharmacodynamics should preferably be evaluated as part of the comparative pharmacokinetic studies using validated assays. There is currently no identified biological marker related to the mechanism by which IFN-β influences the clinical evolution of MS. However, a number of markers of the biological activity of IFN-β are well known and a comprehensive comparative evaluation of some of these markers could be used to support the similarity of the biosimilar and reference medicinal products (“fingerprint approach”).
MxA induction can be measured from peripheral blood leukocytes both at the protein and mRNA level; it is currently considered as one of the most sensitive markers of the biological activity of type I interferons and should be one of the selected markers. Neopterin, which was found to show a consistent and robust dose-response relationship, should also be investigated. Other possible markers include serum (2'-5')oligo-adenylate-synthetase activity, interleukin 10, or TNF-related apoptosis inducing ligand (TRAIL).

Magnetic resonance imaging (MRI) is a useful tool for monitoring CNS lesions in MS. Different MRI derived parameters have been related to clinical activity, e.g. gadolinium-enhancing T1-weighted lesions or new/enlarging T2-weighted lesions have been related to relapses.

Clinical efficacy

Similar clinical efficacy between the biosimilar and reference medicinal product should be demonstrated in an adequately powered, randomised, parallel group, equivalence clinical trial, preferably double-blind. If blinding is technically not feasible, alternative measures should be applied to avoid information bias. The route of administration used in the clinical trial should be the route recommended for the reference product.

According to the Guideline on medicinal products for the treatment of MS (CPMP/EWP/561/98 Rev1) an acceptable primary efficacy variable for a disease modifying agent in RMS is the relapse rate, which has been used in the pivotal trials on medicinal products containing recombinant IFN-β. While in principle this would be the preferred option, such a trial may not be necessary in a biosimilar context, since the focus of this trial is to demonstrate comparable clinical activity of the biosimilar product to the reference product, which then allows bridging to the benefit-risk of the reference product. For demonstrating clinical similarity of a biosimilar and reference product, magnetic resonance imaging of disease lesions in RMS may be sufficient (see Pharmacodynamics). In addition, clinical outcomes such as relapse rate or percentage of relapse-free patients should be used as secondary endpoints in support of the MRI outcomes.

The design of the equivalence trial should ensure assay sensitivity, i.e. the choice of study design, population, duration and MRI endpoints should make it possible to detect a difference between the biosimilar and reference products, if such difference actually exists. Regarding the study design, assay sensitivity could be shown by a three-arm trial including a placebo arm for a short period of time (e.g. 4 months) sufficient to demonstrate superiority of both the biosimilar and reference products over placebo using an MRI endpoint. Patients in the placebo arm could be subsequently switched to the biosimilar product and the trial continued with the two active arms. An alternative design could be a three-arm trial with the reference product and two doses of the biosimilar product, for which differences in MRI and clinical outcomes are expected to be observed over 12 months; if the MRI curves do not differentiate the two doses over time, interpretation of the results would be difficult as
the assay sensitivity of the trial would be questionable. Whatever the design, the duration of the trial should be sufficient to show comparable efficacy on MRI endpoints and provide relevant information on clinical outcomes, i.e. not less than 12 months.

The most sensitive patient population, which would enable to detect differences between the biosimilar and reference products, should be selected. This would be a homogeneous sample of patients with a confirmed diagnosis of relapsing-remitting MS (RRMS) and sufficient disease activity based on relapse frequency and/or MRI criteria to anticipate rapid changes in MRI.

MRI-based variables are acceptable primary endpoints in the context of a biosimilar comparison if backed up by relapse-related clinical outcomes; no formal equivalence test is required for clinical outcomes, which would be expected to show the same trend in effect as the MRI-based variables. A relapse should be differentiated from a pseudo-exacerbation and accurately defined. Repeated MRI scans should be performed during the trial. All possible actions should be taken to ensure high quality MRI data and maximum reliability of measurements. Updated recommendations on appropriate technical facilities and standardized procedures and training should be followed. The reading of the images should be central and blinded. The combined unique active lesions (CUA, defined as new gadolinium-enhancing T1-weighted lesions and new/enlarging T2-weighted lesions without double counting) are the most sensitive documented MRI variable, and therefore, should always be determined; a cumulative estimate over several scans may be calculated. Other MRI variables may also be used as primary endpoint if adequately justified.

The equivalence margin for the primary MRI endpoint should be pre-specified and adequately justified based on MRI data for the reference medicinal product relative to placebo, or if not available, extrapolation from other IFN-β relevant data. Of note, these data are important at the planning stage of the trial but are not essential for the interpretation of the results as assay sensitivity has to be shown within the trial. It should be adequately powered with particular attention paid in the protocol to the potential drop-out rate and the way of handling missing data.

**Clinical safety**

Comparative safety data from the efficacy trial are usually sufficient to investigate the more frequent adverse reactions and provide an adequate pre-authorisation safety database for such reactions but not for rarer adverse reactions, which should be addressed post-authorisation.

As IFN-β products are immunogenic, an assessment of immunogenicity by testing of sera from IFN-β treated patients should be performed according to the principles defined in the Guideline on immunogenicity assessment of therapeutic proteins. Its main objective is the comparison of the immunogenicity profile of the biosimilar and reference products over
time since the antibody characteristics and effects change as a result of affinity maturation of the antibody response and/or epitope spreading. A minimum of 12-month comparative immunogenicity data should be submitted pre-authorisation with further assessment to be continued post-approval for at least 6 months for the biosimilar product. A strategy that includes serum sampling at baseline and at regular intervals is necessary for assessing the comparability of the dynamics of antibody development during therapy, e.g., every month in the beginning of the treatment (first 3 months) followed by every 3 months.

The use of a validated, highly sensitive antibody assay, capable of detecting all antibodies (i.e. of different affinities, class and sub-class) is mandatory. Approaches that avoid specific masking of particular epitope(s) should be considered to avoid false negative results. Following confirmation of antibody positive samples, further characterization including determination of the ability to neutralise the biological activity of IFN-β and cross-reactivity is required. It is recommended that the standardised MxA protein NAb assay or a NAb assay that has been validated against the MxA protein NAb assay is used (EMEA/CHMP/BWP/580136/2007). The approach used to determine assay sensitivity (e.g., by using different cut-off points) should be described but the distribution of titres should also be presented at each time point for each treatment arm. Finally, patients should be categorised according to the evolution of their immune response over time using predefined criteria. For example, the patient’s NAb status may be defined as antibody negative (-ve for all post-treatment samples according to predefined low/high dilutions or titres) or antibody positive, which can be categorised as ‘transiently positive’ (1 or more post-treatment samples +ve, followed by –ve samples at all subsequent and at least 2 sampling time points) or ‘persistently positive’ (2 or more consecutive post-treatment samples consistently +ve). MRI activity and clinical relapses should be compared between these categories for both the biosimilar and reference product. The impact of NAbs on clinical outcomes is unlikely to be sufficiently ascertainable before 12 months of therapy and thus will need to be further evaluated post-authorisation as part of the risk management plan.

