SCIENCE & SOCIETY

An FDA perspective on the assessment of proposed biosimilar therapeutic proteins in rheumatology

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Abstract | Biologic products have revolutionized the management of many rheumatic diseases, but access to these products might be limited by their relatively high costs. The US Biologics Price Competition and Innovation Act of 2009, which is contained within the Patient Protection and Affordable Care Act, established an abbreviated pathway for licensure by the FDA of biologic products that are demonstrated to be biosimilar to or interchangeable with FDA-licensed biologic products, termed reference products. This law allows for the approval of biosimilar biologic products, which are expected to increase access to treatment for patients, and ensuring the implementation of this Act is a high priority for the FDA. In this Perspectives article we describe the considerations for approval of proposed biosimilar products, including those to treat rheumatological conditions, by describing the FDA's rigorous approach to assessment of biosimilarity.

Several therapeutic proteins, mainly monoclonal antibodies and Fc-fusion proteins, have become important treatment options for patients with rheumatoid arthritis (RA) and other rheumatologic diseases (TABLE 1). These biologic therapies have had a major effect on the course of disease for many patients. Various novel biological products targeting different pathways in rheumatic diseases are in development, and expansion of the options for biologic treatment is expected. Nevertheless, the cost of biologic therapies limits access to these important drugs for many patients.

The Biologics Price Competition and Innovation Act of 2009 (BPCI Act), which is part of the Patient Protection and Affordable Care Act, created an abbreviated pathway for licensure of biologic products that are demonstrated to be biosimilar to or interchangeable with a previously approved FDA-licensed biological product (termed reference product), which are expected to improve access to biologic drugs for rheumatologic and other diseases[1]. Although an abbreviated pathway for approval of 'generic' drugs (typically small-molecule products) was established in 1984, in the Drug Price Competition and Patent Term Restoration Act (Public Law 98–417; also known as the Hatch–Waxman Act), this legislation applies only to drugs that have the 'same' active ingredient as an approved drug and effectively copy it. Due to the inherent complexity of biologic products and their manufacturing processes, for any given biologic drug there will be variability between lots. Thus, two biologic products are highly unlikely to be identical, despite having strong structural and functional similarities. This factor underscores the new scientific and regulatory challenges in the implementation of the BPCI Act.

Biosimilarity is defined in the BPCI Act to mean "the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components" and that "there are no clinically meaningful differences between the biological product and the reference product in terms of safety, purity and potency" (REF. 1). The FDA's guidance documents *Scientific Considerations in Demonstrating Biosimilarity to a Reference Product*[1] and *Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product*[2] outline the important scientific principles of demonstrating biosimilarity and the FDA's recommendations to sponsors seeking to do so. Multiple scientific challenges are associated with the development of a biosimilar product, but the FDA can leverage its extensive experience reviewing within-product manufacturing changes, including comparability data for complex novel biologics[3].

The FDA considers the totality of the evidence from analytical, nonclinical and clinical studies provided by sponsors of proposed biosimilar products (FIG. 1). Biosimilarity should be demonstrated with a stepwise approach, in which the foundation evidence comprises an extensive structural and functional characterization of the proposed product compared with the reference product. Clinical studies that assess pharmacokinetic and, if appropriate, pharmacodynamic similarity are important components to show biosimilarity. Nonclinical data may be provided from *in vitro* pharmacology studies and, if necessary, animal toxicity studies. Of note, the goal of a biosimilar development programme is not to establish independently the safety and efficacy of the proposed biosimilar product, because this was previously proven for the reference product; rather, the onus is on proving its biosimilarity to the reference product. Therefore, any additional clinical safety and effectiveness studies, including immunogenicity testing, should be designed to assess whether there are clinically meaningful differences between the proposed biosimilar and the reference product.

Here we provide an overview of the FDA's approach to assessing analytical similarity and describe considerations for the design of clinical studies to support applications for biosimilar products for RA. This approach was reflected in the FDA's approval of three such products in 2016: infliximab-dyyb (Inflectra™, Hospira, USA), a biosimilar to US-licensed Remicade® (Centocor); etanercept-szzs (Erelzi™, Sandoz, USA), a

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Table 1 | **Therapeutic proteins approved for rheumatologic diseases**

