Comparative immunogenicity assessment of biosimilars

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The first anticancer biosimilars have entered clinical use, with many others under clinical development. Like all biologics, biosimilars may elicit unwanted immune responses that can significantly impact clinical efficacy and safety. Head-to-head immunogenicity assessment of biosimilars and their reference biologics should, therefore, be a critical component of a biosimilar's clinical development program. Various bioanalytical platforms may be used to detect and characterize immune responses, each having relative strengths and weaknesses. To fully recognize the clinical relevance of such data, regulators must be able to interpret immunogenicity results in an assay-specific context as well as in perspective of clinical pharmacology, efficacy and safety. Herein, we discuss current challenges imposed by global regulatory requirements for immunogenicity assessment of biosimilars.

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Over the past 20 years, biologic medicinal products (biologics) have transformed treatment landscapes worldwide for patients with hematological or solid malignancies. Today, as data exclusivity periods of first wave biologics approach expiration/have expired, several biosimilar products (i.e., biologics that are considered to be similar in terms of quality, safety and efficacy to an approved 'reference' biologic) are being developed or have already been approved for human use [1].

Like all biologics, biosimilars are structurally complex proteins that are typically manufactured using genetically engineered animal, bacterial or plant cell culture systems. As a consequence of this molecular complexity and the proprietary nature of the manufacturing process, which will inevitably result in the use of different host cell lines and expression systems as well as related differences in manufacturing conditions, it is not possible to manufacture exact copies of a reference biologic [2]. Due to the potential product differences, it follows directly that regulatory approval of biosimilars (in the majority of global jurisdictions) is granted on the basis of rigorous quality, nonclinical and clinical head-to-head assessments that demonstrate 'biosimilarity' to the reference biologic [3–5]. As part of this approval pathway, the clinical immunogenicity profile of the biosimilar must be investigated in a comparative manner with the reference biologic.

When administered to patients, all therapeutic proteins have the potential to induce an unwanted immune response (i.e., to stimulate the formation of antidrug antibodies [ADAs]). The impact of immune responses can range from no apparent effect to changes in pharmacokinetics (PK), loss of effect and serious adverse events [6–8]. Furthermore, the immunogenicity profile of a biologic can be significantly altered by even small differences in its manufacturing process that are accompanied by a change in product attributes, as well as differences in dosing schedules, administration routes or patient populations [3,6,7,9–11]. For instance, during clinical development of Omnitrope[®] (Sandoz Inc., NJ, USA; a somatotropin biosimilar), high levels of host cell protein in a batch used for Phase III studies were associated with up to 60% of patients developing antigrowth hormone antibodies (vs 2% of patients treated with the reference biologic) with no apparent impact on efficacy [12]. At the other end of the scale, several cases of neutralizing anti-erythropoietin antibody development and subsequent adverse events (pure red cell aplasia) have been reported following subcutaneous administration of erythropoietin biologics from different

manufacturers in patients with chronic renal failure; this effect has been attributed to the route of administration as well as product-related factors, such as changes in formulation and in container closure systems [2,7,13,14].

Assessment of immunogenicity is, therefore, of critical importance when establishing the safety profile of all biologics, and head-to-head assessment of immunogenicity with the reference product, ideally demonstrating similarity, is considered a key component of a biosimilar's clinical development program. In this review, we discuss current regulatory guidance and critically evaluate bioanalytical methods for judgement of comparable immunogenicity between biosimilars and their reference products.

Current regulatory guidance for comparative immunogenicity assessments

Guidelines for the development of biosimilars have been issued by the EMA, WHO and US FDA [4,5,15–21]. Over the past decade, many more countries have also implemented national guidance documents based on the same principles as outlined in EMA, WHO and FDA guidances (e.g., Canada, Japan, Brazil, South Africa, the Republic of Korea and many others) [22].

According to these guidelines, the development of biosimilars involves a stepwise demonstration of similarity to a reference biologic, with regulatory approval based on the 'totality of evidence' provided by head-to-head comparisons of analytical, nonclinical and clinical data [1,22,23]. At each step of the biosimilar's development, the sponsor can identify any differences between the biosimilar and reference biologic that contribute to 'residual uncertainties' about product similarity and tailor the scope and extent of subsequent studies accordingly. Thus, provided analytical data demonstrate robust physicochemical and biological similarity, a biosimilar candidate may be granted regulatory approval based on abbreviated nonclinical and clinical data packages [22].