The immune response to the biosimilar and reference medicinal products is expected to be comparable with regard to the incidence and titres of antibodies (neutralising or not) as well as their impact on efficacy; although the clinical impact of binding, non-neutralising antibodies is not clear, an increased frequency of such antibodies for the biosimilar product relative to the reference medicinal product would contradict the concept of biosimilarity. However, lower immunogenicity alone would have to be explained but may not preclude biosimilarity if efficacy is shown to be comparable in the various categories of patients according to their immune response (as previously defined) and provided all other data (quality, non-clinical, PK, PD, and safety) are supportive of biosimilarity.
Pharmacovigilance

Within the authorisation procedure a risk management plan should be presented in accordance with current EU legislation and pharmacovigilance guidelines. The RMP should in particular take into account identified and potential risks associated with the use of the reference medicinal product and safety in indications authorised for the reference medicinal product that are claimed based on extrapolation. The risk management plan should further address rare events such as autoimmune disorders, adverse events of special interest such as hepatotoxicity and depression, potential effects of unwanted immunogenicity, and important missing information such as safety in pregnancy (a pregnancy registry for IFN-β containing products is mandatory). This could be managed through routine pharmacovigilance, supplemented by the extension of the pre-authorisation trial (in particular regarding immunogenicity as previously mentioned), a dedicated observational study or the participate on in an existing registry.

Extrapolation of indication

Extrapolation of clinical efficacy and safety in confirmed RRMS to the other indications of the reference medicinal product in MS is possible on the basis of the totality of the evidence provided from the comparability exercise.
8.9 Guideline on similar biological medicinal products containing monoclonal antibodies– non-clinical and clinical issues (EMA/CHMP/BMWP/403543/2010)

Introduction

Monoclonal antibodies have been established as a major product class of biotechnology-derived medicinal products. Different mAb products share some properties, e.g. being cytotoxic to their target, or neutralizing a cytokine, but differ in aspects like the mechanism of action. They are structurally complex, and may have several functional domains within a single molecule, depending on the isotype (antigen-binding region, complement-binding region, constant part interacting with Fc receptors). Each individual mAb presents a unique profile with respect to the antigen-binding region, the Fc cytotoxic effect or function, and binding to Fc receptors. Various assays have been established in the past years that allow for more in-depth characterisation of complex proteins, both on a physicochemical and a functional level, e.g. with potency assays, and there is experience in the assessment of minor quality differences due to changes in manufacturing processes for monoclonal antibodies. However, it may at the current stage of knowledge be difficult to interpret the relevance of minor quality differences in the physicochemical and biological characterization when comparing a biosimilar mAb to a reference mAb.

This guideline lays down the non-clinical and clinical requirements for monoclonal antibody-containing medicinal products claimed to be similar to another one already authorised, i.e. similar biological medicinal products (biosimilars). The studies described here should be planned with the intention to detect any potential differences between biosimilar and reference medicinal product and to determine the relevance of such differences, should they occur. A biosimilar mAb should be similar to the reference mAb in physicochemical and biological terms. Any observed relevant difference would have to be duly justified and could contradict the biosimilar principle. For quality aspects the principles as laid out in the guidelines on biosimilars including the “Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: Quality issues” (EMEA/CHMP/BWP/49348/2005), and the “Guideline on development, production, characterisation and specifications for monoclonal antibodies and related substances” (EMEA/CHMP/BWP/157653/2007) apply.

Also, Guideline on Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use (EMA/CHMP/BMWP/86289/2010)

While this guidance is specifically related to mAbs, the principles discussed can also be applied to related substances like for example fusion proteins based on IgG Fc (e.g. molecules).

Next-generation mAbs, defined as mAbs that are structurally and/or functionally altered (for example, glyco-engineered mAbs with higher potency), in comparison to already licensed reference medicinal products, to achieve an improved or different clinical performance, are not biosimilars and therefore beyond the scope of this guideline.

Non-clinical studies

As regards non-clinical development, a step-wise approach is applied to evaluate the similarity of biosimilar and reference mAb.
Non-clinical studies should be performed before initiating clinical trials. *In vitro* studies should be conducted first and a decision then made as to the extent of what, if any, *in vivo* work will be required. The approach taken will need to be fully justified in the non-clinical overview.

**In vitro studies = step 1**

In order to assess any difference in biological activity between the biosimilar and the reference medicinal product, data from a number of comparative *in vitro* studies, some of which may already be available from quality-related assays, should be provided.

*In vitro* non-clinical studies should be performed with an appropriate number of batches of product representative of that intended to be used in the clinical trial. These studies should include relevant assays on:

- Binding to target antigen(s)
- Binding to representative isoforms of the relevant three Fc gamma receptors (FcγRI, FcγRII and FcγRIII), FcRn and complement (C1q)
- Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation or blockade)
- Fc-associated functions (e.g. antibody-dependent cell-mediated cytotoxicity, ADCC; complement-dependent cytotoxicity, CDC; complement activation)

These studies should be comparative in nature and should be designed to be sensitive enough to detect differences in the concentration–activity relationship between the similar biological medicinal product and the reference medicinal product, and should not just assess the response per se. It should be noted that an evaluation of ADCC and CDC is generally not needed for mAbs directed against non-membrane bound targets. As indicated in the ICH S6 (R1) guideline, tissue cross-reactivity studies are not suitable to detect subtle changes in critical quality attributes and are thus not recommended for assessing comparability.

Together these assays should broadly cover the functional aspects of the mAb even though some may not be considered essential for the therapeutic mode of action. As the *in vitro* assays may be more specific and sensitive than studies in animals, these assays can be considered paramount in the non-clinical comparability exercise.

If the comparability exercise using the above strategy indicates that the test mAb and the reference mAb cannot be considered biosimilar, it may be more appropriate to consider developing the product as a stand alone.