International nonproprietary name	Therapeutic area and year first approved*	Description
Monoclonal antibodies		
Rituximab	Oncology (1997), rheumatology (2008)	Anti-CD20 mAb
Infliximab	Gastroenterology (1998), rheumatology (1999), dermatology (2006)	Anti-TNF mAb
Adalimumab	Rheumatology (2002), gastroenterology (2007), dermatology (2008)	Anti-TNF mAb
Certolizumab pegol	Gastroenterology (2008), rheumatology (2009)	Anti-TNF mAb
Golimumab	Rheumatology (2009), gastroenterology (2013)	Anti-TNF mAb
Canakinumab	Rheumatology (2009)	Anti-IL-1β mAb
Tocilizumab	Rheumatology (2010)	Anti-IL-6 receptor mAb
Belimumab	Rheumatology (2011)	Anti-BAFF mAb
Ustekinumab	Dermatology (2009), rheumatology (2013)	Anti-IL-12/23 mAb
Secukinumab	Dermatology (2015), rheumatology (2016)	Anti-IL-17a mAb
Fc-fusion proteins		
Etanercept	Rheumatology (1998), dermatology (2004)	TNFR–Fc fusion protein
Abatacept	Rheumatology (2005)	CTLA4–Fc fusion protein
Rilonacept	Rheumatology (2008)	IL-1 receptor and IL-1 receptor accessory protein Fc fusion protein
Other therapeutic proteins		
Anakinra	Rheumatology (2001)	IL-1 receptor antagonist

BAFF, B-cell activating factor (also known as TNFSF13B or BLyS); CTLA4, cytotoxic T-lymphocyte protein 4; mAb, monoclonal antibody; TNFR, TNF receptor.
*General therapeutic areas are listed and do not reflect that many products might be approved for multiple indications within each therapeutic area.

biosimilar to US-licensed Enbrel® (Amgen); and adalimumab-atto (Amjevita™, Amgen, USA), a biosimilar to US-licensed Humira® (Abbvie)[4].

Analytical similarity

Categorization of similarity

The first step towards achieving biosimilarity is to show that the proposed biosimilar and reference product are highly similar. The final conclusion about similarity, which excludes minor differences in clinically inactive components, is made when the FDA reviews the biologics license application (BLA). A biosimilar sponsor may submit analytical data earlier in the development process, and the FDA review of these data may lead to designation of one of four categories within a development-phase continuum[5]: 'not similar', when characteristics differ between products that require modifications to the manufacturing process before further development through the 351(k) regulatory pathway can be recommended; 'similar', where products have differences that require further information from analytical or other studies to determine whether they are within an acceptable range (for instance, if there are differences in glycosylation, which is important for pharmacokinetics of certain protein products, but can be affected by manufacturing processes); 'highly similar', when the proposed biosimilar product meets the statutory standard for analytical similarity and results of comparative analytical characterization studies suggest that targeted and selective animal and/or clinical studies would resolve residual uncertainty; and 'highly similar with fingerprint-like analytical similarity', when the proposed biosimilar product meets the statutory standard for analytical similarity based on integrated, multiparameter approaches that are extremely sensitive in identifying analytical differences, and any residual differences are likely to be resolved by more-targeted animal and/or clinical studies.

The FDA assessment of analytical data at the time of the development-phase determination should inform the next steps in the demonstration of biosimilarity. Early in development, a proposed biosimilar might be determined to be 'similar' for various reasons. For example, the proposed product's biochemical, biophysical and biological attributes might seem to be highly similar to those of the reference product, but an insufficient number of lots of the proposed biosimilar and/or reference product were assessed. Characterization of additional lots would be needed to provide confidence that the proposed biosimilar will consistently demonstrate high similarity with the reference product. Second, some minor differences between products might need additional justification, such as presentation of supporting data in the scientific literature and/or further characterization. Even with additional justification, however, the FDA might still request further studies or information to determine whether the proposed biosimilar product could reach 'highly similar' status.

If the results of the comparison between the proposed biosimilar product and the reference product early in development endow confidence that they are either analytically 'highly similar', or 'highly similar with fingerprint-like similarity', nonclinical and clinical development programmes can proceed with targeted and selective studies to support the demonstration of biosimilarity overall. Analytical studies on additional lots of the proposed biosimilar product should continue throughout development to confirm high analytical similarity in lots produced under scaled-up manufacturing processes for clinical study and commercial lots.

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Analytical methods

Analytical similarity studies are expected to use orthogonal analytical methods to assess quality attributes. This approach includes the following methods: assessment of primary, secondary and higher-order structures of the proteins; extensive analysis of post-translational modifications, such as the glycan structure (if relevant), deamidation, oxidation or other modifications of amino residues that have the potential to affect the function, safety or stability of the molecule, or intentional post-translational modifications, such as the addition of polyethylene glycol; size and charge variants; and biological activity and other functional assays. If a molecule has multiple biological activities, where feasible each should be demonstrated to be highly similar to that in the reference product, especially in cases where the mechanism or mechanisms of action for a specific indication are not clear.

