Pharmacological approaches to demonstrating biosimilarity

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Biosimilars offer increased treatment options for patients and physicians, and optimize efficiencies across different healthcare systems. Therefore, biosimilars have the potential to provide lower cost alternatives, and offer greater access to biologics, allowing increased use of biologic therapies.

By definition, and in contrast with small-molecule generic products, it is impossible to manufacture identical copies of biologic products (The World Health Organization, WHO; 2016). However, this does not mean that the biosimilar is less effective or has greater adverse effects, when compared to the original drug. Information from the literature, the United States (US) Summary Basis of Approval, European Public Assessment Reports and requests via the Freedom of Information Act (EMA, 2017; FDA, 2015a) can be used to identify key studies and methodologies pertaining to reference products.

Additionally, because biosimilar development likely began several years after the development of the reference product, the relevant technologies may have evolved and improved meanwhile. The regulatory expectation is that the biosimilar manufacturer must apply contemporary technologies to product development, and also adhere to current industry standards and which may also have evolved from the first time the reference product was developed and approved. The approval of biosimilars is a highly regulated and detailed process. The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) guidance documents stipulate that a biosimilar manufacturer must perform a series of extensive similarity assessments to demonstrate biosimilarity to the reference product, in order to ultimately gain regulatory approval or licensure (EMA, 2015; FDA, 2015b). WHO has also published general guiding principles for the development of biosimilars, to provide coherent approaches for national regulatory guidelines (WHO; 2016).

Biosimilars must be highly similar to the reference product, in terms of structure, biological and physico-chemical properties, efficacy and safety.

For these reasons, processes leading to biosimilar approval encompasses several comparability exercises between the biosimilar and the original drug, consisting of three fundamental steps;

- comparative quality studies;
- comparative non-clinical studies;
- comparative clinical studies.

In this chapter, we focus on quality comparative studies and non-clinical comparative studies.

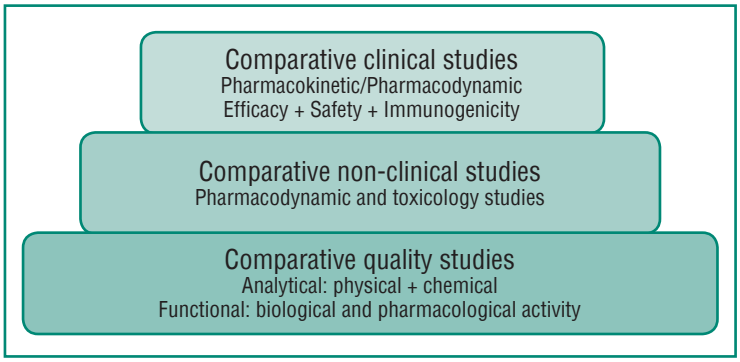

FIGURE 1.1 Adapted from the EMA Biosimilars in the EU Information guide for healthcare professionals.

Quality comparative studies are *in vitro* studies that compare protein structure and biological function, using techniques that identify even the smallest clinically relevant difference between a biosimilar and its reference drug. Differences that affect clinical safety, efficacy or immunogenicity must be further analyzed (*e.g.* with non-clinical or clinical comparative studies).

Non-clinical comparative studies include *in vitro* drug-dynamic studies which analyze relationships between activation (or inhibition) of physiological targets, and characterize immediate physiological effects in cells. *In vivo* pharmacodynamics studies (animal models) are performed only in the absence of viable *in vitro* alternatives. *In vivo* toxicology studies are only necessary in some cases, for example when the biosimilar is produced in a new cell or organism, or when the formulation includes new excipients not previously used.

In terms of quality, biosimilars must provide the same quality standards as all other authorized medicines: companies must demonstrate significant data to show that the biosimilar is manufactured to agreed standards, and that it is suitable for clinical use.

This is the concept of "pharmaceutical quality" which according to legislation, is declined in the study aimed at providing detailed data on:

- structural characterization and other physical-chemical properties;
- purity (residue traces from the manufacturing process must be checked and must not exceed fixed acceptable levels);
- biological activity;
- excipients and source materials;
- strength and formulation;
- control of the production process (to ensure the active substance and the finished product comply with accepted ranges for technical specifications);
- stability of the active substance and finished product, up to the expiration date under defined storage conditions.