**Determination of the need for in vivo studies = step 2**

It is acknowledged that some mAbs may mediate effects that cannot be fully elucidated by *in vitro* studies. Therefore, evaluation in an *in vivo* study may be necessary, provided that a
relevant in vivo model with regard to species or design is available. Factors to be considered when the need for additional in vivo non-clinical studies is evaluated, include but are not restricted to:

- Presence of relevant quality attributes that have not been detected in the reference product (e.g. new post-translational modification structure)
- Presence of quality attribute significantly different amounts than those measured in the reference product
- Relevant differences in formulation, e.g. use of excipients not widely used for mAbs.

Although each of the factors mentioned here do not necessarily warrant in vivo testing, these issues should be considered together to assess the level of concern and whether there is a need for in vivo testing.

If the comparability exercise in the in vitro studies in step 1 is considered satisfactory and no factors of concern are identified in step 2, or these factors of concern do not block direct entrance into humans, an in vivo animal study may not be considered necessary.

If there is a need for additional information, the availability of a relevant animal species or other relevant models (e.g. transgenic animals or transplant models) should be considered.

Due to the specificity of mAbs, the relevant species studied is in most cases a non-human primate. In all cases the limitations of an in vivo study (such as sensitivity and variability) should be taken into account.

If a relevant in vivo animal model is not available the applicant may choose to proceed to human studies taking into account principles to mitigate any potential risk.

**In vivo studies = step 3**

If an in vivo study is deemed necessary, the focus of the study (PK and/or PD and/or safety) depends on the need for additional information. Animal studies should be designed to maximise the information obtained. In addition, depending on the endpoints needed, it may not be necessary to sacrifice the animals at the end of the study. The principles of the 3Rs (replacement, refinement, reduction) should be considered when designing any in vivo study. The duration of the study (including observation period) should be justified, taking into consideration the PK behaviour of the mAb and its clinical use.

When the model allows, the PK and PD of the similar biological medicinal product and the reference medicinal product should be quantitatively compared, including concentration-response assessment covering the therapeutic doses in humans.

The conduct of repeated dose toxicity studies in non-human primates is usually not recommended.

Also, the conduct of toxicity studies in non-relevant species (i.e. to assess unspecific toxicity only, based on impurities) is not recommended. Due to the different production processes used by the biosimilar and reference product manufacturers, qualitative differences of process related impurities will occur (e.g. host cell proteins). Such impurity level should be kept to a minimum, which is the best strategy to minimise any associated risk. Qualitative or quantitative difference(s) of product-related variants (e.g. glycosylation patterns, charge variants) may affect biological functions of the mAb and are expected to be evaluated by
appropriate in vitro assays. These quality differences may have an effect on immunogenic potential and potential to cause hypersensitivity. It is acknowledged that these effects are difficult to predict from animal studies and should be further assessed in clinical studies.

Immunogenicity assessment in animals is generally not predictive for immunogenicity in humans, but may be needed for interpretation of \textit{in vivo} studies in animals. Blood samples should be taken and stored for future evaluations if then needed.

Studies regarding safety pharmacology and reproduction toxicology are not required for non-clinical testing of biosimilar mAbs. Studies on local tolerance are usually not required. If excipients are introduced for which there is no or little experience with the intended clinical route, local tolerance may need to be evaluated. If other \textit{in vivo} studies are performed, evaluation of local tolerance may be part of the design of that study instead of the performance of separate local tolerance studies.

\textbf{Clinical studies}

Comparative clinical studies between the biosimilar and reference medicinal product should always be conducted. The number and type of studies might vary according to the reference product and should be justified based on a sound scientific rationale. A stepwise approach is normally recommended 1 “Safety” in this context does not usually refer to a complete repeated dose toxicity study, but rather an in-life evaluation of safety parameters such as clinical signs, body weight and vital functions. throughout the development programme, and the extent and nature of the clinical programme depends on the level of evidence obtained in the previous step(s). During the clinical development programme, patients are normally enrolled commensurate with the level of evidence obtained from preceding steps which support comparability.

\textbf{Pharmacokinetics (PK) = step 1}

The comparison of the pharmacokinetic properties of the biosimilar product and the reference medicinal product forms normally the first step of a biosimilar mAb development. The design of the study depends on various factors, including clinical context, safety, the PK characteristics of the antibody (target-mediated disposition, linear or non-linear PK, time-dependencies, half-life, etc.) and should take into account the recommendations as outlined in the “Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins” (CHMP/EWP/89249/2004) and the “Guideline on the investigation of bioequivalence” (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr). Furthermore, bioanalytical assays should be appropriate for their intended use and adequately validated as outlined in the “Guideline on bioanalytical method validation” (EMEA/CHMP/EWP/192217/2009).

\textbf{Study design}

The primary objective of the pharmacokinetic studies performed to support a MAA for a biosimilar is to show comparability in pharmacokinetics of the biosimilar with the reference medicinal product in a sufficiently sensitive and homogeneous population. This is expected to reduce variability, and thus the sample size needed to prove equivalence, and can simplify
interpretation. Healthy volunteers are likely to have less variability in PK as target-mediated clearance may be less important than in patients. Hence, if feasible, a single dose study in healthy volunteers is recommended, which could provide important information on biosimilarity. From a pharmacokinetic perspective, a single dose cross-over study with full characterisation of the PK profile, including the late elimination phase, is preferable. A parallel group design may be necessary due to the long half-life of mAbs and the potential influence of immunogenicity.

A study in healthy volunteers may not be possible in case of a toxic mechanism of action, or in case the information obtained would not be sufficient to establish biosimilarity. Under these circumstances a study in patients may be a better option. If a single dose study is not feasible in patients, a multiple dose study should be conducted.

It may be necessary to perform the PK study in a different population from that selected to establish similar clinical efficacy, since the most sensitive population where PK characteristics can be compared may not be the same as the most sensitive population where similar efficacy and safety can be demonstrated. In such scenarios, population PK measurements during the clinical efficacy trial are recommended since such data may add relevant data to the overall database to claim comparability. The choice of the patient population for the PK study should be fully justified, based on a comprehensive survey of scientific literature as regards its sensitivity, and also the possibility to infer PK results to other clinical indications where the reference mAb is licensed.

In case a PK study in healthy volunteers is conducted to support bioequivalence, supportive PK data from clinical studies in patients are encouraged and could provide highly supportive evidence of a similar PK behavior.