As part of this development pathway, head-to-head clinical immunogenicity testing in an adequately sensitive study population (i.e., the patient population in which the study biologics are most likely to elicit an immune response) is recommended by the EMA, WHO and FDA as a key criterion for the regulatory evaluation of biosimilarity [3,5,24–26]. To assess clinical immunogenicity, a fully-validated, tiered approach for detection of ADAs is recommended. The four main assessment stages involve: ADA screening, ADA confirmation assays, ADA characterization and titration, and assessment of neutralizing capacity. Each of these stages may be fulfilled using a variety of bioanalytical platforms, and results of these assays should be considered as part of an integrated pharmacology, safety and efficacy analysis, critical for elucidating the clinical relevance of immunogenicity data [26–28].

While the need for comparative immunogenicity assessments is accepted among regulators, of note, regulatory opinions on what constitutes a clinically meaningful difference in immunogenicity can diverge. This matter is illustrated by the case example of Flixabi[®] (Samsung Bioepis UK Ltd, Middlesex, UK), an infliximab biosimilar which was issued a positive opinion by the EMA Committee for Medicinal Products for Human Use for all licensed indications of the reference biologic, Remicade[®] (Janssen Biotech, Inc., PA, USA), in April 2016 [29,30]. EU approval was granted although a substantial number of committee members considered the benefit/risk ratio negative on account of higher ADA rates observed in Flixabi versus Remicade treated subjects in Phase I and Phase III clinical equivalence studies (Phase I: 9.5% difference by study day 71; Phase III: 5–12% difference at individual timepoints). The committee members noted that the higher ADA rates offered a potential explanation for the lower (but within prespecified equivalence margins) efficacy of Flixabi versus Remicade. Additionally, the sensitivity of the Phase III population (patients with rheumatoid arthritis [RA] in receipt of immunomodulatory therapy) was questioned. Notably, limitations of the immunoassay could not be ruled out as the cause of variation in observed ADA rates, highlighting the need for further standardization of both bioanalytical approaches and regulatory definitions of similar immunogenicity.

Variability in immunogenicity assays

Immunogenicity data are highly dependent on the assay used to measure ADAs (including reagents, standards, validation criteria, etc.). Furthermore, immunoassays for screening and confirming ADAs can be conducted using a variety of formats and detection systems, each of which has relative strengths and weaknesses (Table 1; Figure 1) [11,27,28]. While there is no single assay format suitable for assessing the immunogenicity of all biologics, regardless of the method selected, all assays must be thoroughly validated according to parameters that include cut-point, sensitivity, specificity, selectivity, precision, dilution linearity and limit of drug tolerance (see Table 2 for definitions) [26,28,31–34]. However, because human ADAs are of limited availability, international reference sera/standards for immunoassay validation and calibration are generally unattainable, with the exception of the

Table 1. Strengths and weaknesses of immunoassays.