Biosimilar approval processes are stringent and are based on a "*totality of evidence*" approach, which is accepted worldwide by all legislators. This approach considers analytical, preclinical and clinical evidence as a whole and does "not independently establish safety and effectiveness of the proposed biosimilar" as reported in the CHMP/ICH guidelines (available at http://www.ema.europa.eu) Human medicines > Scientific guidelines > Multidisciplinary > Biosimilar and, at FDA reports in the US Food and Drug Administration (FDA), Biosimilar development, review and approval (2017) document.

COMPARATIVE QUALITY STUDIES

ANALYTICAL STUDIES

The main purpose of analytic similarity studies is to identify attributes (defined by the FDA as critical quality attributes - CQAs) which impact on clinical outcomes, in terms of mechanism of action, pharmacokinetics and pharmacodynamics, and to evaluate the relationship with the latter. CQAs are required by all regulatory agencies.

CQAs can be identified at any stage of the manufacturing process, because any small change can lead to big changes in clinical outcomes. For instance, biologics are highly sensitive to environmental exposition during manufacturing, therefore responses to this factor must be carefully evaluated.

CQAs (ICH Q9 Quality risk management, 2006) are defined as "any chemical, physical, biological and microbiological attribute that can be measured, and continually monitored to ensure final product outputs remain within acceptable quality limits". For this reason, CQAs are indirect indicators of the manufacturing process quality. CQA identification serves as an evaluation and control process for those product characteristics that impact on quality, purity, efficacy and safety.

CQAs include product specific variants (*e.g.*, size, charge, glycans, oxidation, isomerization, aggregation and sequence variation), process related impurities (*e.g.*, host cell proteins, DNA, raw material and leachables), composition and strength (pH, excipients, quantity/concentration and osmolarity) and adventitious agents (potential viruses, bioburden and endotoxins).

A first critical point in quality assessment is to understand how CQA modifications translate to changes in clinical outcomes. To understand the connection of specific CQAs with clinical outcomes, on the basis of the pharmacokinetic, pharmacodynamic and mechanism of action of the drug.

Legislators classify CQAs into three tiers, based on the criticality and degree of CQA associated risks. The most relevant CQA to clinical outcome is classified as tier 1, mild-to moderate CQAs to tier 2, and least relevant CQAs to clinical outcomes as tier 3.

To reach "*totality of evidence*" and to demonstrate similarity between the biosimilar and the reference, the tier approach suggests that sponsors examine similarity through an equivalence test for CQAs in tier 1, a quality range approach for CQAs in tier 2, and raw data or graphical comparisons for CQAs in tier 3.

The equivalence test measures the equivalence of the mean between the biosimilar and the reference, while quality range approach attributes are assessed in relation to an interval (the mean and a multiple of the standard deviation). Finally, tier 3 is accepted as a descriptive comparison of raw and graphical data, presented side by side.

Extensive analytical data obtained with the analytical similarity assessment put the sponsor in the position of demonstrating the highly similarity between the proposed biosimilar and the reference product, but also the capacity of manufacturing the biosimilar in a well-controlled and consistent way.

Extensive analytical data obtained from analytical similarity assessments, places the sponsor in the position of demonstrating high similarity between the biosimilar and the reference, but also manufacturing the biosimilar in a well-controlled and consistent manner.

As described, the analytical consideration of quality comparability exercises must focus on physicochemical properties, biological activities, immunochemical properties and purity and impurities.

Analytical comparability exercises classifies the product in one of the following categories:

- insufficient analytical similarity;
- analytical similarity with residual uncertainty;
- tentative analytical similarity;
- fingerprint-like analytical similarity.

As previously described, approval process phases are considered as continuum developmental pathways, and only at the end of this pathways biosimilarity determined.

More specifically, a physicochemical analysis program should include composition determination, physical properties and primary and higher order biosimilar structures, using appropriate methodologies. Biosimilar amino acid sequences must be confirmed and be identical to the reference. N- and C-terminal amino

acid sequences, free SH groups and disulfide bridges should also be compared. Quantification of modifications and truncations must be performed, and any system-related variability has to be comprehensively described. Justification, with respect to micro-heterogeneous patterns/differences of the reference (*e.g.* C-terminal lysine variability), must be provided.

