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EXPERT OPINION

1. Biosimilars
2. Monoclonal antibodies
3. Biosimilar monoclonal antibodies
4. Expert opinion

Biosimilar monoclonal antibodies: a science-based regulatory challenge

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Monoclonal antibodies (MAs) are complex biotherapeutics as their molecular mechanism of action depends on multiple domains. Consequently regulatory approval of biosimilars of MAs is subjected to specific, science-based guidelines. An extensive comparative *in vitro* characterization to evaluate the biosimilarity of the various functional domains is required. The exquisite species specificity of MAs precludes reliable *in vivo* non-clinical evaluations and means that adequately designed clinical studies are extremely critical to confirm the biosimilarity. To date no biosimilar MAs have been approved. Taking into account the expected high development costs for biosimilar MAs, their use may well be superseded by alternative antibody formats and next-generation MAs.

Keywords: biosimilars, biotherapeutics, monoclonal antibody, regulatory

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1. Biosimilars

After the expiration of patent(s) for the first approved biopharmaceuticals, "copying" and marketing of these biologicals can be offered by other biotech companies and might possibly, as with generics, reduce cost to patients and social security systems. However, biopharmaceuticals are made by living cells. Because of their intrinsic complexity and because no two cell lines, developed independently, can be considered identical, biopharmaceuticals cannot be fully copied. The final biopharmaceutical product is influenced by many variables, such as the type of expression system (e.g., bacteria, yeast, and mammalian cells), the growth conditions, the purification process, the actual formulation and the conditions during storage and transport. Post-translational modifications such as glycosylation, phosphorylation, sulfation, methylation, acetylation and hydroxylation may affect biological activity and result in an intrinsic molecular heterogeneity. Importantly, and in contrast to traditional chemical drugs, biopharmaceuticals are potentially immunogenic. In this respect it is important to note that subtle structural differences (e.g., consequent to small differences in the number and type of product variants) may significantly affect the immunogenic potential of the drug product [1]. Additionally, product- or process-related impurities can provoke an immune response [2].

The European regulatory authorities have introduced the term "biosimilar" in recognition of the fact that biosimilar products are similar to the original product, but never exactly the same [3,4]. Therefore, the European medicines agency (EMA) has issued a number of general as well as product-specific guidelines to be taken into account when developing biosimilars [5]. The concept of biosimilarity is based on comparability studies: extensive full comparison (relative to an approved reference product) at the level of structural biochemical and functional properties and reduced non-clinical and clinical evaluation. To date, only biosimilars of relatively small (20 – 30 kDa) biologicals have been authorized: somatropin, epoetin alfa, and filgrastim. No biosimilar monoclonal antibodies (MAs) (size 150 kDa) have

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been approved yet. Two biosimilar MAs (reference product: Infliximab, Remicade®) are currently under evaluation at the European Medicines Agency.

2. Monoclonal antibodies

The development of hybridoma technology by Kohler and Milstein in 1975 [6] is a major landmark in the generation and production of MAs. Even though initially mainly used for research purposes it became soon clear that the properties of MAs opened up new, unexplored therapeutic possibilities. Firstly, MAs are directed against one particular epitope in one particular target molecule and therefore they are highly specific ("magic bullet"); secondly, MAs exhibit particular effector functions through the Fc region; thirdly, MAs can be raised and selected against virtually any putative target. The first approved therapeutic monoclonal antibody was Muromonab-CD3 (Orthoclone Okt3®, anti-CD-3) authorized for the reversal of kidney transplant rejection [7]. Intrinsically associated with the procedure of the hybridoma technology Muromonab-CD3 was from murine origin. Indeed, the hybridoma technology is based on the fusion between two cell types, i.e., antibody producing B cells isolated from an immunized mouse and a murine myeloma cell. Successful fusion leads to a hybrid cell combining properties from the B cell (e.g., antibody production) and from the myeloma cell (i.e., immortal). At that time, and also still valid to date, this technology is mainly restricted to the use of mice as the source of B cells because the most efficient and compatible fusion partners are of murine origin. Despite of their high specificity toward the human target against which they are raised, MAs of murine origin suffer from a high degree of immunogenicity in humans and lack adequate Fc-mediated effects in humans. Undoubtedly, the widespread therapeutic application of MAs to date has been made possible through concomitant significant developments in rDNA technology. This allowed the cloning of the monoclonal antibody encoding sequences from hybridomas as well as from any other origin, including human. Subsequently, sequences encoding the antigen-binding portion of the (mouse) monoclonal could be recombined with cloned sequences encoding the human Fc-portion resulting in the production of chimeric antibodies (e.g., abciximab, rituximab, infliximab). More advanced cloning strategies resulted in the generation of "humanized" antibodies in which the CDR regions of the murine monoclonal are grafted into a human framework (e.g., palivizumab, trastuzumab, alemtuzumab). Parallel developments in "transgenic" technology led to the generation of transgenic mice containing the corresponding human antibody genes. Combination of the latter with hybridoma technology then allowed the direct generation of fully human antibodies (e.g., panitumumab). Alternatively, phage-displayed human antibody fragment libraries combined with cloning technology also allows the construction of fully human MAs (e.g., adalimumab).