The following factors impact on the strategy of designing PK evaluations:

**Disease and patient characteristics**
Factors that may influence the choice of the patient population are age of usual manifestation and age range (since lower age may be less prone to presence of concomitant clinical conditions), number of previous treatments, concomitant treatments, or expression of antigen (which may be related to disease stage). For mAbs which are indicated for both monotherapy and in combination with immunosuppressant or chemotherapy, it may be sensible to study the comparative PK in the monotherapy setting in order to minimize the sources for variability. However, a first line setting, where patients are in a better clinical condition, or an adjuvant setting in patients with early cancer, where the tumour burden is low, may be preferable; in these instances, the mAb is typically administered in combination with other therapies.

**PK characteristics of the reference mAb**
Pharmacokinetics of anticancer mAbs may be time dependent, as the tumour burden may change after multiple dosing (e.g. increased half-life with multiple dosing) and this should be taken into account in the design of the study.

The existence of target-mediated clearance in addition to nontarget-mediated clearance may affect the number of studies needed. In case target-mediated clearance is not relevant, one
comparative PK study may be sufficient. If the reference mAb is eliminated both by target-mediated and non target-mediated mechanisms, comparable PK should be demonstrated where each mechanism of clearance predominates: preferably one study in healthy volunteers for non target-mediated clearance and one supportive study in patients, which can be part of the efficacy trial, to investigate comparability in target-mediated clearance.

For mAb targets that involve receptor shedding, it is advisable to measure shed receptor levels at baseline and, if relevant, during the conduct of the study, in order to verify the baseline comparability of the treatment groups. Stratification by tumour burden or receptor shedding, if possible, may help to ensure baseline comparability. An exploratory statistical analysis on post-baseline comparability at the time point relevant to the conclusion of PK equivalence could be helpful.

For mAbs licensed in several clinical indications, it is not generally required to investigate the pharmacokinetic profile in all of them. However, if distinct therapeutic areas are involved for one particular mAb (e.g. autoimmunity and oncology), separate PK studies may be needed if different target-mediated clearance exists for different therapeutic areas.

**Doses**

In principle, it is not required to test all therapeutic dosage regimens; the most sensitive dose should be selected to detect potential differences in PK between the biosimilar and the reference products. When limited data are available to know which dose is the most sensitive it is recommended to investigate a low or the lowest recommended therapeutic dose where it is assumed that the target-mediated clearance is not yet saturated and a high or the highest therapeutic dose where it is believed that the non specific clearance mechanism dominates. A single dose study with the lowest therapeutic dose in patients is considered the most adequate design to investigate the differences in target-mediated clearance, if any.

**Routes of administration**

If the reference product can be administered intravenously and subcutaneously and if both routes are applied for, it is preferable to investigate both routes of administration. However, as the evaluation of subcutaneous administration covers both absorption and elimination, it may be possible to waive the evaluation of intravenous administration if comparability in both absorption and elimination has been demonstrated for the subcutaneous route using additional PK parameters such as partial AUCs (see 5.1.2).

**Sampling times**

In single dose studies, the sampling times should be selected to characterise the whole profile, including the late elimination phase. For those products administered as two (or more) consecutive doses useful information can be obtained from both the first and last administrations since the first administration is preferred for comparative purposes and the last one can provide information on the final elimination phase that cannot be observed after the first dose.

If a multiple dose PK study in patients is used to show similarity between the biosimilar and
reference medicinal product and if elimination after the last dose cannot be characterised, sampling should normally be undertaken to characterise the concentration-time profile both after the first dose and later, preferably at steady state. Characterisation of the full concentration-time profile at steady state is especially important in case of non-linear PK of the reference mAb (e.g. many anticancer mAbs with cellular targets exhibit dose- or time-dependent PK or immunogenicity-related changes in distribution or elimination kinetics).

**PK parameters of interest**

In a single dose study, the primary parameter should be the AUC\(_{(0\text{--}\infty)}\). Secondary parameters such as C\(_{\text{max}}\), t\(_{\text{max}}\), volume of distribution, and half-life, should also be estimated. In case of subcutaneous administration, C\(_{\text{max}}\) should be a co-primary parameter. In addition, if no data are provided for the intravenous route, partial AUCs should be assessed to ensure comparability of both absorption and elimination.

In a multiple dose study, the primary parameters should be the truncated AUC after the first administration until the second administration (AUC\(_{0\text{--}t}\)) and AUC over a dosage interval at steady state (AUC\(_{\text{inf}}\)). Secondary parameters are C\(_{\text{max}}\) and C\(_{\text{trough}}\) at steady state.

Anti-drug antibodies should be measured in parallel of PK assessment using the most appropriate sampling time points. Comparability margins have to be defined a priori and appropriately justified. For some reference mAbs, inter-subject variability for some parameters was reported to be considerable. This may have to be accounted for in the choice of the comparability margin at least for such parameters. As a principle, any widening of the conventional equivalence margin beyond 80-125\% for the primary parameters requires thorough justification, including an estimation of potential impact on clinical efficacy and safety. For secondary parameters, confidence intervals (CI) for ratio or differences can be presented together with descriptive statistics but no acceptance range needs to be defined. The clinical relevance of estimated differences and associated confidence intervals should be discussed.

**Timing of the PK evaluation**

Usually, proof of similar PK profiles should precede clinical efficacy trials. However, in certain scenarios, e.g. for mAbs where PK is inevitably highly variable even within one clinical indication, it may, for feasibility reasons, be necessary to explore PK comparisons as part of a clinical study that is designed to establish similar clinical efficacy (as only this trial will be large enough to demonstrate PK equivalence). To start with a comparative clinical efficacy trial that includes PK evaluation, without a formal preceding comparative PK study, could be problematic with no former human exposure to the biosimilar mAb, together with potentially limited non-clinical in vivo data, depending on the mAb. Therefore, such a plan could only be justified on a case by case basis depending on the product profiles observed in the quality and non-clinical data.

**Pharmacodynamics (PD)**

Pharmacodynamic parameters may contribute to the comparability exercise for certain mAbs and in certain indications. Depending on the mAb and availability of PD endpoints, the following scenarios are, theoretically, possible:
PD markers as support to establish comparability
Pharmacokinetic studies can be combined with pharmacodynamic (PD) endpoints, where available. This could add valuable information for the overall comparability exercise. PD markers are especially valuable if they are sensitive enough in order to detect small differences, and if they can be measured with sufficient precision. The use of multiple PD markers, if they exist, is recommended. With regard to pharmacodynamic evaluation, there is often a lack of specific PD endpoints. The emphasis may then have to be on non-clinical PD evaluations, e.g. *in vitro* testing.