Biological activities should be evaluated using sensitive, specific and sufficiently discriminatory biological assays, in compliance with appropriate European Pharmacopoeia. Biological activities must be measured by different and complementary approaches, using ligand or receptor binding, enzymatic, cell-based and functional assays.

Finally, the purity profile of the biosimilar and the reference should be qualitatively and quantitatively compared by a combination of analytical procedures. This comparison should include the evaluation of specific degradation pathways (*e.g.* oxidation, deamidation and aggregation) of the biosimilar, and potential protein post-translational modifications. To further support similarity of degradation pathways, quality attributes comparison, tests at selected time points and storage conditions (*e.g.* accelerated or stress conditions) should be implemented.

FUNCTIONAL EVALUATIONS

Evaluation of the pharmacologic activity of the biosimilar must be performed using *in vitro* and/or *in vivo* functional essays. Those include biological assays, binding assays and enzyme kinetics, but also animal models of disease to evaluate functional effects on PD markers, or to test efficacy. Through functional assays, biological activity and potency is evaluated, potentially demonstrating high similarity or no clinically meaningful differences between the reference and the biosimilar. Comparisons between MOAs and product performances are also necessary to support biosimilarity demonstrations.

For example, in procedures to assess Ontruzant prior to market authorization, a trastuzumab biosimilar *in vitro* assays includes an anti-proliferation assays and ADCC and ADCP assays. In addition, binding properties are compared to HER2 binding, Fc receptor (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb and FcRn) binding and C1q binding.

Additional biological assays include surface HER2 expression measurements, HER2 ECD shedding, inhibition of AKT phosphorylation, *in vitro* angiogenesis and combination treatment with chemotherapy. These assays complete *in vitro* similarity assessments.

COMPARATIVE NON-CLINICAL STUDIES

After extensive structural and functional characterization, animal toxicity and pharmacodynamic studies are required. The purpose and extent of toxicity studies will depend on information obtained from previous phases, and on the degree of similarity from analytical and functional comparability exercises. Based on consistent analytical and functional data and similarity results, legislators foresee the possibility of foregoing toxicity assessments in animals. To date, all approved biosimilars in the US and Europe, with the exception of one, have been performed in non-clinical animal studies.

According to a recent paper evaluating non-clinical animal studies, conducted for 55 biosimilars approved in the US and/or the European Union (EU), the following techniques were performed: primary PD, PK, safety pharmacology, single-dose toxicity, repeat dose toxicity, toxico-kinetics, local tolerance and immunogenicity. Repeat-dose toxicity studies, along with toxico-kinetic studies are the most frequently conducted non-clinical studies, during the approval process. Primary PD and PK studies are conducted infrequently. Animal models used in the

repeat-dose toxicity and PK studies include mouse, rat, dog and monkey. For example, in a trastuzumab biosimilar toxicology program, a sponsor performed a comparative one month repeat-dose monkey toxicology study, a 14 day repeat-dose rat toxicology study and two tissue cross-reactivity studies with frozen human tissues. In line with guidelines on biosimilars, single dose toxicity, genotoxicity, carcinogenicity, developmental and reproductive toxicity studies were not performed.

The assessment of immunogenicity in animals should support the interpretation of animal study results. Animal immunogenicity assessments assist in the interpretation of animal study results, where general immune responses of biosimilar products in humans are not predicted. However, for differences in manufacturing between the reference and the biosimilar, that may produce differences in immunogenicity, the measurement of anti-drug antibodies in animal models could be a useful information for the biosimilar dossier. Furthermore, differences in immunogenicity profiles could highlight structural and functional differences between the reference and the biosimilar.

PK/PD STUDIES TO SUPPORT BIOSIMILARITY

A key point in the approval pathway of biosimilars is demonstrating pharmacodynamic and pharmacokinetic equivalence.

Given the intrinsic complexity of biologic drugs, instruments for functional and structural characteristic analyses are insufficient to guarantee no significant clinical differences between the biosimilar and the reference. For this reason, pharmacokinetic and pharmacodynamic studies are required. These represent the first steps towards clinical development and they consist in the demonstration of pharmacokinetic similarity and in the conduction of safety and immunogenicity studies.

