Review

Biosimilar monoclonal antibodies: preclinical and clinical development aspects

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ABSTRACT

Biological drugs and their originated biosimilars are large, highly complex molecules derived from living cells or organisms. Traditional medicines, by contrast, are usually simple molecules of low molecular weight, synthesised by chemical means. The distinct complexities and methods of manufacture create an important difference between biosimilars and conventional generic drugs: while chemical generics can be fully characterised as identical to the originator product, biosimilars cannot. In addition, biological therapies are inherently variable, creating unavoidable differences between even subsequent batches of the same product. An expiring patent does not necessarily mean that the manufacturing process of the originator product becomes available to the biosimilar developers (for instance, the relevant cell line clone and growth medium). Therefore, it cannot be guaranteed that biosimilar products are identical to their reference product on a molecular level. This difference has important implications for the regulation and licensing of biosimilars. While conventional generic drugs require only a limited comparison and demonstration of identical chemical structure to the reference product, biosimilars require far more rigorous testing. In general, there must be a thorough comparison of structural and functional characteristics between biosimilar and originator drug. Stepwise nonclinical in vitro and in vivo approaches are recommended to evaluate the similarity of both drugs and any identified micro-heterogeneities must then be assessed for their impact on safety and clinical performance. Subsequently, clinical pharmacokinetic (PK) studies need to be performed in order to demonstrate a similar PK profile, prior to conducting clinical efficacy trials.

Introduction

The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) define biosimilars as biological products that are highly similar to an already authorised biological medicine (reference product). FDA details the definition by adding "with no clinically meaningful differences in terms of safety and effectiveness from the reference product" in their definition (1, 2).

In the European Union (EU), biosimilars are licensed through a thorough comparability exercise with the reference product, including clinical studies to ensure equivalence of efficacy and safety. Guidelines produced by the EMA detail manufacturing process requirements, and the range of protein structure, isoform, aggregate, receptor binding and biological activity assays necessary to demonstrate biological equivalence (3, 4). EMA guidelines also outline the required clinical and non-clinical pharmacokinetic (PK), pharmacodynamic (PD) and pharmaco-toxicological evaluations necessary to assess safety and efficacy before approval (2, 5). EMA guidelines have served as a starting point for development of licensing procedures in the US, where the FDA released draft guidance for the regulatory review of biosimilars in early 2012 (6).

The initial regulatory experience of EMA involved biologicals of relatively small size (insulin, interferon, filgrastim, epoetin and somatropin) (7). Amongst the biosimilars that underwent EMA review from 2006–2011, six produced unexpected results that were not foreseen during non-clinical testing. Of these biosimilars, four failed to demonstrate comparable efficacy and/or safety against the originator products, and were rejected or withdrawn. For the remaining two, one had its manufactur-

ing process modified and its clinical trial repeated, and the other had its label extension for subcutaneous use denied, prior to marketing authorisation (7).

For monoclonal antibodies, the EMA "Guideline on similar biological medicinal products containing monoclonal antibodies: non-clinical and clinical issues" came into effect in 2012 (8). Due to the nature of monoclonal antibodies, PK is often highly variable even within the same disease, for example in adjuvant versus metastatic breast cancer, where comorbidities may alter PK. Therefore, PK studies are a necessary component of the clinical programme to establish similar efficacy to the originator antibody. In addition to PK, PD studies are also important for assessing comparability. For some drugs, such as filgrastim and epoetin, absolute neutrophil counts and haemoglobin concentration/reticulocyte counts are established and validated markers of drug activity. For other drugs, particularly anti-neoplastic antibodies, no validated PD markers exist to indicate anti-tumour activity (9).

The purpose of this article is to review relevant pre-clinical and clinical issues in the development of biosimilar monoclonal antibodies given the recent EMA endorsement of the first biosimilars for the treatment of inflammatory rheumatic diseases.