PD markers as pivotal proof of comparability

Sponsors should always explore possibilities to study dose-concentration-response relationships or time-response relationships, since this approach, if successful, may provide strong evidence of comparability, provided that the selected doses are within the linear part of the dose-response curve.

The following prerequisites need to be met to accept that PD markers can constitute the pivotal evidence for the efficacy comparability exercise:

- A clear dose-response relationship is shown.
- At least one PD marker is an accepted surrogate marker and can be related to patient outcome to the extent that demonstration of similar effect on the PD marker will ensure a similar effect on the clinical outcome variable.

If that is not the case, then proceed to Step 2 (i.e. clinical efficacy).

When PD markers are planned as pivotal evidence to establish similarity, it is recommended to discuss such approach with regulatory authorities. This should include a proposal of the size of the proposed equivalence margin and its clinical justification as regards lack of a clinical meaningful difference.

A comparative single or repeat dose study in the saturation part of the dose-concentration-response curve is unlikely to discriminate between different activities, should they exist, and a dose in the linear part of the dose-response curve may result in treating a patient with a too low dose. It is also acknowledged that dose-response data may not exist for the reference mAb, and that exposing patients to a relatively low dose of the mAbs, in a worst case scenario, might also sensitize them to develop anti-mAb antibodies, and, consequently, may make them treatment resistant. However, for some reference mAbs clinical conditions may exist where such studies are feasible.

Clinical Efficacy – step2

If dose comparative and highly sensitive PD studies cannot be performed convincingly showing comparability in a clinically relevant manner, similar clinical efficacy between the similar and the reference product should be demonstrated in adequately powered, randomised, parallel group comparative clinical trial(s), preferably double-blind, normally equivalence trials.

For most of the clinical conditions that are licensed for mAbs, specific CHMP guidance on
the clinical requirements to demonstrate efficacy exists. However, to establish comparability, deviations from these guidelines (choice of endpoint, timepoint of analysis of endpoint, nature or dose of concomitant therapy, etc) will be warranted in some circumstances. Such deviations need to be scientifically justified on the basis that the proposed clinical concept is designed to establish biosimilarity by employing PD markers, clinical outcomes, or both. The guiding principle is to demonstrate similar efficacy and safety compared to the reference medicinal product, not patient benefit \textit{per se}, which has already been established by the reference medicinal product. Therefore, in general the most sensitive patient population and clinical endpoint is preferred to be able to detect product-related differences, if present and, at the same time, to reduce patient and disease-related factors to a minimum in order to increase precision and to simplify interpretation. For example, patients with different disease severity and with different previous lines of treatment might be expected to respond differently, and thus differences between the study arms may be difficult to interpret, and it may remain uncertain whether such differences would be attributable to patient or disease related factors rather than to differences between the biosimilar mAb and reference mAb.

Comparability should be demonstrated in scientifically appropriately sensitive clinical models and study conditions (whether licensed or not), and the applicant should justify that the model is relevant as regards efficacy and safety, and sensitive to demonstrate comparability in the indication(s) applied for.

The safety of patients should not be compromised by a comparability exercise, and patients should only be treated as medically warranted. In case there are no endpoints that are sufficiently sensitive to detect relevant differences, applicants need to implement additional measures to enable sufficient sensitivity of the overall clinical dataset obtained from the clinical study. For example, the study could be combined with a multiple dose study, or applicants could measure pharmacodynamic markers in addition to clinical endpoints in order to further establish comparability.

Clinical studies in special populations like the paediatric population or the elderly are normally not required since the overall objective of the development programme is to establish comparability, and therefore the selection of the primary patient population is driven by the need for homogeneity and sensitivity.

The inclusion of patients from non-European countries is generally possible if there are no intrinsic differences, but it may increase heterogeneity. Knowledge of efficacy and safety of the reference mAb in a particular region may be necessary in order to prospectively define an equivalence margin.

Stratification and appropriate subgroup analyses are normally expected if patients from different global regions are included in order to demonstrate consistency with the overall effect. Diagnostic and treatment strategies should be comparable in order to prevent the influence of extrinsic factors.

**Additional considerations for mAbs licensed in anticancer indications**

Establishing similar clinical efficacy and safety of biosimilar and reference mAb may be particularly challenging in an anticancer setting: According to the “Guideline on the
evaluation of anticancer medicinal products in man” (CHMP/EWP/205/95) the preferred endpoint to prove efficacy in cancer indications would be either progression free / disease free survival (PFS / DFS) or overall survival (OS).

Such endpoints are important to establish patient benefit for a new anticancer drug, but may not be feasible or sensitive enough for establishing comparability of a biosimilar mAb to a reference mAb, since they may be influenced by various factors not attributable to differences between the biosimilar mAb and the reference mAb, but by factors like tumour burden, performance status, previous lines of treatments, underlying clinical conditions, subsequent lines of treatment (for OS), etc. They may therefore not be suitable to establish similar efficacy of the biosimilar and the reference mAb.

Again, the focus of the comparability exercise is to demonstrate similar efficacy and safety compared to the reference medicinal product, not patient benefit per se, which has already been established by the reference medicinal product. In general the most sensitive patient population and clinical endpoint is preferred to be able to detect product-related differences, if present and, at the same time, to reduce patient and disease-related factors to a minimum in order to increase precision. A clinical trial in a homogeneous patient population with a clinical endpoint that measures activity as primary endpoint may be considered. An example may be Overall Response Rate (ORR, proportion of patients in whom a Complete Response (CR) or Partial Response (PR) was observed). It may also be worthwhile to explore ORR measured at a certain timepoint (i.e., ORR at x months) or percentage change in tumour mass from baseline or pathological Complete Response (pCR) in certain clinical settings.

Applicants should engage in efforts for a standardized assessment and clear definitions of endpoints with patients evaluated at appropriate intervals. PFS and OS should be recorded, where feasible. It is acknowledged that data on survival may have to be interpreted with caution due to numerous factors influencing survival beyond the performance of the biosimilar mAb or the reference mAb. However, in case PFS is likely to be more sensitive than ORR as outcome measure, this is the preferred option even though this will prolong the clinical study.
Novel endpoints may be tested on an exploratory basis (e.g. time to response) and may add supportive evidence for biosimilarity.

**Clinical Safety**

Clinical safety is important throughout the clinical development programme and is captured during initial PK and/or PD evaluations and also as part of the pivotal clinical study establishing comparability. Care should be given to compare the type, severity and frequency of the adverse reactions between the biosimilar mAb and the reference mAb, particularly those described for the reference product.