Assay	Description	Strengths	Weaknesses
ADA screening and confirmatory assays[†]			
ELISA	ADAs captured by immobilized biologic and detected using a colorimetric labeled antibody	<ul style="list-style-type: none"> – Convenient (ease of use) – High-throughput – Inexpensive 	<ul style="list-style-type: none"> – High chance of false-positive results – Loss of low-affinity ADAs during washes – High background – Not suitable for detection of ADAs against mAbs
Bridging ELISA	ADAs captured by immobilized biologic and detected with colorimetric labeled biologic	<ul style="list-style-type: none"> – More specific and selective than ELISA – Can be used where the biologic is a mAb – High-throughput 	<ul style="list-style-type: none"> – Loss of low-affinity ADAs during washes
ECL	ELISA or Bridging ELISA technique with a ruthenium-conjugated antibody or biologic for detection, respectively	<ul style="list-style-type: none"> – Increased sensitivity vs. colorimetric conjugates – Bridging format can be used where the biologic is a mAb – High-throughput 	<ul style="list-style-type: none"> – Reagents can be expensive – Loss of low-affinity ADAs during washes
RIA	ADAs captured on protein A beads and detected with a radiolabeled antibody or biologic	<ul style="list-style-type: none"> – More sensitive than ELISA – Moderate throughput – Inexpensive 	<ul style="list-style-type: none"> – Radioactivity may deter investigators – Reagents require specific equipment and safety measures – Loss of low-affinity ADAs during washes
SPR	Sensor with immobilized biologic detects changes in mass following ADA binding	<ul style="list-style-type: none"> – Can detect low-affinity and rapidly dissociating ADAs – Can be used to determine ADA isotype 	<ul style="list-style-type: none"> – May be less sensitive than other assays – Not for high-throughput – Expensive equipment and reagents
NAb detection			
Cell-based assay	Measures neutralization of cell responses using relevant cell lines	<ul style="list-style-type: none"> – Provides a functional biological readout – May be the preferred NAb assay of regulatory authorities 	<ul style="list-style-type: none"> – High variability – Matrix interference – Low sensitivity – Low free drug tolerance – Longer assay time than ligand binding assays
Ligand binding assay	Measures neutralization of drug to target (e.g., cellular receptor) binding	<ul style="list-style-type: none"> – Easier to implement and more sensitive and drug-tolerant than cell-based assays – Different detection systems (e.g., ELISA and ECL) can be used 	<ul style="list-style-type: none"> – Does not provide the functional readout of cell-based assays

[†]ADA confirmatory assays employ the same design as screening assays but involve the addition of competing, unlabeled reference or biosimilar drug to measure response inhibition.
ADA: Antidrug antibody; ECL: Electrochemiluminescence; ELISA: Enzyme-linked immunosorbent assay; mAb: Monoclonal antibody; Nab: Neutralizing antibody; RIA: Radioimmunoassay; SPR: Surface plasmon resonance.

Table 2. Glossary of immunoassay validation parameters.

Validation parameter	Definition
Cut-point	The threshold at which a sample is categorized as ADA-positive/negative. Cut-point should be determined, based on negative control signals, before commencing clinical trials
Sensitivity	The lowest ADA concentration that consistently produces a positive signal or a signal equal to the cut-point. Sensitivity is dependent on the binding affinity of the positive control
Selectivity	The ability of the assay to detect biologic-specific ADAs in a matrix, like plasma, that may contain interfering substances. Selectivity can be assessed by measuring responses in drug-naïve negative control samples Of note, target molecules of the biologic (e.g., target antigens of mAbs) can interfere with immunoassays and reduce selectivity
Precision	The ability of the assay to produce consistent results between runs (i.e., the measure of intra- and inter-assay variability)
Dilution linearity	The ability of the assay to maintain a linear, positive relationship between the ADA concentration and the signal. Samples with ADA concentrations above the linear range of detection should be analyzed using the appropriate dilutions
Specificity	The ability of the assay method to detect ADAs that bind to the biologic but not to other assay components (e.g., plates) or reagents. Specificity can be assessed by demonstrating that binding is blocked by excess unlabeled biologic
Drug tolerance limit	The concentration of biologic that blocks ADA detection in positive control samples

ADA: Antidrug antibody; mAb: Monoclonal antibody.

WHO erythropoietin antibody reference panel (the only international ADA standard currently available, consisting of nine human monoclonal antibodies against erythropoietin with various characteristics) [35]. Furthermore, because human ADAs are complex analytes (i.e., they are usually polyclonal, bind to different domains of the biologic and have variable isotypes and binding affinities) reference sera/standards may not accurately represent ADA heterogeneity within a study population [11,28,31]. As such, there is currently no consensus among experts regarding

Figure 1. Antidrug antibody detection assays. Refer to Table 1 for specific assay descriptions.