Quality and pre-clinical development of biosimilar monoclonal antibodies

Quality by design

The current regulatory frameworks and manufacturing processes for biosimilar products are revolutionary because they imply that there can be biosimilar development with no major focus on clinical testing. This new strategy, termed Quality by Design (QbD), implements a stepwise, knowledge and science-driven approach of biological production, further guiding non-clinical and clinical development of biosimilars toward the goal of employing suitable test systems, especially those that will give the best results for establishing biosimilarity (10-12).

The manufacturing steps used to create biologicals are complex and technology has greatly evolved in the last 15 years since the first therapeutic antibodies were introduced. Owing to the complicated nature of the cell-based expression systems, isolation methods and endogenous "drift" inherent to biological systems, as well as intentional changes introduced in the production processes, all biologicals demonstrate some degree of variability over time. Unlike small-molecule therapeutics, which are synthesised to obtain homogenous molecules, biologicals (reference and biosimilars) are produced by living cells and expression results in a heterogeneous mixture of similar molecules, with the same primary amino acid sequence and a range of quality attributes (which may include subtle differences in molecule glycosylation patterns) (13).

Extensive quality and pre-clinical analysis

As for all biologicals, the pharmaceutical quality of a biosimilar must be established including a complete description of the manufacturing process and full characterisation of the quality attributes (amino acid sequence, secondary and tertiary structure and post-translational modifications). In addition, comparability of the range of these attributes between the biosimilar and reference product must be shown. This interval is determined from historical data of batches of innovative monoclonal antibodies present in the market in recent years, allowing the implementation of a range where the quality of the biosimilar is included.

A critical feature of the development of biosimilars is that instead of establishing the clinical efficacy and safety of the product *per se*, it should be demonstrated that the product is (highly) similar to the reference product for which substantial clinical evidence already exists (14, 15). In addition to the comparability of the quality attributes, the comparability and similarity of the biological activity is determined using assays relevant to the mechanisms of action of the reference biological in all indications (Table I) (16).

Most biologicals are likely to be modified throughout their life cycles

All biological pharmaceutical agents are likely to be modified several times throughout their life cycles (17, 18). Therefore, after several changes to the original manufacturing process, biologicals are no longer identical to the original version that underwent clinical testing at the time of marketing authorisation (17). When submitting a modification to a biological agent, the manufacturer must conduct comparability testing as required by the regulatory authorities to ensure that quality, efficacy and safety are not adversely affected (16-19).

Over the past decades of biotechnology development, regulators have accumulated extensive experience in the assessment of such changes. Once approved, the new version is expected to have the same efficacy and safety in all therapeutic indications, without undergoing further clinical testing (17). For example, with original infliximab, the addition of manufacturing facilities and continuous process optimisation has required 50 major submissions to the regulatory authorities using the Comparability Protocol (CP) as a regulatory filing strategy. The CP allows for downgrades to a lesser reporting category with no clinical testing requirement (20). With each of these process alterations, no label changes were necessary. There were no reported safety or efficacy problems. Batch-to-batch variability in the reference product is often minimal, but larger variations can be found after manufacturing changes. Both pre and post change products are often used concurrently in the same patients, thus the range of variation between them with respect to product attributes is presumed to be acceptable to regulators and judged as not impacting safety, purity, or potency. This means that pre and post change products are used interchangeably and can be found on the market at the same time. Several switches can and do occur between these products during the course of a treatment regimen (16). For example, commercial batches of rituximab with expiry dates from September 2007 to October 2011 were recently characterised using glycan mapping, cation exchange chromatography and antibody-dependent cellular cytotoxicity

(ADCC) *in vitro* bioactivity. In 2008, a sudden change in the quality profile became apparent for batches with expiry dates in 2010 or later, probably due to a manufacturing change. The difference was found in the amount of the C-terminal lysine and N-terminal glutamine variants when analysed by cation exchange chromatography. However, as noted, these changes do not impact strongly in antibody activity. Another physicochemical difference was detected in the glycan map for unfucosylated G0 glycans. The abundance of unfucosylated G0 glycan in rituximab increased in the antibody structure and the measured ADCC potency also showed an increase (21).