Where no homogeneous definition exists for safety parameters (e.g., measurement of cardiotoxicity) it is recommended to use the same definitions as that used for the reference mAb in its original development programme (if known) or the definitions used during post-authorisation follow-up. Comparison of pharmacologically mediated adverse reactions (e.g., cardiotoxicity), i.e., safety-related pharmacodynamic markers, could also be used as further supportive evidence for clinical comparability, and could be analysed in a similar way to that discussed for efficacy-related PD markers.
In cases where comparative and highly sensitive PD studies are suitable to provide the pivotal evidence for equivalence in clinical efficacy, applicants will have to provide sufficient reassurance of similar clinical safety, including immunogenicity. Actively controlled safety data should normally be collected pre-authorisation, depending on the mAb and the number of exposed patients, and duration of treatment. The duration of safety follow up pre authorisation should be justified.

It might be decided to collect part of the safety data, or also additional safety data, in the post-authorisation setting as described below. Rare events such as progressive multifocal leukencephalopathy are unlikely to be detected in a pre-authorisation setting. Therefore, applicants need to propose pharmacovigilance and risk management activities for the post-authorisation phase at the time of the marketing authorisation application (see section 7). Usually, similar pharmacovigilance activities as those of the reference medicinal product would be required, rather than a direct comparison with the reference medicinal product, since comparative data will most likely be difficult to interpret due to their rarity of occurrence and consequent lack of precision for estimated differences.

Applicants should reflect upon how re-treatment of patients would be handled. Concepts should be presented at the time of marketing authorisation application on how to systematically measure safety of repeat exposure of patients, for example in oncological indications where patients undergo several treatment cycles. It is highly encouraged to extend the clinical study as a post-authorisation follow-up study to a full treatment cycle, where relevant and feasible.

As regards immunogenicity assessment, applicants should refer to existing CHMP guidance. Systematic and comparative evaluation and discussion of immunogenicity is important, due to clinical consequences like loss of efficacy and also likely resistance against further treatment with the reference mAb. It may be advisable not to include patients previously treated with the reference mAb where possible, or to pre-specify a subgroup analysis for patient previously treated (in order to explore if pre-treatment impacts immunogenicity), as previous treatment could have resulted in an anti-drug antibody response that could hamper interpretation of the safety data and thus also decrease sensitivity for detecting differences. Comparative assessment of unwanted immune responses against the biosimilar and the reference mAb are normally undertaken as part of the clinical study establishing similar clinical efficacy and safety, using the same validated assay(s) (see relevant CHMP guidelines on immunogenicity assessment). A population PK approach with sparse sampling and determination of drug concentration together with anti-drug antibody detection is acceptable. However, for some mAbs, antibodies can be better detected in healthy volunteers, who develop a strong immune response after a single dose within a few days. The dose of mAb administered is also an important factor to consider when investigating immunogenicity: some mAbs inhibit antibody formation when administered at high doses, and therefore studies conducted with low doses, if medically possible, are more sensitive to compare the immune response of the biosimilar and reference medicinal products.

Investigation of unwanted immunogenicity is especially important when a different expression system is employed for the biosimilar mAb compared to the reference mAb which might, for example, yield in relevant quality attributes that have not been detected in the reference product (e.g. new post-translational modification structure) that could result in a higher immunogenicity. This is particularly important if there is limited experience with this
expression system in humans. It is recommended that such approaches are discussed in advance with regulatory authorities.

A higher immunogenicity as compared to the reference mAb may become an issue for the benefit/risk analysis and would question biosimilarity. However, also a lower immunogenicity for the biosimilar mAb is a possible scenario, which would not preclude biosimilarity. Here, the efficacy analysis of the entire patient population could suggest that the biosimilar is more efficacious (since fewer patients developed an immune response and thus more patients may show a treatment effect with the biosimilar mAb). It is therefore recommended to pre-specify an additional exploratory subgroup analysis of efficacy and safety in those patients that did not mount an anti-drug antibody response during the clinical trial. This subgroup analysis could be helpful to establish that the efficacy of the biosimilar and the reference mAb are in principle similar if not impacted by an immune response.

Additional long-term immunogenicity and safety data might be required post-authorisation, e.g. in situations where the study duration for establishing similar clinical efficacy is rather short. The need for additional long-term immunogenicity and safety data should be discussed in the risk management plan and, if considered needed, applicants should go beyond routine pharmacovigilance and perform post-authorisation safety studies. As regards safety and immunogenicity across different indications licensed for the reference mAb and claimed by the biosimilar mAb, a post-authorisation concept for obtaining further indication-specific safety data may be needed as described in section 7.

**Extrapolation of indications**

Extrapolation of clinical efficacy and safety data to other indications of the reference mAb, not specifically studied during the clinical development of the biosimilar mAb, is possible based on the overall evidence of comparability provided from the comparability exercise and with adequate justification. If pivotal evidence for comparability is based on PD and for the claimed indications different mechanisms of action are relevant (or uncertainty exists), then applicants should provide relevant data to support extrapolation to all claimed clinical indications. Applicants should support such extrapolations with a comprehensive discussion of available literature including the involved antigen receptor(s) and mechanism(s) of action. For example, if a reference mAb is licensed both as an immunomodulator and as an anticancer antibody, the scientific justification as regards extrapolation between the two (or more) indications is more challenging. The basis for such extrapolation forms an extensive quality and nonclinical database, including potency assay(s) and *in vitro* assays that cover the functionality of the molecule, supplemented by relevant clinical data as described further in this document. The possibility of extrapolating safety including immunogenicity data also requires careful consideration, and may have to involve more specific studies (see sections 5 and 7). For the mechanism of action, e.g. the depletion of immune cells, several mechanisms may play a role in the various clinical conditions. For example, ADCC appears to be more important in some indications than in others. To provide further evidence about the mechanism of action, it may also be helpful to perform a literature search to identify what is known, e.g. about potential signalling inhibition by the reference mAb that would not be covered by ADCC/CDC tests, in particular direct induction of apoptosis. This could provide more knowledge on potential read-outs that could be used to support comparability on a molecular level.
7. Pharmacovigilance

For the marketing authorisation procedure the applicant should present a risk management plan/pharmacovigilance plan in accordance with current EU legislation and pharmacovigilance guidelines. Risk minimisation activities in place for the reference medicinal product may have to be implemented as well into the Risk Management Plan of the biosimilar.