Currently, 40 MAs for therapeutic or diagnostic use are approved in the European Union or the United States [7,8]. These are used mainly in the area of oncology, arthritis and immune and inflammatory disorders, but also in other therapeutic areas such as infectious and respiratory diseases, in ophthalmology and hemostasis. Many more MAs are in the pipeline [9,10]. MAs exert their pharmacological and therapeutic effects through a variety of functions. They can neutralize the action of and sequester soluble targets (e.g., anti-TNFa: infliximab, adalimumab, certolizumab, golimumab; anti-IL1b: canakinumab; anti-VEGF: bevacizumab, ranibizumab). When targeting cell-bound receptors MAs can be used to deliver a toxin or radioactive label (e.g., tositumomab-I^{131}, ibritumomab-tiuxetan), to block the function of a receptor (e.g., anti-IL2R: basiliximab, daclizumab; anti-RANK-L: denosumab) or to induce apoptosis (e.g., anti-CD20: rituximab, ofatumumab). In addition, therapeutic effects of MAs may also be mediated by antibody-dependent cell cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).

3. Biosimilar monoclonal antibodies

When considering the development of biosimilar MAs, it is important to realize that they are composed of multiple domains that contribute to their mode of action and affect their clinical properties. The Fab region contains the variable domains responsible for the specific interaction with the target. The Fc region plays an important role in antibody-dependent cell-mediated cytotoxicity, in complement-dependent cytotoxicity and can exert other general regulatory effects on the cell cycle by triggering signaling pathways. Importantly, the Fc region is glycosylated and both type and extent of glycosylation play an important role in the effector function and on the clearance. Even though to a lesser extent, the glycosylation of the Fab region should also not be ignored [11]. Therefore, evaluation of biosimilar MAs (including comparability exercises of quality attributes) should not only include antigen-binding (Fab) but also Fc-mediated functions (e.g., binding to FcγR, FcRn, complement). Fab-associated functions should not be restricted to antigen binding but also include the expected functional effects on the target (e.g., neutralization, receptor blocking, and receptor activation). Because of this complexity biosimilarity of MAs should not be demonstrated merely on *in vitro* biochemical evaluation but requires also extensive *in vivo* functional evaluation. The latter, however, is very much limited because of the lack of appropriate animal models (i.e., species specificity of antigen and of Fc-binding partners hampers a full evaluation). Taking all these factors into account it is clear that the development of a biosimilar monoclonal antibody is much more demanding than the development of a simple biological as has been the case until to date.

Not surprisingly, the EMA has issued, very recently, specific guidelines on biosimilar MAs [12,13]. As for any other biosimilar they need to comply with the relevant general guidelines. In addition, because of their particular complexity and multi-domain composition, they need to be subjected to a set of specific analyses.

According to the guideline [12], a first step is the evaluation of particular quality attributes with respect to binding and functional characteristics. Therefore, *in vitro* studies are required in which the biosimilar and the reference are compared to each other with respect to

1) *binding* to the target antigen,
2) *binding* to representative isoforms of the relevant three Fc gamma receptors (FcγRI, FcγRII, and FcγRIII), FcRn and complement (C1q),
3) Fab-associated *functions* (e.g., neutralization of a soluble ligand, receptor activation or blockade) and
4) Fc-associated *functions* (e.g., ADCC, CDC, complement activation).

It is important to note that the guideline specifies that the functional assays should be designed to allow the detection of minute differences in the concentration–activity relationship between biosimilar and reference. In view of the fact that animal models may not be adequate, these extensive *in vitro* characterization assays (target antigen, Fc-receptors, and cells all of human origin) are of crucial importance since they are usually more specific, more sensitive and more representative for the "human situation." The guideline explicitly states that "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" [12].