ADA: Antidrug antibody; ECL: Electrochemiluminescence; ELISA: Enzyme-linked immunosorbent assay; RIA: Radioimmunoassay; SPR: Surface plasmon resonance.

the implementation of reference standards, and several key assay parameters (e.g., sensitivity and limit of drug tolerance) are relative values determined by the choice of surrogate positive control samples, often generated via hyperimmunization of animals or phage display [11,31,36]. As a result, the vast majority of immunoassays used for detecting ADAs currently cannot be universally validated nor calibrated, limiting the value of comparisons between different studies as well as the ability to provide meaningful, quantitative data [11,28,31,36,37].

Additionally, in recent years, more sensitive and specific assays have been developed. For example, as ADAs bound to excess biologic in patient serum samples usually cannot be detected by traditional ADA assays, novel ‘drug-tolerant’ assays (e.g., acid-dissociation radioimmunoassay) have been established. In such assays, complexes of ADA and the biologic drug are dissociated prior to ADA detection, minimizing the possibility for false-negative results [28]. As such, modern immunoassays often provide higher readouts compared with traditional assay formats, and historic data cannot be used for comparative purposes or for establishing margins of clinically acceptable difference in ADA rates between biosimilars and reference products. The increasing sensitivity of modern immunoassays is highlighted by the case example of adalimumab (a TNF- α inhibitor approved for treatment of various inflammatory disease), where ADA rates in patients with RA have been reported in the range of 1–66% depending on the choice of assay [28]. Similarly, the US product labeling of etanercept (also a TNF- α inhibitor) sets ADA rates at up to 6% in patients with RA, yet recent data from Emery *et al.* reported a rate of 13.1%, which has been attributed to the high sensitivity and drug tolerance of the chosen electrochemiluminescence (ECL) assay [38–42]. Given the limitations of data comparisons across studies, the immunogenicity of a biosimilar must instead be investigated using head-to-head comparative assays with the reference biologic.

In addition to the choice of screening and confirmatory assays, multiple options for detection of neutralizing antibodies (NAb) in ADA positive samples also exist [43,44]. Depending on the biologic’s mechanism of action (MoA), the selected assay may be cell-based or non-cell-based (Table 1). While the non-cell-based (competitive ligand binding) approach offers greater precision and sensitivity and an easier set up in terms of establishing validation parameters, these assays are considered appropriate only when the biologic’s MoA involves blocking of a soluble

target or cellular receptor [3,11,26,44]. Additionally, cell-based approaches may be favored by certain regulatory authorities as they may offer better insight into the functional and biological activity of the product [11,26,44]. However, cell-based experiments should not be considered the default option if sufficient sensitivity, precision and robustness cannot be achieved; in these situations, further dialogue with regulators may be necessary to define an appropriate non-cell-based method [3,26].

Similar to ADA assays, NAb assays are validated according to assay-specific control signals [44]. Additionally, during the development of the reference product, the NAb assay is likely to have been developed according to key MoAs that contribute most therapeutic efficacy as well as contemporaneous technology limitations; yet, with advancements in technology and knowledge of biological processes, it is accepted that NAb assays for biosimilars can be based on a distinct MoA, which should be a major efficacy contributor but also the most sensitive MoA for detecting differences between products [44]. As such, historic NAb data are also of limited value when assessing a biosimilar's immunogenicity profile.

Currently, no specific regulatory guidelines are available for the conduct of NAb confirmatory or titration assays as it is widely accepted that the NAb assay is a qualitative (positive or negative) experiment used for further characterization of ADA assays, which already include a confirmatory step. However, if unexpected or nonspecific responses are observed during qualitative experiments, further practical considerations for NAb confirmation (e.g., use of an agonist molecule to assess signal inhibition) or titration experiments will be required [44].