Further to safety considerations as discussed above, applicants should provide at the time of MAA a comprehensive concept how to further study safety in a post-authorisation setting including also the following aspects:

- Safety in indications licensed for the reference mAb that are claimed based on extrapolation of efficacy and safety data, including long term safety data unless otherwise justified.

- Occurrence of rare and particularly serious adverse events described and predicted, based on the pharmacology, for the reference mAb. The pharmacovigilance plan should be proportionate to identified and potential risks and should be informed by the safety specification for the reference mAb in addition to relevant knowledge regarding similar biological products as appropriate.

- Detection of novel safety signals, as for any other biological medicinal product.

- Activities to obtain additional immunogenicity data, if considered needed.

The concept is likely to have to exceed routine pharmacovigilance, and may have to involve more pro-active pharmacovigilance activities, e.g. where possible, registries or large population based databases, in which data is captured in a standardised way to ensure accurate and consistent data capture/review. In addition, participation in already existing registries is recommended and should be presented as part of the Risk Management Plan. The adequacy of such proposals will have to be assessed in the context of the safety data at the time of approval, the overall data from the comparability exercise, and the known safety profile of the reference mAb. The need for additional risk minimisation activities should be clearly evaluated taking into account the requirements for the reference medicinal product.

For suspected adverse reactions relating to biological medicinal products, the definite identification of the concerned product with regard to its manufacturing is of particular importance. Therefore, all appropriate measures should be taken to identify clearly any biological medicinal product which is the subject of a suspected adverse reaction report, with due regard to the name of the medicinal product and the batch number.

Depending on the handling of biosimilars and reference medicinal products in clinical practice at national level, ‘switching’ and ‘interchanging’ of medicines that contain a given mAb might occur. Thus, applicants are recommended to follow further development in the field and consider these aspects as part of the risk management plan.
9.0 APPENDICES

9.1 Appendix I (Abbreviations and Acronyms)

9.4 Appendix II (Glossary of terms)

9.3 Appendix III (references )

APPENDIX I:

Abbreviations and Acronyms

BWP Biologics Working Party
CMC Chemistry, Manufacturing and Controls
CHMP Committee for Medicinal Products for Human Use
CDCR Control of Drugs and Cosmetic Regulations
DNA Deoxyribonucleic Acid
EU European Union
EMA European Medicines Agency
GMP Good Manufacturing Practice
ICH International Conference of Harmonisation
INN International Non-proprietary Names
NCE New Chemical Entity
NPCB National Pharmaceutical Control Bureau
PK/PD Pharmacokinetic/Pharmacodynamic
Ph Eur European Pharmacopeia
PSUR Periodic Safety Update Reports
QWP Quality Working Party
RMP Risk Management Plan
WHO World Health Organisation
**APPENDIX II:**

**GLOSSARY OF TERMS**

**Antibody**
A spectrum of proteins of the immunoglobulin family that is produced, in the human (or animal) body, in response to an antigen (e.g., a virus or bacterium, or a foreign protein unknown to the body's immune system). Antibodies are able to combine with and neutralize the antigen, as well as to stimulate the immune system for defense reactions. Retrieved from "http://www.biology-online.org/dictionary/Antibody" :This page was last modified at 21:16, October 3, 2005.

**Antigen**
A substance that reacts with the products of a specific immune response.

**Biologic (Biological medicinal product)**
Biological products include a wide range of products such as vaccines, blood and blood components, allergens, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins. Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues. Biologics are isolated from a variety of natural sources - human, animal, or microorganism - and may be produced by biotechnology methods and other cutting-edge technologies. They often are at the forefront of biomedical research and may be used to treat a variety of medical conditions for which no other treatments are available. (Taken from: http://www.fda.gov/Cber/faq.htm#3).

**Biotechnology**
A set of tools that employ living organism (or part of organism) to make or modify products, to improve plants and animals, or to develop microorganisms for specific uses. or A collection of technologies that use living cells and/or biological molecules to solve problems or make useful products (http://www.ncbiotech.org/biotech101/glossary.cfm) Accordingly, modern technology includes the use of the new genetic tools of recombinant DNA to make a new genetically modified organism.

**Biotherapeutics**
Therapeutic biological products, some of which are produced by recombinant DNA technology

**CMC (Chemistry, Manufacturing, and Control)**
The section of a submission dealing with the substance properties, manufacturing and quality control, intended for evaluating the provided information in the context of the current standards in chemical science and technology, and the current regulations.
Comparability
A conclusion that a given product has highly similar quality attributes before and after manufacturing process changes, and that no adverse impact on the safety or efficacy, including immunogenicity, of the drug product occurred. This conclusion can be based on an analysis of product quality attributes. And additional non-clinical and clinical data are usually necessary for the demonstration of comparable efficacy and safety to the reference medicinal product.

Comparability Exercise
The activities including study design, conduct of studies, and evaluation of data, that are designed to investigate whether the products are comparable.

Drug substance
Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body. Also termed active pharmaceutical ingredient (API). (http://www.fda.gov/CDER/guidance/4286fnl.htm).

Drug product

Equivalent
Equal or virtually identical in the parameter of interest. Small non-relevant differences may exist. Equivalent efficacy of two medicinal products means they have similar (no better or no worse) efficacy and any observed differences are of no clinical relevance.

Follow-on Biologic
Term used to describe similar biological medicinal products (biosimilars) in the U.S.

Glycoform
A glycoform is defined as an isoform of a glycosylated protein with identical polypeptide sequence, but with different sugar (saccharide) structures attached to the sites of glycosylation by either post-translational or co-translational modification. Such differences in glycosylation may affect properties of the glycoprotein such as biological activity, half-life, receptor binding, etc.

Glycosylation
Glycosylation is the process or result of enzyme-catalyzed addition of sugar residues (saccharides) to proteins and lipids. The process is one of the principal co-translational and post-translational modification steps in the synthesis of membrane and secreted proteins.
ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)
ICH is a project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration. The purpose is to make recommendations on ways to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for product registration in order to reduce or obviate the need to duplicate the testing carried out during the research and development of new medicines.
For more information, see http://www.ich.org/.

Immunogen
Any substance that is recognized as foreign by the immune system in a (particular) higher organism and induces an immune response which may include the formation of antibodies and developing immunity, hypersensitivity to the antigen, and tolerance.

Impurity
In drug substance: Any component of the new drug substance that is not the chemical entity defined as the new drug substance.
In drug product: Any component of the new drug product that is not the drug substance or an excipient in the drug product.