Safety evaluation

Toxicity studies are used for the safety evaluation of pharmaceuticals, including biologicals. Like efficacy, predictable adverse effects of biologicals are related to exaggerated pharmacology. Once comparable pharmacological activity has been established *in vitro*, there is no need to confirm these properties *in vivo*. Some types of adverse effects are not predicted by animal studies and can be designated as unpredictable adverse effects, as they only occur at a low rate. As an example, infusion reactions, which are associated with the release of cytokines, may be triggered by different causes, including process-related impurities acting through Toll-like receptors (TLRs), which are part of the innate immune system.

When there is a concern related to the presence of process-related impurities, *in vitro* TLR assays may detect and identify such contaminants. An additional type of adverse effect is due to unexpected toxicity, or 'off-target toxicity'. Unlike new chemical or protein entities, where it is imperative to perform toxicology studies, for biosimilars of older agents such as infliximab, this is not the case. Examples of unexpected toxicity are scarce and none have involved biosimilar products, but rather biologicals that were still in development. For example, thrombocytopenia occurred after administration of a monoclonal antibody under development, whereas this did not happen with four other monoclonal antibodies directed at the same pharmacological target. In this case, monoclonal antibodies had the same target, but were not biosimilars. Subsequently, it was shown that the functional differences between these antibodies had a structural basis due to variances in amino acid sequence. This unexpected toxicity was driven by protein or platelet aggregation. Obviously, such structural differences are not within the scope of a biosimilar development, since the amino acid sequence is similar to the reference drug and structural and functional similarity have already been shown by analytical and *in vitro* methods. Therefore, although unexpected toxicity may be encountered in animal studies in rare cases during the development of new biological entities, it has never been shown to occur during the development of a biosimilar. There is no scientific data to indicate realistic safety concerns to extrapolated indications. When analytics demonstrate that the active moieties in two products are "highly similar," then the only "unknowns" – or residual uncertainty - are PK and Immunogenicity (16).

Table I. Required comparability testing for biosimilar products.

Attribute	How is similarity demonstrated?
Protein structure and manufacturing quality	Extensive laboratory analyses of molecular characteristics (multiple batches)
Pharmacokinetics, pharmacodynamics and toxicity (animal) *vitro* experiments)	*In vitro* and in vivo assays (in a relevant animal species, only if additional info is needed after *in*
Pharmacokinetics, pharmacodynamics and toxicity (human)	Early clinical studies
Clinical efficacy and safety	Pivotal clinical trials
Safety in routine practice	Risk management plan Phase IV study Routine AE reporting / pharmacovigilance systems

Pharmacology assessment

The challenges that manufacturers face in establishing clinical efficacy and safety similarity of a biosimilar and reference monoclonal antibody in the anti-cancer setting are well recognised. Preferred endpoints for confirming efficacy, such as progression-free, disease-free and overall survival may not be feasible to establish biosimilarity as they may be influenced by factors unrelated to differences between the biosimilar and reference monoclonal antibodies, such as tumour burden, performance status and previous therapy (22-25). The non-clinical and clinical biosimilar guideline therefore acknowledges that surrogate endpoints such as overall response rate or change in tumour mass may be more appropriate (26).

In the context of immunomodulatory monoclonal antibody biosimilars, the mechanism of action of infliximab in rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA) is the inhibition of the inflammatory activity mediated by TNF signaling via binding and blocking of both soluble and membrane TNF.

In inflammatory bowel disease (IBD), additional mechanisms have been proposed for the efficacy of monoclonal antibodies (such as infliximab and adalimumab). These include the ability of these agents to induce reverse signaling through membrane-bound TNF, resulting in reduced cytokine production and increased T-cell apoptosis. The ability of monoclonal antibodies to induce ADCC has also been hypothesised to contribute to the efficacy in IBD. Besides the binding of the complementarity-determining region (CDR) to its primary target, the Fc portion of the molecule also contains binding sites to different Fc receptors (FcyR and FcRn), which may elicit several effector functions, notably complement activation, complement-dependent cytotoxicity (CDC) and ADCC. These Fc-related binding properties and effector functions can also be evaluated *in vitro*. This knowledge was not present during the

initial stages of reference infliximab's commercial life, therefore, they were not analysed when the manufacturing changes occurred.