Influence of dosing, sampling & patient populations

Aside from the assay that is used to detect ADAs, there are additional factors to consider which can influence immunogenicity data, such as the dosing schedule, the timing of clinical sample collection for the analyses and patient-related factors. As highlighted by the case of infliximab treatment in patients with Crohn's disease, incidence of ADAs may increase when using an episodic versus regular dosing schedule (30 vs 8% of patients, respectively) [9]. Additionally, sampling should account for temporal ADA formation patterns. For instance, ADA formation may occur rapidly, gradually or transiently, and sampling schedules should be designed to capture this information accordingly. As such, it is generally recommended that samples are taken more frequently during early use of the product (i.e., when an immune response is more likely to occur), and post-treatment sampling should be long enough to determine the persistence of immune responses [17,25]. As recommended in EMA guidance, sampling should also take into account product half-life and drug tolerance of the ADA assay [3]. Differences in ADA rates can also occur among patient populations, e.g., in patients with Crohn's disease who received infliximab with versus without concomitant immunomodulators (46 vs 73%, respectively) [3,9]. Head-to-head comparisons of ADA rates between biosimilars and their reference products must therefore be performed in adequately sensitive patient populations, using an appropriate dosing and sampling schedule. It is particularly important for regulators and sponsors to consider these aspects of study design when evaluating the potential to extrapolate immunogenicity data to other indications, under which patient immune status and dosing schedules may vary. Furthermore, as a biologic may have a unique MoA in different patient populations, additional data may be required to demonstrate that the formation of neutralizing antibodies is similar between patients treated with the reference biologic versus the biosimilar in all intended therapeutic indications [4,17]. As specified in current regulatory guidance, extrapolation is not self-evident and always requires scientific justification [4,5,17].

Limitations of comparative immunogenicity assessments

Immunogenicity assessment strategies for biosimilars are established on a case-by-case basis. The aim of the assessment is to characterize potential differences between the biosimilar candidate and the reference product in terms of the incidence and severity of human immune responses. To achieve this aim, bioanalytical assays used to determine similar immunogenicity must have capacity to detect both biosimilar and reference product ADAs (or NAb if assessing neutralization) in a comparable manner. However, a general approach for comparative assessment of immunogenicity is not well defined, nor is there full consensus among experts [28,37]. As such, the use of a two-assay or one-assay approach for comparing ADA rates remains a matter of debate.

The two-assay approach uses two parallel assays, one with reagents that detect ADAs against the reference product and one with reagents for detection of ADAs against the biosimilar (Figure 2A) [28,45]. While two separately optimized assays may offer better representation of the individual immunogenicity profiles of the biosimilar and reference product, this approach is challenging from an operational perspective as samples either must be run twice (once with each assay) or, alternatively, unblinded [37,44]. Additionally, the use of separate assays/reagents can be expected

Figure 2. Head-to-head comparative immunoassays. (A) Two-assay ECL approach. (B) One-assay ECL approach. ADA: Antidrug antibody; ECL: Electrochemiluminescence.

to decrease reliability of data comparisons due to inter-assay variability. Comparable inter-assay performance and sensitivity must therefore be demonstrated to employ a two-assay approach.

In contrast, the one-assay approach uses a single assay to minimize bioanalytical bias. ADA binding is first measured in a screening assay that uses biosimilar reagents only; a confirmatory step is then implemented to assess inhibition of binding using both unlabeled reference and biosimilar product as competing antigens (Figure 2B) [28,45]. With this approach, detection of biosimilar ADAs is optimized and inter-assay variability minimized, but less information concerning immunogenicity of the reference product is obtained. However, as the generation of new information concerning the reference product is not the purpose of comparative immunogenicity assessment, a validated one-assay approach, rather than a two-assay approach, is generally recommended by regulators and experts in the bioanalytical industry [28,37,44].

Nonetheless, if a one-assay approach is chosen, additional experiments to confirm the suitability of the assay to detect both reference product and biosimilar ADAs are required [37]. While several methods for substantiating the one-assay approach have been proposed (e.g., using increasing concentrations of biosimilar and reference biologic to generate and compare positive control signal inhibition curves), no standardized technique or regulatory guidance is currently in place to direct such experiments [37,45–48]. Furthermore, there is little unanimity on whether one or two positive controls should be used to validate the assay (i.e., antibiosimilar only or both antibiosimilar and antireference product controls) nor on the stage of assay development at which these experiments should be implemented [37].

