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White Paper

Recommendations for the Development and Validation of Immunogenicity Assays in Support of Biosimilar Programs

Francesca Civoli,[1,15] Aparna Kasinath,[2] Xiao-Yan Cai,[3] Meenu Wadhwa,[4] Andrew Exley,[5] Philip Oldfield,[6] Safa Alvandkouhi,[7] Gregor Schaffar,[8] John Chappell,[9] Ronald Bowsher,[10] Viswanath Devanarayan,[11] Joseph Marini,[12] Shannon Rebarchak,[12] Michael Anderson,[13] Vera Koppenburg,[14] and Todd Lester[7]

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Abstract. For biosimilar drug development programs, it is essential to demonstrate that there are no clinically significant differences between the proposed biosimilar therapeutic (biosimilar) and its reference product (originator). Based on a stepwise comprehensive comparability exercise, the biosimilar must demonstrate similarity to the originator in physicochemical characteristics, biological activity, pharmacokinetics, efficacy, and safety, including immunogenicity. The goal of the immunogenicity assessment is to evaluate potential differences between the proposed biosimilar product and the originator product in the incidence and severity of human immune responses. Establishing that there are no clinically meaningful differences in the immune response between the products is a key element in the demonstration of biosimilarity. An issue of practical, regulatory, and financial importance is to establish whether a two-assay (based on the biosimilar and originator respectively) or a one-assay approach (based on the biosimilar) is optimal for the comparative immunogenicity assessment. This paper recommends the use of a single, biosimilar-based assay for assessing immunogenic similarity in support of biosimilar drug development. The development and validation of an ADA assay used for a biosimilar program should include all the assessments recommended for an innovator program (10–16, 29). In addition, specific parameters also need to be evaluated, to gain confidence that the assay can detect antibodies against both the biosimilar and the originator. Specifically, the biosimilar and the originator should be compared in antigenic equivalence, to assess the ability of the biosimilar and the originator to bind in a similar manner to the positive control(s), as well as in the confirmatory assay and drug tolerance experiments. Practical guidance for the development and validation of anti-drug antibody (ADA) assays to assess immunogenicity of a biosimilar in comparison to the originator, using the one-assay approach, are described herein.

KEYWORDS: Immunogenicity; Anti-drug antibodies; ADA; Biosimilar.

Views should not be misconstrued as representing those of the MHRA or the EMA

[1] Coherus BioSciences, Redwood City, CA, USA.
[2] Syngene International Ltd, Bangalore, India.
[3] Accurant Biotech, Inc., Cranbury, New Jersey, USA.
[4] Medicines and Healthcare Products Regulatory Agency (MHRA), National Institute for Biological Standards and Control (NIBSC), Hertfordshire, UK.
[5] Regulatory Division, Medicines and Healthcare Products Regulatory Agency (MHRA), London, UK.
[6] Philip Oldfield Bioanalytical Consulting, Quebec, Canada.
[7] BioAgilytix Labs, Durham, North Carolina, USA.
[8] Hexal AG , Oberhaching, Germany.
[9] Gyros Protein Technologies, Uppsala, Sweden.
[10] B2S Life Sciences, Franklin, Indiana, USA.
[11] GlaxoSmithKline, Collegeville, Pennsylvania USA.
[12] Janssen Research & Development, LLC, Spring House, Pennsylvania, USA.
[13] Immunologix Laboratories, Tampa, Florida, USA.
[14] Hexal AG, Holzkirchen, Germany.
[15] To whom correspondence should be addressed. (e–mail: fcivoli@coherus.com)

INTRODUCTION

A biosimilar is defined as a biological therapeutic product that is highly similar to an already approved biological drug (reference product, i.e., originator), notwithstanding minor differences in clinically inactive components, with no clinically meaningful differences between the biological product and the originator in purity, potency, and safety of the product (1–3). Biosimilarity assessment is based on stepwise assessments of analytical, non-clinical, and clinical characteristics, with detailed physicochemical and functional evidence of similarity obtained before the initiation of the clinical program (3,4,35,36). A key requirement in the development of biosimilars is the demonstration of similar immunogenicity in a comparative (head-to-head) clinical study, as per United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) Guidelines (1–3,34). These guidance documents outline general principles for handling the scientific challenges related

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to demonstrating biosimilarity between a proposed biological product and a reference product.

Immunogenicity is the humoral or cell-mediated immune response to a biological product. The majority of biological or biotechnology-derived proteins (biotherapeutics) induce an immune response that may be triggered by patient-, disease-, and product-related factors. The clinical consequences of an immune response to biotherapeutics may range from no effect to benign reactions, loss of efficacy, and even serious life-threatening events (5–9). Therefore, testing of unwanted or undesirable immunogenicity is critical for any biotherapeutic drug development program, including those for biosimilars.

Immunogenicity evaluation in the clinical setting is conducted using anti-drug antibody (ADA) assays, to detect the presence of antibodies that bind to the biotherapeutic product in a biological matrix (generally serum or plasma), and neutralizing antibody (NAb) assays which demonstrate whether the binding ADAs neutralize the biological activity of the biotherapeutic. While recent progress has been made in describing and understanding the challenges of the assessment of ADAs in support of biosimilar programs (17–24,27), there is little if any specific regulatory guidance on development and validation requirements for immunogenicity assessments of biosimilars and reference biologics in comparability studies. The lack of specific guidance has led to different approaches used in different biosimilar programs with respect to the use of one assay or two assays to compare the immunogenicity of the biosimilar and the originator molecule, and to different approaches to assay validation.