Because the biological properties of a biological drug can be characterised *in vitro*, there is little – if any – further information that would be gained by *in vivo* models. Thus, there was no need to re-establish the pharmacodynamic response in an *in vivo* model. Moreover, using cellular systems, more extensive, precise and thus more sensitive comparisons can be made using *in vitro* assays, which further strengthens the *in vitro* approach in the evaluation of biosimilar infliximab. These assays are more sensitive than clinical trials to evaluate mechanisms of action.

As mentioned, analytical and PK/PD assessments are the most robust scientific basis for comparing independently sourced biologics. However, it is important to acknowledge that quality attributes of a biological may also affect its PK behavior, which is not covered by the *in vitro* assay. Therefore, it is necessary to establish PK similarity in human volunteers or patients.

Immunogenicity

The primary safety concern for biosimilars, as for all biological medicines, is immunogenicity. Formation of anti-drug antibodies (ADA) in patients is an important issue that needs to be evaluated for all biologicals – both originator products and biosimilars – before they enter the market. Binding antibodies may affect the PK and neutralising antibodies can lead to loss of efficacy. Knowing that the presence of aggregates may increase the immunogenicity, their levels should be similar (or lower) in a biosimilar compared with the reference product. This can be determined by analytical methods.

Most biological therapies elicit an immune response, in most cases with no clinical consequences. However, there are some biologicals for which immune responses have been linked to serious safety issues, notably the pure red-cell aplasia (PRCA) caused by cross-reacting neutralising antibodies against erythropoietin. Even small structural alterations may have an impact on immunogenicity and analytical or animal data cannot always predict human immune responses. To mitigate this unavoidable risk, extensive clinical trial data demonstrating no increase in immunogenicity of the biosimilar compared with the reference product are required before a biosimilar can be licensed. In fact, the risk for detection of a new and serious adverse effect after licensing is considered by some to be much lower for a biosimilar than for a biological containing a new or modified active substance (27). Furthermore, the new technologies used in manufacturing biosimilars mean that the products are generally of higher purity and quality, and more consistent potency, than their originator reference products (28). Unfortunately, inadequately produced copies exist and can lead to major issues, as recently exemplified by numerous cases of PRCA in Thailand (29).

Pharmacokinetic analysis of biosimilars

Although the route to market for classic generics is well defined and has been successfully applied for many drugs over the years – typically a small number of studies in healthy volunteers are sufficient to prove physicochemical and PK equivalence – the corresponding route to market for biosimilars is relatively new and considerably more complex. There are some key issues that should be considered when performing PK analyses to prove biosimilarity:

Study design

The most common designs associated with bioequivalence studies are cross-over designs; however, as biologicals tend to have much longer half-lives than classic drugs, a cross-over approach is generally not practical (the washout period which would be required is often prohibitively long) (4, 6, 30). Furthermore, the potential for biologics to illicit an immune response also limits the use of cross-over studies; if a patient was to develop an immune response in the first period of a cross-over study, the same patient's ability to participate in the second treatment period would be compromised.

To overcome these issues, it is common to use a parallel group design when conducting biosimilar studies. Only one treatment period is required for each subject, removing the need for a washout period and the potential problematic effect caused by a patient developing an immune response is limited. However, it is important to note that parallel designs may have other problems: large sample sizes are required to ensure that there is sufficient statistical power to prove biosimilarity and as treatment differences are estimated between subjects (rather than within subjects), it is important to account for covariates (such as age, weight and sex) in the statistical assessment. In addition, this design limits conclusions regarding the switch between the originator and the biosimilar.