Notably, the use of one or two positive controls is a central issue for the validation of both the one-assay and two-assay approach. While the use of both positive controls may be considered the more conservative option for a one-assay set up, it should be noted that no two positive controls can have the same binding affinity, even when generated in the same species according to the same immunization schedule [31,37]. As such, use of multiple controls

can introduce analytical complexities, such as two different sensitivities and, subsequently, two sets of acceptance criteria [37]. As discussed in a publication by Islam *et al.*, a single antibiosimilar positive control may be used for a two-assay set up, allowing for a more uniform establishment of validation parameters [36]. However, if structural differences (e.g., significant differences in glycosylation) between the biosimilar and reference product are identified or expected, use of both antibiosimilar and antireference positive controls may be necessary to better represent potential immune responses against each biologic [36]. Nonetheless, the need to evenly represent ADAs against both the originator and biosimilar using two positive controls can be questioned, as no surrogate positive control can be expected to mimic the full spectrum of immune responses observed in patients [37]. As such, many experts would agree that one positive control is sufficient as controls should only be perceived as a means to demonstrate system suitability [37].

Due to the wide range of factors that can prevent detection of ADAs (ranging from assay design to sampling schedules), a lack of difference in detected ADAs cannot automatically exclude the potential existence of clinically meaningful differences in immunogenicity. In line with regulatory recommendations, ADA rates must therefore be considered in integrated analyses of clinical pharmacology, safety and efficacy. Additionally, subgroup efficacy and safety analyses (in patients with and without immune responses) may be recommended by regulatory authorities [17]. However, given the reduced clinical data requirements for approval of biosimilars, the ability to place ADA rates into a clinically meaningful context and to perform subgroup analyses on pre-approval data may be limited. Collection of postmarketing data is, therefore, recommended by regulators.

Postmarketing immunogenicity assessment

Results of short-term immunogenicity analyses may not reflect real-world experience with biologics, including biosimilars. In particular, rare ADA-related adverse events may not be detected in the premarketing phase due to the limited size of the population exposed and the greater scrutiny of patient care in the clinical trial setting. As such, EMA, WHO and FDA guidelines recommend consideration of immunogenicity in pharmacovigilance and risk management plans of biologic medicinal products [4,5,17].

Continuous product traceability by ensuring unique product identification is critical for the successful management of postmarketing surveillance activities. To accurately report and attribute adverse drug reactions (ADRs) to the correct biologic, physicians must know which particular product was given to a patient and must be able to differentiate safety signals between products or among different batches of the same product. To ensure correct attribution of ADRs in the postmarketing setting, regulators have responded to concerns regarding use of the same international nonproprietary name (INN) for biosimilars and their reference biologics by issuing mandates on nomenclature conventions [49,50]. To clearly distinguish between products, the FDA require a unique four-letter suffix in the INN of all biologics, including biosimilars, and the Japanese Pharmaceuticals and Medical Devices Agency specify that the nonproprietary names of biosimilars should contain the INN of the reference product, plus 'biosimilar' and a number indicating the order in which the biosimilar was approved in Japan [51]. The EMA on the other hand indicate that biosimilars should use the same INN as the originator but state that the capture of brand name and batch number during ADR reporting should be ensured to be able to distinguish between products.

However, for these measures to be effective, appropriate systems must be in place to ensure that physicians, pharmacists and dispensaries are aware of reporting obligations. As suggested in a recent survey of Dutch hospital physicians and pharmacists, reporting of biologic brand names may not be consistent, and only 76% of European ADR reports for biologics contained brand names [52].

Notably, the stringent definitions and regulatory requirements related to the approval and pharmacovigilance of biologics, including biosimilars, as stipulated by relevant bodies such as the EMA, the FDA and the WHO are not equally applied globally. Therefore, ADRs reported for 'follow-on' biologics that did not undergo the same stringent regulatory evaluation (also called 'noncomparable biologics') may not be representative of biologics approved in highly regulated markets [23,53]. A clear distinction of safety profiles between products approved in these jurisdictions is essential. However, in resource-limited settings, it may be challenging to increase the capacity of regulatory authorities to achieve a rigorous oversight of biologics throughout their life-cycle [54].