In-process control (or: Process control)
Checks performed during production to monitor and, if appropriate, to adjust the process and/or to ensure that the intermediate or API conforms to its specifications.
(Taken from: ICH Q7A, http://www.fda.gov/CDER/guidance/4286fnl.htm#P1272_96843)

Interchangeability
A product is interchangeable with another if both products are approved for the same indication, and can be used for the said indication. For interchangeable products, one or the other can be used (prescribed) but these products cannot be substituted with one another during a treatment period. Hence, interchangeability does not imply substitutability.

Isoform
A protein isoform is a version of a protein with some small differences, e.g. a splice variant or the product of some posttranslational modification (such as glycosylation).

Originator product
An originator product is defined as the product for which a marketing authorization is granted to a given marketing authorization holder (MAH) for a given active substance based upon a complete dossier. (Taken from: http://medagencies.org/mrfg/docs/rec/rec annexII.pdf)

Pegylation
Pegylation is the covalent (chemical) attachment of polyethylene glycol (abbreviated PEG),
a chemically inert and non-toxic polymer, to another substance or material, e.g. to a protein. In drug development, pegylation is an established method to improve on the pharmacokinetic profile of therapeutic compounds. Pegylation has been very successfully applied to the development of second-generation biotherapeutics, such as pegylated interferon-alpha.

**Pharmacovigilance**
According to the WHO definition, pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug related problems. The decision to approve a drug is based on its having a satisfactory balance of benefits and risks within the conditions specified in the product labeling. This decision is based on the information available at the time of approval. The knowledge related to the safety profile of the product can change over time through expanded use in terms of patient populations and the number of patients exposed. In particular, during the early post-marketing period the product might be used in settings different from clinical trials and a much larger population might be exposed in a relatively short timeframe. Detailed evaluation of the information generated through pharmacovigilance activities is important for all products to ensure their safe use.

**Pre-clinical (non-clinical)**
During preclinical drug development (which precedes the clinical trials in patients), a sponsor evaluates the drug's toxic and pharmacologic effects through *in vitro* and *in vivo* laboratory animal testing. Generally, genotoxicity screening is performed, as well as investigations on drug absorption and metabolism, the toxicity of the drug's metabolites, and the speed with which the drug and its metabolites are excreted from the body. (Taken from: [http://www.fda.gov/cder/handbook/preclin.htm](http://www.fda.gov/cder/handbook/preclin.htm))

**Similarity**
If a company chooses to develop a new biological medicinal product claimed to be similar to a reference medicinal product, comparative studies are needed to generate evidence substantiating the similar nature, in terms of quality, safety and efficacy, of the new similar biological medicinal product and the chosen reference medicinal product.

**Specification**
A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use.

Conformance to specification means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval.
**Structure (primary, secondary, tertiary, quaternary)**
Terms used to describe the two- and three-dimensional arrangement of the polypeptide chain in a protein. Primary structure is a synonym for the sequence of amino acid residues; the secondary structure is formally defined by hydrogen bonds between backbone amide groups (forming structure elements such as the a-helix and the b-pleated sheet), whereas tertiary structure describes the proteins overall shape, also known as its fold. The arrangement of multiple folded protein subunits which are assembled in a multi-subunit complex is called quaternary structure.

**Substitution, generic**
Generic substitution is the dispensing of a different brand or an unbranded drug product for the drug product prescribed; i.e., the exact same chemical entity in the same dosage form but distributed by a different company. (Taken from: World Medical Association Statement on Generic Drug Substitution, 1989/2005, see [http://www.wma.net/e/policy/d9.htm](http://www.wma.net/e/policy/d9.htm))

**Substitutability**
Two products are substitutable with each other if they can both be used in lieu of the other during the same treatment period. Substitutable products are interchangeable with each other. Cross-over studies are required to demonstrate substitutability.

**Validation**
The process of demonstrating that the system (or process) under consideration meets in all respects the specification of that system or process. Also, the process of evaluating a system or component during or at the end of the development process to determine whether it satisfies specified requirements. In the manufacturing of medicinal products, production processes, cleaning procedures, analytical methods, in-process control test procedures, and computerized systems all have to be validated according to the ICH guidelines for Good Manufacturing Practice. (see [http://www.ich.org/LOB/media/MEDIA433.pdf](http://www.ich.org/LOB/media/MEDIA433.pdf))

**Well-characterized biologic**
A well-characterized biologic is a chemical entity whose identity, purity, impurities, potency and quantity can be determined and controlled. Most of these products are recombinant DNA-derived proteins or monoclonal antibodies. For DNA-derived proteins, determining identity requires establishing the primary and secondary structures, including amino acid sequence, disulfide linkages (if possible), and post-translational modifications such as glycosylation (the attachment of carbohydrate side chains to the protein). Monoclonal antibodies can be identified with rigorous physicochemical and immunochemical assays. Purity and impurities must be quantifiable, with impurities being identified if possible; the biological activity and the quantity must be measurable. (see [http://pubs.acs.org/hotartcl/ac/96/nov/fda.html](http://pubs.acs.org/hotartcl/ac/96/nov/fda.html))
Appendix III References:

- WHO - GUIDELINES ON EVALUATION OF SIMILAR BIOThERAPEUTIC PRODUCTS.

- ICH guidelines:
  - ICH S6 - Pre-clinical safety Evaluation of Biotechnology-derived pharmaceuticals.
  - ICH E8 - General consideration for clinical trials.
  - ICH E9 - Statistical principles for clinical trials.
  - ICH Q5C - Quality of Biotechnological products: Stability testing.
  - ICH Q5D - Derivation and characterization of cell substrates used for production of Biotechnological /Biological products.
  - ICH Topic Q5E, Step 4 Note for Guidance on Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process.
  - 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 Q11 - Development and manufacture of drug substances (chemical entities and biotechnological/biological entities.

- EMA guidelines:
  It should be noted that the Committee for Medicinal Product for Human Use (CHMP) has or may develop additional guidance documents addressing both (the quality, non-clinical and clinical aspects, also for product-class specific documents) to be conducted for the development of biosimilars & will be made progressively available on EMA website:

- EMA - Overarching biosimilar guidelines
- EMA- Product-specific biosimilar guidelines
- EMA- Other guidelines relevant for biosimilars
- EMA- Scientific Guidelines on Biological Drug substances
- EMA- Scientific Guidelines on Biological Drug Products
- FDA - Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product
- FDA- Scientific Considerations in Demonstrating Biosimilarity to a Reference Product