Acceptance limits

In classic bioequivalence studies, PK equivalence is demonstrated using bioequivalence limits of (0.80, 1.25); the test and reference products are considered to be equivalent if the 90% confidence interval of the ratio of geometric least-squares means lies entirely within (0.80, 1.25) (9). There are currently no such limits defined for biosimilars by EMA or FDA. Therefore, it may be possible to justify wider acceptance limits; however, it is still very common for biosimilar studies to apply the same (0.80, 1.25) criteria used in equivalence studies (31, 32).

Anti-drug antibodies (ADA)

Due to protein nature and a complex manufacturing process (often resulting in impurities), biologics have the potential to illicit an immune response. Indeed, nearly all biologicals induce ADA; however, the incidence differs widely among products and between individuals (10). In most instances, ADA have no clinical significance, but high levels can interfere with PK and PD properties of the drug (*e.g.* increasing clearance and thus reducing the extent of systemic exposure and desired drug effect). It is therefore important to have an assay in place to test for the presence of ADA at suitable intervals during the study, so that subjects who elicit an immune response can

be identified. A clinically meaningful and sensitive comparative assessment of ADA is a regulatory requirement in the development of biosimilars. However, the study design and type of immunogenicity assay are still a matter of debate since currently available assay formats have technical limitations, which may compromise reliable ADA detection (33-35).

Elimination characteristics

Whereas bioequivalence studies are typically limited to the area under the concentration-time curve (AUC) and maximum concentration (Cmax) as measures of the overall extent of systemic exposure and rate of absorption, respectively, elimination characteristics must also be considered when comparing biologicals due to their long half-lives and potential to develop immune responses (which can significantly alter clearance). Indeed, current EMA guidelines state that "the design of comparative PK studies should not necessarily mimic that of the standard "clinical comparability" design, since similarity in terms of absorption/bioavailability is not the only parameter of interest. In fact, differences in elimination characteristics between products *e.g.* clearance and elimination half-life should be explored" (4, 6, 30).

In this context it is important to note that to fully characterise elimination kinetics in biosimilarity studies, the sampling schedule guidelines associated with equivalence (or clinical comparability) studies should still be applied (31, 32). The sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if AUC (0-t) covers at least 80% of AUC (0-∞). At least three to four samples should be needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant, which is needed for a reliable estimate of AUC (0-∞) (4, 6, 30). Thus, the last sampling time point may be many weeks after dosing.

Extrapolation of indications

Confirming comparability

EMA states, in its Guideline on Similar Biological Medicinal Products Containing Monoclonal Antibodies, that 'Extrapolation of clinical efficacy and safety data to other indications of the reference monoclonal antibody, not specifically studied during the clinical development of the biosimilar monoclonal antibody, is possible based on the overall evidence of comparability provided from the comparability exercise and with adequate justification (9). Comparability, as depicted in Figure 1 (same dose, same efficacy and safety) is mostly demonstrated by the extensive pre-clinical assessment, whereas clinical studies are needed for confirmation of these data.

The QbD totality-of-evidence approach means that all accumulated data, gathered since the reference product was first approved, have been considered. Regarding the most recent example of biosimilar infliximab, current knowledge about the mechanism of action in its several clinical indications exceeds the inactivation of circulating TNF and includes the induction of membrane-bound TNF reverse signaling (36, 37) and possibly CDC and ADCC (38). For RA and AS, the synovial effects of infliximab are mainly derived from the prevention of circulating TNF binding to its receptors (13-15, 36). However, in IBD, infliximab's binding to membrane-bound TNF may also be essential to achieve maximal efficacy. All of these proposed mechanisms of action were analysed in the pre-clinical comparability studies and the biosimilar was deemed comparable to the reference product in all of them. In fact, EMA's assessment report concludes that in the exhaustive pre-clinical comparability exercise, only a small potentially relevant difference in the mechanism of action was found between the