Another key consideration for the real-world setting is the possibility for unwanted immune responses to occur when clinically stable patients who are treated with a reference biologic are switched to treatment with a biosimilar (or vice versa). As clinical switching studies are not a prerequisite for regulatory approval of biologics, the type and amount of switching-related immunogenicity data submitted in the premarketing phase will be limited or nonexistent [55]. Thus, physicians may be confronted with residual uncertainty when assessing the potential impact

of switching in their patient populations [55]. Furthermore, the availability of switching data may vary according to indication, with switching data for therapeutic monoclonal antibodies in the oncology setting being generally scarce [56]. Accordingly, further clinical data from well-designed switching studies, which take into account the above-mentioned criteria in study design and test systems to allow for reliable immunogenicity assessment, are required to support physicians' decision making in this important context [56].

Conclusion

To conclude, ADA assay results need to be understood in the context of the assay that was used and its validation. Regulatory agencies must have in-depth understanding of the merits and limitations of individual immunogenicity assessments to appropriately interpret immunogenicity data generated in the prelicensure studies, and physicians should be aware of the relevance of these data for their specific patient populations. Further guidance from regulatory authorities on the details of comparative assay validation should facilitate the standardization of bioanalytical approaches and help to harmonize global definitions of similar immunogenicity.

Executive summary

- When administered to patients, all biologics, including biosimilars, may induce unwanted immune responses (e.g., the formation of antidrug antibodies [ADAs]), the impact of which can range from no apparent effect to severe adverse events.

Current regulatory guidance for comparative immunogenicity assessments

- Head-to-head clinical immunogenicity testing of biosimilars versus the reference biologic is recommended by regulators as a key criterion for demonstrating 'biosimilarity' in the premarketing setting.
- A tiered approach involving ADA screening assays, confirmatory assays, characterization and assessment of neutralizing capacity is recommended.

Variability in immunogenicity assays

- Immunoassays for detecting and characterizing ADAs can be conducted using a variety of formats, all of which have strengths and weaknesses and must be validated according to parameters such as sensitivity and limit of drug tolerance.
- The current lack of suitable reference standards means that these validation parameters cannot be universally calibrated. Considering that modern immunoassays have shown improved sensitivity and drug tolerance, immunogenicity comparisons across studies are of limited value.
- Immunogenicity of biosimilars must, therefore, be assessed in a head-to-head manner as per regulatory requirements.

Influence of dosing, sampling & patient populations

- In addition to the choice of assay, dosing schedules, timing of clinical sample collection and patient immune status can influence the detectability of ADAs.
- Regulators must evaluate these aspects of study design carefully when considering extrapolation of immunogenicity data to other patient populations with different dosing schedules.

Limitations of comparative immunogenicity assessments

- A general approach for head-to-head assessment of immunogenicity is not well defined, and debates concerning use of a one-assay or two-assay set-up are ongoing.
- While the one-assay set-up is emerging as the preferred option among regulators, there is currently no standardized method to demonstrate adequate sensitivity for ADAs against both biosimilar and reference products, nor is there consensus on the type(s) of positive control to be used.

Postmarketing immunogenicity assessment

- Collection of immunogenicity data must continue in the real-world setting.
- To assign postmarketing safety events to the correct biologic, product nomenclature conventions must be followed for full traceability.
- Immunogenicity should also be monitored closely in real-world studies where patients on stable treatment with a reference biologic are switched to a biosimilar.
- Currently, immunogenicity data from switching studies are limited, especially for oncological indications.

Conclusion

- Comparative immunogenicity results must be understood by regulators and physicians in the context of the assays and population(s) used and their associated limitations.
- Further efforts are required to standardize comparative immunoassays.

Future perspective

A number of anticancer biosimilars are under development and expected to enter clinical use in the near future. As bioanalytical platforms for detecting unwanted immune responses against biologics continue to advance, regulators must stay abreast of these developments in order to accurately interpret the risk of immune responses associated with biologic treatments, including biosimilars. Clinicians must also be aware of the relevance of evolving immunogenicity tests when choosing from an ever-increasing pool of biologic therapies. In addition to standardization of regulatory requirements for comparative immunogenicity assessments of biosimilars, future accumulation of real-world and fully traceable pharmacovigilance data should facilitate the evaluation of the potential risks and benefits of a biosimilar treatment.

Author contributions

All authors contributed equally to the manuscript and all approved the final article.

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