The design of the analytical assessment should be the first step in developing a biosimilar product (FIG. 1). Knowledge of the structure–function relationship of the reference product and the basic biology of the molecule and its intended target provide the starting points for selecting the relevant methods. A sponsor's analysis of reference product lots can be used to establish the quantitative analytical criteria for each attribute that the proposed biosimilar product should match. Early in development, before conducting nonclinical or clinical studies, clone selection and development of the manufacturing process should be iterative to give the proposed biosimilar product the highest chance of meeting the criteria for 'highly similar' when manufacture is scaled up.

Most of the therapeutic proteins approved for rheumatologic diseases are monoclonal antibodies. These have the advantage that knowledge of the general properties of one IgG1 antibody are applicable to other IgG1 monoclonal antibodies. In particular, how specific antibody glycan isoforms impact pharmacokinetic or biological functions is generally well understood. Therefore, the following discussion illustrates how to assess analytical similarity for a monoclonal antibody, although the general approach is applicable to other important product attributes.

The mechanism of action of a therapeutic monoclonal antibody might include antibody effector functions, such as complement-dependent and antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis and Fc-mediated induction of apoptosis. The antibody glycan, which is attached to an asparagine residue in the heavy-chain CH2 region (FIG. 2), is necessary to activate Fcγ receptors and the C1 component of complement[6]. Specific glycan structures (FIG. 2) are important for complement-dependent and antibody-dependent cell-mediated cytotoxicity[6,7].

The core heptasaccharide contains *N*-acetyl glucosamine and mannose residues. The addition of galactose, fucose and sialic acid is heterogeneous, but the presence or absence of these monosaccharides can alter the function or pharmacokinetics of a monoclonal antibody. For example, the glycan structure on each constant region can have no, one or two galactose residues, designated G0, G1 and G2, respectively, where increased levels of G0 glycans can reduce complement-dependent cytotoxic activity[8,9].

Fucose inhibits antibody-dependent cell-mediated cytotoxic effects specifically for natural killer (NK) cells[10,11] due to the interaction between the fucose on the monoclonal antibody glycan and the glycan moiety of Fcγ receptor IIIa (FcγRIIIa) leading to reduced binding affinity[12]. Fucose, however, might have a lesser effect on antibody-dependent cell-mediated cytotoxic activity by other effector cell types[13], because it has no impact on binding to other FcγR family members[14] and because NK cells only express FcγRIII and FcγRIIC, whereas cells of the myeloid and granulocyte lineages express most FcγR family members[15]. Nevertheless, some data suggest that afucosylated monoclonal antibodies increase effector functions of cells expressing multiple FcγR family members through improved binding to FcγRIIIa[16]. Thus, differences in the levels of galactose and fucose could be addressed by assays testing for complement-dependent and antibody-dependent cell-mediated cytotoxic effects. However, although afucosylation has the largest impact on antibody-dependent cell-mediated cytotoxic activity and galactosylation has the largest impact on complement-dependent cytotoxic activity, recent literature suggests that specific glycan structures containing galactose and sialic acid monosaccharides may also enhance complement-dependent or antibody-dependent cell-mediated cytotoxic activity[17–19].