Particular attention should be paid to PD biomarker choice. Legislators[4] allow the use of a single biomarker or a composite of biomarkers, as long as they effectively test the characteristics of the effects on the target of the drug. When determining which biomarkers should be used, it is important to consider the following points:

- the time of onset of change in the PD biomarker relative to dosing, and its return to baseline with discontinuation of dosing;
- the dynamic range of the PD biomarker over the exposure range to the biological product;
- the sensitivity of the PD biomarker to differences between the biosimilar and the reference;
- the relevance of the PD biomarker to the mechanism of drug action (to the extent that the mechanism of action is known for the reference);
- the analytical validity of the PD biomarker;
- clinical pharmacology data can then be used to support extrapolation of indications.

For example, the pharmacodynamic similarity of a rituximab biosimilar was shown by a whole blood depletion assay, by measuring an *in vitro* concentration-dependent decrease in B cells, after incubation with different concentrations of a rituximab originator and a rituximab biosimilar.

From a clinical perspective, the evaluation of PK and PD similarity should be based on two study designs: crossover and parallel.

Generally, a crossover design is preferred, due to its greater sensitivity in evaluating similarity, therefore identifying differences in exposure, with a minimum number of subjects. This design is recommended especially for products with a short half-life (less than five days), a rapid pharmacodynamic effect, and low expected immunogenicity. For pharmacokinetic similarity assessments, a single dose study may be sufficient, while a multiple dose study is required for pharmacodynamic evaluations, if delayed exposures are expected.

The use of a parallel design is more appropriate for drugs with long half-lives,

which trigger immune responses, or for cases where repeated exposure over time could trigger immune responses and alter PK/PD similarity.

Biosimilar monoclonal antibodies

Monoclonal antibodies (mAbs) represent a major product class of biotechnology-derived medicinal products. 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 region interacting with Fc receptors). Each individual mAb presents a unique profile with respect to the antigen-binding region, the Fc cytotoxic effector function and binding to Fc receptors.

NON-CLINICAL STUDIES

As reported in the EMA guidelines on similar biological medicinal products containing mAbs, non-clinical development, a step-wise approach is applied to evaluate the similarity of biosimilars and reference mAbs.

Non-clinical studies should be performed before initiating clinical trials. The comparative properties of mAbs must be studied using *in vitro* and *in vivo* studies.

In vitro *studies*

Relevant essays evaluate:

- 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 sensitive to detect differences in concentration-activity relationships between the biosimilar and the reference, and they should not just assess the responses per se.

In vivo *studies*

In vivo evaluations may be necessary if mAbs mediate effects have not been fully elucidated by *in vitro* studies.

Factors for additional *in vivo* non-clinical studies include, but are not restricted to:

- the presence of relevant quality attributes that were not detected in the reference (*e.g.*, new post-translational modifications);
- the presence of quality attributes in significantly different amounts than those measured in the reference;
- relevant differences in formulation, e.g. the use of excipients not widely used for mAbs.

If there is a need for additional information, the availability of a relevant animal species or other relevant model (*e.g.* transgenic animals or transplant models) should be considered. Due to mAb specificity, the relevant species is in most cases a non-human primate. In all cases however, the limitations of an *in vivo* study (*i.e.* sensitivity and variability) should be seriously considered. Animal studies should be designed to maximize the information obtained. When the model allows it, the PK and PD of the biosimilar and the reference should be quantitatively compared, including concentration-response assessments covering therapeutic doses in humans. For example, to evaluate the anti-tumor efficacy of Ontruzant, a trastuzumab biosimilar in the orthotopic BT-474 human breast cancer cell xenograft model was used. Ten groups of BT-474 xenograft mice, each consisting of 12 females (nine weeks old) received intrave-