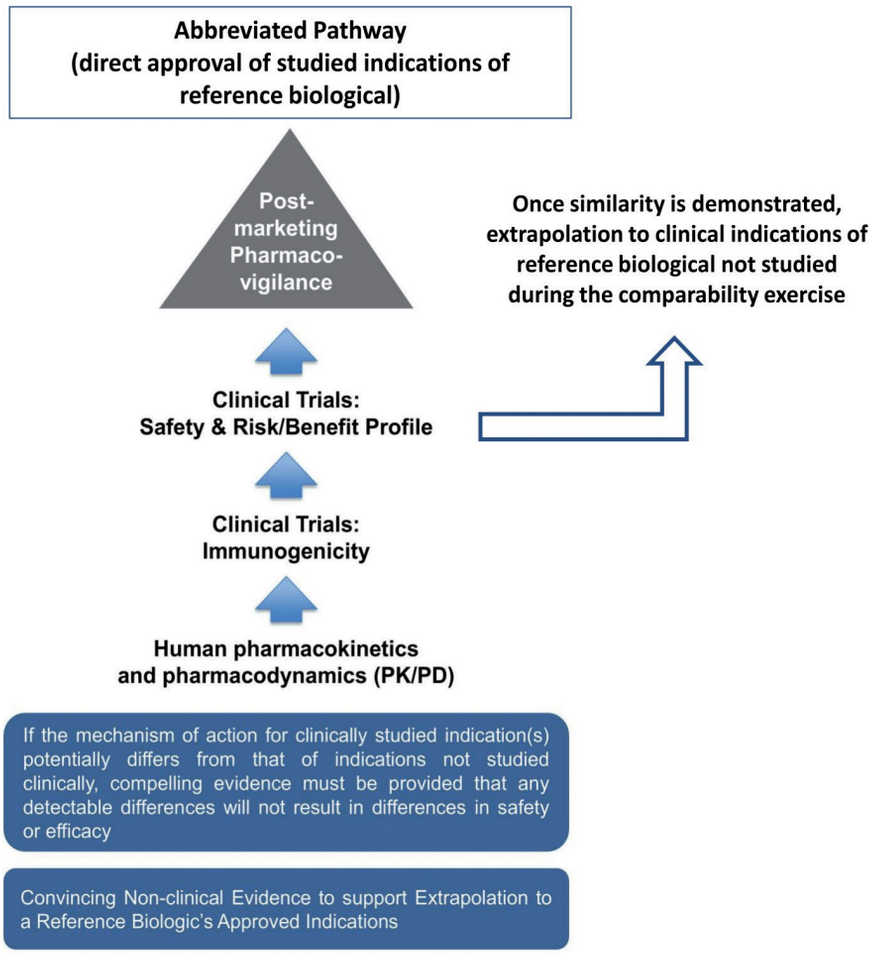

Fig. 1. Overall evidence of comparability required to support extrapolation to indications not clinically studied (33).

biosimilar and the originator infliximab (37-39). The biosimilar showed a lower amount of afucosylated moieties, which led to a significantly lower binding affinity to isolated Natural Killer (NK) cells FcγRIIIa and FcγRIIIb receptors, in the high affinity FcγRIIIa receptor 158V/V and V/F genotypes. This difference translated into lower ADCC activity, which hypothetically might affect the clinical efficacy of the biosimilar in IBD, while its efficacy in patients with RA would remain unchanged (38). This small difference, found only in a subgroup of patients and in the most sensitive preclinical assay was not replicated when conditions closer to reality were studied and it seems extremely unlikely that it will have any clinical impact (38-40). Nevertheless, the extrapolation of indications for the treatment of IBD granted by the EMA has received initial criticism by some scientific societies. The use of biosimilar infliximab in this context is regarded with caution due to residual uncertainty on the clinical implications of FcγRIIIa affinity discrepancies, but also to the fact that RA has been challenged as the most sensitive model to assess similarity and extrapolate immunogenicity, efficacy and safety data to a distinct disease, such as IBD (17, 41). However, it should be said that the importance of this mechanism of action for IBD is still a matter of debate, since no publish reports describe the induction of ADCC by anti-TNF inhibitors and there is no firm evidence that the FcyRIIIa polymorphism have an impact on the clinical course of Crohn's disease. The efficacy and safety of biosimilar infliximab in IBD patients has been assessed in several observational studies and a few open-label trials (42-52). These studies have shown biosimilar efficacy in patients with IBD, however, further data are welcome, and additional studies that will support the validity of extrapolation to IBD are ongoing (53). Many of the concerns raised with regard to extrapolation appear to be hypothetical, and will likely not be problematic in the long term.