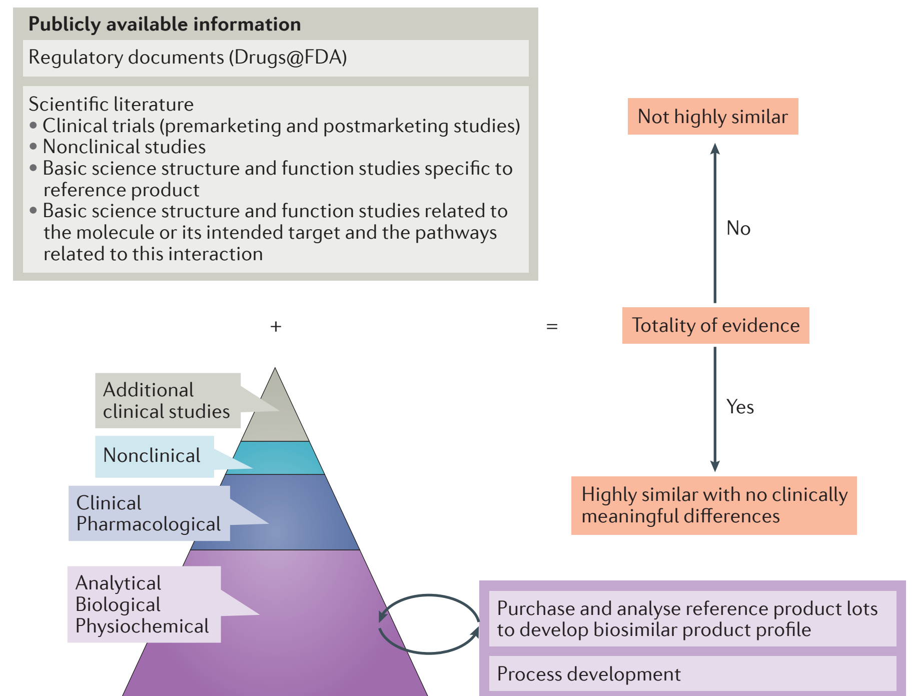

Figure 1 | **Overview of biosimilar product development.** Biosimilar product development should start with searches of publicly available information on the reference product, its target and the known biology. The protein structure, post-translational modifications and biological activity of the reference product should be analysed by the biosimilar sponsor to determine the product profile. Development of the expression system (expression construct and host cell line) and the manufacturing process should be extensive to provide a firm foundation for demonstration of biosimilarity. If FDA categorization of a proposed biosimilar product of 'highly similar' can be made on the basis of these data early in development, a selective and targeted approach to clinical testing might be possible.

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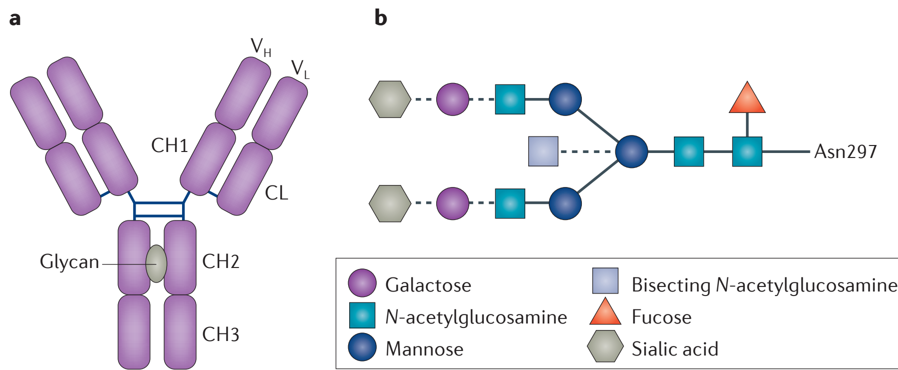

Figure 2 | **Features of antibody structure important for biosimilar cytotoxic effects. a** | The basic structure of an IgG antibody contains a light chain with a variable and constant region and a heavy chain with a variable region, a hinge and three constant region domains, each with different functions. The glycan is attached to Asn297 in the CH2 domain. **b** | The antibody glycan contains a core heptasaccharide with four *N*-acetylglucosamine and three mannose residues. Human antibodies may contain a bisecting *N*-acetylglucosamine, but this structure is not as common in other species. Fucose and galactose residues are added heterogeneously. Sialic acid is only added on structures with galactose.

Incompletely processed high mannose forms are cleared faster than monoclonal antibodies containing the core heptasaccharide and other terminal monosaccharides[20–22]. Differences in levels of high mannose forms can be assessed in pharmacokinetic studies. The pharmacokinetic properties of Fc-fusion proteins, where the non-Fc portion is glycosylated, or of other therapeutic proteins could be altered by different types of glycan structures[23,24].

Culture conditions and the host-cell substrate can influence which monoclonal antibody glycan variants are present in the final product, and should be taken into account when developing biosimilar glycoproteins. Cell lines from different species synthesize the core heptasaccharide with typical heterogeneous addition of galactose, fucose and sialic acid, but the amounts of these residues might differ between the mouse and hamster cell lines most commonly used to produce therapeutic monoclonal antibodies[25,26]. Plant cell substrates can also synthesize the basic monoclonal antibody glycan structure, but some terminal saccharides might be added using different linkages, and xylose, which is not used by mammalian cell substrates, will be incorporated[27]. A proposed biosimilar product with novel glycan variants is unlikely to be deemed 'highly similar'.