In a second step, it needs to be considered if there is a need for *in vivo* non-clinical testing. This evaluation is based on, e.g., the presence of relevant quality attributes that have not been detected in the reference product (e.g., new post-translational modification structure), presence of quality attributes in significantly different amounts than those measured in the reference product, relevant differences in formulation. If no concerns are identified one may consider *in vivo* animal studies unnecessary. If critical elements have been detected in the *in vitro* comparability exercises then relevant animal models should be looked for. A major hurdle is the search for a suitable species because of the high (species) specificity of MAs. In most cases either non-human primates, transgenic animals or (human) transplant models may be appropriate, even though still including limitations [14]. In the event animal studies are deemed necessary, the design will depend on the required information (pharmocokinetics/pharmacodynamics/safety) and on the properties of the model. Repeated dose toxicity studies are usually not informative and not required. Potential toxicity risks consequent to differences in process-related impurities should be minimized by keeping these impurities to low levels. Differences in product-related variants that could affect biological functions are assumed to be detected in the *in vitro* tests. One of the major concerns of adverse reactions is immunogenicity. On the one hand the immunogenicity is significantly influenced by the clinical context; on the other hand the clinical impact of immunogenicity very much depends on the binding site, titer, affinity and duration of immune response. To date, there is no appropriate animal model to predict immunogenicity in humans. Therefore, initial immunogenicity assessment is mainly based on a risk-based approach and requires an important postmarketing vigilance plan [15].

Clinical evaluation of the biosimilar monoclonal antibody requires a comparative analysis between the biosimilar and the reference product. According to the guidelines, the study design for clinical pharmacokinetic analysis should take numerous factors into account (e.g., long half-life, immunogenicity, disease and patient characteristics, and pharmacokinetics of reference). The dosing should be selected on the basis of the sensitivity to detect possible differences and preferably all routes of administrations should be investigated. Pharmacodynamic markers might be sensitive to detect differences and preferably a set of different markers should be evaluated to provide evidence of comparability. Similar to the requirements of the *in vitro* evaluation, a dose–response or time–response may provide a pivotal proof of comparability. Ultimately, clinical efficacy needs to be evaluated. Importantly the guiding principle is to demonstrate similarity of the biosimilar to the reference, and not to evaluate the patient benefit. Consequently, the selection of the patient population is mainly based on the need of homogeneity and sensitivity. Extrapolation of clinical safety and efficacy data to other indications can be considered only upon scientific justification based on the relative contribution of Fc and Fab and their relative interactions as well as on the molecular mechanism of action of the monoclonal antibody.

For all biologicals in general and biosimilars in particular, pharmacovigilance is of utmost importance and needs to be focused particularly on safety in indications authorized based on extrapolation and on immunogenicity.

Whereas two biosimilars of infliximab are currently under evaluation at the European Medicines Agency, two companies developing a biosimilar of rituximab have suspended their plans for Phase III clinical trials. It is not clear whether this decision is purely strategic or based on the detection of significantly different properties that may compromise biosimilarity. In the latter case, it may be more appropriate to develop the product as a stand-alone biological. At least four other companies are currently conducting clinical trials (Phase I, II, or III) for biosimilar versions of rituximab, infliximab, or trastuzumab [16].

4. Expert opinion

Biosimilar monoclonal antibodies constitute a unique class of biosimilars. MAs comprise multiple domains each contributing to the pharmacological and therapeutic properties of the molecule as well as to the safety profile. Stringent regulatory requirements specific for biosimilar monoclonal antibodies result from this scientific complexity. In the absence of any approved biosimilar monoclonal antibody and in the absence

of available scientific evaluation reports of submitted applications, it is currently unclear if and how biosimilar MAs will penetrate in healthcare. Even though it might be expected that in the near future biosimilar MAs will be approved for marketing, some obstacles could hinder their fast and long-term use: i) In view of their complexity the development costs can be expected to be significantly higher compared to that of "regular" biosimilars. Consequently, price reductions compared to the reference may be very small; ii) biosimilar MAs are also expected to experience significant competition by next-generation MAs exhibiting improved properties, e.g., through glycoengineering [17]; iii) alternative antibody-based therapeutics such as bispecific MAs and Fc-linked fusion proteins, exhibiting improved therapeutic potential and improved pharmacodynamics may also compete with biosimilar MAs; and iv) other antibody formats currently under development such as nanobodies, produced in bacteria and thus expected to be much cheaper may well form an alternative for reducing costs in healthcare.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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