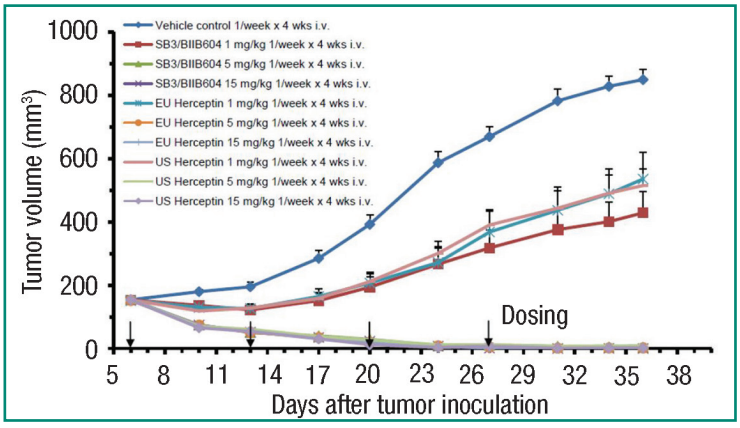

FIGURE 1.2. Tumor weight of mice between SB3 and herceptin treated groups on day 36.

nous Ontruzant formulation buffer (vehicle), Ontruzant, or EU or US Herceptin at 1, 5, and 15 mg/kg weekly doses for four weeks. (Figure 1.2).

The results showed that Ontruzant exerted similar effects in tumor growth inhibition on day 36 when compared to herceptin at the same doses of 1, 5, and 15 mg/kg. Tumor growth inhibition was measured by differences in tumor volume for treated versus vehicle tumors on the last day of therapy, or harvest day. (Assessment report for Ontruzant. Procedure No. EMEA/H/C/004323/0000).

CONCLUSIONS

Despite many challenges, the development of biosimilars is characterized by rigorous biotechnological processes. Biosimilarity is established based on the "*totality of evidence*", from structural and functional assessments through to non-clinical and clinical studies, thereby adopting a tailored, comprehensive approach throughout development.

The arrival of biosimilars challenges the healthcare community to learn and understand the scientific basis of biosimilars production and development when compared to long standing, widely used reference products.

Clinicians must understand that analytical assessments and preclinical studies are very sensitive tools in assessing similarity. Clinical studies are valuable tools for establishing biosimilarity.

In conclusion, when a biosimilar is established by regulatory agencies, there can be no doubt that the drug is safe in terms of efficacy and patient safety.

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2 Clinical approaches to demonstrating and evaluating biosimilarity

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The advent of biologic agents has transformed the treatment of several cancers, including breast cancer, hematologic malignancies, colorectal cancer, lung cancer. Despite the benefits of these biologic therapies and regulatory approval, not all patients for whom their use is indicated have access to the treatment. The high costs of these therapies is one of the most important barriers to patient access to treatment; some healthcare systems ration high-cost treatments by limiting the number of therapies or restricting the use to specific patient populations. To date, worldwide access to highly effective cancer treatments remains an unmet medical need in many countries, and this may partly explain the remarkable difference in survival rates observed in less developed countries for many cancer types. The use of biosimilars may expand access to newest therapies by offering a comparable, more affordable alternative to the increasing costs of cancer treatment. Moreover, the use of biosimilars for cancer patients is expected to increase in the near future with impending patent expiration of a number of biological agents.

Given the complexity in structure of biologics, even minor changes in the manufacturing process can produce post-translational structural differences that can affect safety and potency of the product. As a result, it is not possible to manufacture identical molecules or "generics" for biologic agents. The term biosimilar indicates a biologic product that is developed to be highly similar to an existing licensed biologic product with "no clinically meaningful differences between the biological product and the reference product in terms of safety, purity, and potency of the product.[1] Due to the variability and more complex manufacturing of biosimilars, the generic approach, which involves demonstration of bioequivalence with a reference medicine, applicable to most chemically-derived medicines, is not sufficient to demonstrate the similarity of biological-derived products.

In 2004-05, the European Medicines Agency (EMA)/European Commission (EC) was the first major regulatory authority to introduce a framework for approval and marketing authorization of biosimilars.[2] Subsequently, the EMA Committee for Medicinal Products for Human Use (CHMP) and the US Food and Drug Administration (FDA) released specific guidelines to inform biosimilars development, outlining data requirements and studies necessary to demonstrate similarity. Regulatory requirements are usually consistent across different guidelines (FDA, EMA, WHO, Health Canada etc).