Levels of major antibody glycan isoforms can be quantified and assessed in functional assays, but the correlation of any differences between these isoforms *in vitro* and *in vivo* is not understood. If the functional bioassays and FcγR-binding studies show different results for the proposed and the reference product, there would be residual uncertainty about the behaviour of the proposed biosimilar *in vivo*. In addition, for any given indication, the specific effector function, effector cell type and engagement of different FcγRs might not be well understood. Therefore, these features in particular should show high biosimilarity to the reference product.

A biosimilar development programme that puts substantial early effort into the design of the expression construct, development of the clonal cell line, cell culture conditions and purification process, as well as analysing multiple lots of the proposed biosimilar and reference products, increases the chance of creating a product that will receive a preliminary categorization of 'highly similar'.

Clinical studies

Clinical data are intended to resolve residual uncertainty from limitations in the data or observed structural and functional differences from the reference product that might lead to clinical consequences. Uncertainties might arise due to the nature and complexity of the therapeutic protein, which, with the array of current advanced analytical methods available, are unlikely to be shown to be structurally identical. The assessments might also yield analytical outcomes in a range of similarity determinations and, therefore, have varying degrees of residual uncertainty.

Clinical studies should be tailored to address these specific uncertainties, rather than being aimed at independently establishing the safety and efficacy of the proposed biosimilar product, which represents a new paradigm in drug development. To achieve the goal of demonstrating that no clinically meaningful differences exist between the proposed biosimilar and reference product, human pharmacokinetic and, if appropriate, pharmacodynamic data, as well as clinical immunogenicity assessments, would generally be necessary. The need for additional clinical safety and effectiveness data would depend on other factors causing residual uncertainty, such as the degree of understanding of mechanisms of action and disease pathology, the extent to which pharmacokinetic and pharmacodynamic outcomes predict relevant clinical outcomes, the extent of clinical experience with the reference product and its therapeutic class and the nature of its risk–benefit profile, and the extent of clinical experience with the proposed biosimilar product.

Clinical pharmacology studies

As detailed in FDA draft guidance[5], clinical pharmacology studies comprise a critical part of the stepwise approach to demonstrating biosimilarity. Such studies, when well-designed, should enable evaluation of the similarity in pharmacokinetic (exposure) and pharmacodynamic profiles between the proposed biosimilar and the reference product. The data should inform decisions about whether additional clinical studies are needed to address any residual uncertainty and how they should be designed.

In certain cases, human pharmacodynamic data might add to the totality of the evidence to support a biosimilarity determination. For instance, in the development programme for filgrastim-sndz, the first biosimilar product approved by the FDA under the 351(k) pathway, clinical pharmacology studies in healthy volunteers were undertaken to evaluate pharmacokinetic similarity compared with filgrastim. Comparisons of absolute neutrophil counts and $CD34^+$ cell counts were also done, as these cell counts are relevant and sensitive pharmacodynamic markers for these drugs[28,29].

Unfortunately, no pharmacodynamic markers have yet been identified for RA that reliably predict clinical outcomes or provide a meaningful measure of clinically relevant pharmacological activities. Comparative pharmacodynamic information, therefore,

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would be unlikely to address residual uncertainty for proposed biosimilar products for this condition. Nevertheless, the use of pharmacodynamic markers is not precluded if they are relevant in other indications, although it should be borne in mind that the mechanism or mechanisms of action might not be the same for all indications intended for the proposed biosimilar and for which the reference product is licensed. The data, therefore, might or might not be deemed relevant.

Comparative clinical studies

Additional clinical studies, such as comparative clinical studies, should be tailored to evaluate the potential for clinically meaningful differences between the products. Comparative clinical studies in biosimilar development programmes should be designed to address residual uncertainty about biosimilarity between the proposed biosimilar product and the reference product on the basis of structural and functional characterization, animal testing, human pharmacokinetic and pharmacodynamic and clinical immunogenicity findings. However, keeping in mind that clinical studies often comprise the most expensive and lengthy portion of a development programme, many sponsors have sought guidance on clinical studies before full knowledge is obtained about the residual uncertainties to be addressed. As a result, the FDA encourages clinical study designs that are likely to optimize sensitivity to detect differences.