These examples demonstrate how the comprehensive preclinical comparability of different drugs reduced the need for clinical studies after manufacturing changes and provided the grounds for the abbreviated clinical pathway supporting extrapolation of clinical indications and interchangeability within each clinical indication.

Clinical development of biosimilars of monoclonal antibodies

As already highlighted, the term biosimilar refers to a biologic drug that is developed to be highly similar to an existing licensed reference biologic (originator). The aim is to create a product with no clinically meaningful difference and of course with a lower cost. Biosimilars are intended to treat the same diseases as the reference biological using the same doses. Therefore, their greatest advantage is to offer an increased accessibility to targeted therapies through more affordable treatment alternatives with similar efficacy and safety (54). Biosimilars are currently developed or being developed for infliximab, etanercept, trastuzumab, rituximab, adalimumab, bevacizumab and cetuximab (55).

Due to the molecular complexity of biosimilars, the clinical development programme implies phase I and III trials in order to capture any possible clinical consequences of the subtle molecular differences that exist in comparison with the originator.

The clinical development of biosimilars of monoclonal antibodies should be based on equivalence trials, as they can identify the theoretical possibility of a biosimilar candidate having higher efficacy than the originator. However, at present, this is contrary to the concept of similarity and indeed a drug that has more activity/potency might be associated with more adverse events. In addition, as previously discussed, the design should be parallel, due to the long half-life of monoclonal antibodies and also to allow for adequate monitoring of immunogenicity, which would be hampered by a crossover design. EMA also recommends selection of clinical endpoints and patient populations that would facilitate detection of differences between the products, thus selecting the most sensitive scenario for detecting possible differences. EMA positioning regarding extrapolation has prevented the need for trials in all indications of the originator. Justification for extrapolation depends on clinical experience, published data and on the homogeneity of the mechanism of action across indications (54).

The patient position

As already mentioned, all biosimilars are expected to be introduced in the market at a lower price than their originator reference, and price is based on market forces, national competent health authorities and possibly some competition between manufacturers of originators and their biosimilars. This may lead to patient misconception that the availability of lower-priced biologicals, combined with pressure from health authorities and insurers to prescribe on the basis of cost alone, may reflect lower efficacy/quality of biosimilars. Recently, the rheumatic patient association PARE within the European League Against Rheumatism (EULAR) produced a position document stating that they strongly believe that "decisions about prescribing biosimilars should be made on clinical grounds and not solely on financial grounds" (56).

Conclusion

Biosimilars offer a highly attractive strategy for reducing medical costs and increase accessibility to targeted biological therapies. Therefore, both prescribers and patients must be correctly informed about the biosimilar option. Unlike small molecules, biologicals are large and complex tridimensional structures and the development and production of candidate biosimilars can be hampered by unpredictable variability that should be tackle as far as possible during the pre clinical development process.

However, full guaranty of similarity is only granted after equivalence parallel trials assessing PK, efficacy, safety and immunogenicity comparing the biosimilar candidate and the originator. A landmark event for the future widespread use of biosimilars, at least in the field of rheumatic diseases, was the EMA approval of infliximab biosimilars (57). The post marketing surveillance, with special emphasis on the role

of national registries, will be crucial for the confidence on the use of these biosimilars and will pave the way for the approval, in the near future, of other biosimilar candidates of trastuzumab, rituximab, adalimumab, etanercept, bevacizumab and cetuximab.

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