The key challenge is how best to investigate potential clinically meaningful differences between the proposed biosimilar product and the reference product. An equivalence design for a comparative clinical study might be justifiable and informative, but selecting doses, end points, and time points of assessment for such studies is typically based on publicly available data derived from studies designed to assess efficacy and safety. For example, historically, biologic drugs for RA were approved on the basis of data from randomized, placebo-controlled trials that were 6–12 months long and used ACR20 response criteria, a composite index, as a primary end point[30]. Although ACR20 is useful to distinguish the effects seen with an active treatment from those seen with placebo, it may not be sensitive enough to distinguish between active treatments, even those with different mechanisms of action[31]. Furthermore, the historical studies typically employ doses in or near the therapeutic plateau and assess the primary end point at only one time point. This approach potentially limits the ability to detect any temporal clinically meaningful differences between products. If, however, an equivalence study design is judged to be appropriate, the primary end point and equivalence margin should be scientifically justified and based on all relevant publicly available information. For example, assessment with ACR20 might provide a more robust and consistent treatment effect for one product, whereas the 28-joint disease activity score, another composite endpoint, might be more sensitive to detect any differences for another. Sensitivity analyses should be performed to account for missing data, end points should be assessed at multiple time points, and secondary end points (such as the components of a composite primary end point) should be evaluated, as supportive data might bolster this type of study design and add to the totality of the evidence to support a demonstration of biosimilarity.

Safety and immunogenicity

Clinical safety data, including immunogenicity assessments, are typically expected because of the likelihood of small differences between proteins and the concern that they could result in increased immunogenicity and hypersensitivity. The proposed biosimilar product may also differ from the reference product in formulation, impurities, excipients and clinically inactive components, which could result in clinically meaningful immunogenic differences, substantial enough to preclude licensure as a biosimilar. When contemplating what data are necessary, consideration should be given to the populations who are likely to use the product, the likelihood of immune responses in those populations and the risks associated with immune response.

The risk of an immunogenic response might be heightened in patients who have had previous exposure to the reference product. In such a scenario, safety data from patients who undergo a single transition from the reference product to the proposed biosimilar product should be collected and descriptively compared with those for patients who continue taking the reference product. In development programmes for proposed biosimilar products for rheumatological indications, this type of study would be expected. As a scientific matter, it is expected that immunogenicity and effects on exposure (pharmacokinetics), safety and efficacy will be similar between the proposed biosimilar and reference products.

Extrapolation

Licensing of a biosimilar product for multiple conditions for which the reference product is licensed might be possible on the basis of the data in only one condition of use when supported by a scientific justification. In the demonstration of biosimilarity in conditions of use not directly studied in the development programme, the applicant may include a scientific justification to support extrapolation of data to support biosimilarity in one condition of use to other conditions of use where any differences between the proposed biosimilar and the reference product would need to be assessed and scientifically justified from the standpoint of the other conditions of use. As discussed in more detail in FDA guidance[32], the scientific justification should address the following factors for each condition of use and population of patients: the mechanism or mechanisms of action; the pharmacokinetics and biodistribution; immunogenicity; different expected toxicities; and any other factors that might affect safety and efficacy. As a scientific matter, the FDA has determined that differences between conditions of use with respect to these factors do not necessarily preclude extrapolation of data to support the licensure of the biosimilar to those additional indications. In some cases, however, additional clinical studies might be required for a specific condition of use to address pertinent residual uncertainties.

Interchangeability

In addition to biosimilarity, the BPCI Act establishes a standard for interchangeability, whereby applicants must submit information to show that the biological product is biosimilar to the reference product, and can be expected to produce the same clinical result as the reference product, in any given patient, and, for a product that is administered more than once to an individual, the risk in terms of safety or diminished efficacy of alternating or switching between use of the product and the reference product is not greater than the risk of using the reference product without such alternation or switch. Interchangeable products may be substituted for the reference product without the intervention of the prescribing health-care provider and, therefore, the standard for interchangeability has expectations in addition to biosimilarity. The FDA is currently evaluating what additional data would be needed to meet the interchangeability standard.

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Conclusions

The process of demonstrating biosimilarity is likely to pose novel scientific challenges due to the larger and typically more complex structure of biological products. In contrast to a development programme for novel biological products, where the goal is to demonstrate efficacy and safety, programmes for proposed biosimilar products should aim to demonstrate biosimilarity between the proposed biosimilar product and the reference product. The foundation for the demonstration of biosimilarity is extensive structural and functional characterization, and clinical data to address residual uncertainty and to ensure that there are no clinically meaningful differences between the proposed biosimilar product and its reference product in terms of safety, purity, and potency. A stepwise approach facilitates an abbreviated licensure pathway by tailoring the development programme to the scientific questions at hand.

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Author contributions

Both authors researched data for article and made substantial contributions to discussions of the content, writing and review/editing of manuscript before submission.

Competing interests statement

The authors declare no competing interests.

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