A Biosimilars Action Program Committee (APC) was formed within the former AAPS Ligand Binding Assay Bioanalytical Focus Group (LBABFG), which combines the expertise of key industry leaders to provide a forum to discuss the issues surrounding biosimilar bioanalytical assay requirements. In 2014, a white paper from the APC subcommittee was published with a focus on pharmacokinetic (PK) assays to support biosimilar comparability studies (25). In 2017, a white paper by the American Association of Pharmaceutical Scientists (AAPS) biosimilar focus group on non-clinical and clinical assays was published to guide the industry on best practices and testing strategies when developing neutralizing antibody (NAb) assays for biosimilar programs (24).

The objective of this paper is to offer our collective insights into the merits of the one-assay versus two-assays approach for assessing the immunogenicity of a biosimilar and its reference product. In this paper, we recommend the adoption of the one-assay approach as the default strategy for harmonizing the design and implementation of ADA assays to support biosimilar drug development programs, and we describe the considerations and experiments for assay development and validation.

ADA ASSAY METHODOLOGY

Success of any anti-drug antibody evaluation strategy is linked to the corresponding assay design. Assays should be designed to be sensitive, specific, precise, and robust. Clinical aspects to consider include the trial design, half-life of the tested drugs, sampling schedule to accommodate the assay's drug tolerance, cross reactivity of antibodies, pre-existing antibodies, and the variability in patient population. Standard platforms for ADA evaluation exploit the specific interaction of an antibody with its antigen in various analytical formats such as indirect, bridging, and competitive immunoassays, including enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence assay (ECL), radioimmunoassay (RIA), radioimmunoprecipitation assay (RIPA), and surface plasmon resonance assay (SPR) (27).

The widely adopted multi-tiered strategy for evaluation of immunogenicity recommended by FDA and EMA (10–13) is also applicable to biosimilars and consists of a sensitive Screening Assay (tier 1) for the detection of all potential antibody-positive study samples, followed by a Confirmatory Assay (tier 2) where screen-positive samples are tested for specificity to the therapeutic. The confirmatory assay is generally based on inhibition of the assay signal by excess antigen (drug product). Confirmed positive samples are then tested in a Titer Assay to determine the magnitude of positivity, and in a Neutralizing Antibody (NAb) assay to evaluate their neutralizing capacity. Further characterization of responses, following regulatory advice on a case-by-case basis, may include assays to detect particular isotypes (e.g., antigen-specific IgE if anaphylaxis is a clinical concern, or rapidly dissociating antibodies such as IgM) or analysis of cross reactivity to an endogenous counterpart. Potential strategies for the assessment of novel multi-component biological products such as fusion proteins, pegylated proteins, and antibody-drug conjugates include immunoassays for each moiety or dissection of ADA responses using the confirmatory assay to test binding to each individual moiety.

All ADA methods are expected to be sensitive, specific, precise, and robust, and should be fully validated for their intended use. In the biosimilarity assessment, the ADA methods should demonstrate a similar ability to measure the immune response against the therapeutic administered, irrespective of whether the therapeutic was the originator or the biosimilar.

The development of an ADA assay to support a biosimilar program should follow current and standard bioanalytical guidance documents, recent white papers, and other ADA assay-related publications (10–15, 29). It is recommended to also refer to, and consult, regulatory guidelines for biosimilar drug development for specific comparability requirements (1–3). It is generally advisable to perform all critical assessments in assay development mode, to ensure a smooth assay validation phase. Typically, the timelines for clinical development of a biosimilar are shorter than for an originator molecule, as fewer clinical studies are generally required, and can be performed in parallel. During development of an originator molecule, assay improvements throughout the course of the clinical program are not unusual. In contrast, assay improvements are usually not possible during the development of a biosimilar program. Therefore, it is imperative that the initial assay development is thorough and assay validation is complete at the beginning of the clinical program. It is recommended that the strategy for assay development and validation incorporates all necessary testing to assess critical program characteristics (such as patient population, version of reference used—such as US or EU, etc.) early in the program. For example, certain disease

state factors may confound ADA assay results based on their specific or non-specific nature. Several sample pretreatment options are available for optimizing assay steps and reagents to reduce assay background from contributing disease factors or interference from circulating drug or target (26,27,30–33). It is helpful to establish at the outset a method development plan outlining development goals, milestones, strategies, and design of experiments, to ensure a focused assay development execution.

ONE-ASSAY VERSUS TWO-ASSAYS—ADVANTAGES AND DISADVANTAGES

In a biosimilar drug development program, two drug products, the biosimilar and the originator, are tested in head-to-head studies for comparison. Depending on the submission strategy, a clinical study can also include three treatment groups, where the biosimilar is compared to the originator manufactured in the United States (US) and the originator manufactured in the European Union (EU). One might use independent assays to assess immunogenicity of each product (resulting in two or more assays—i.e., two-assays approach) or a single assay (one-assay approach) to assess immunogenicity of the biosimilar and the originator(s). The one-assay approach includes the use of a single screening assay and one confirmatory assay to analyze clinical samples, regardless of the treatment group. As per regulatory recommendations, the ADA assay should detect antibodies against both the biosimilar and the originator similarly, and where any bias occurs it should favor greater sensitivity for detecting antibodies developed against the biosimilar. Therefore, in the "one-assay approach," the biosimilar should be used as capture and detection reagent, to ensure that any potential novel immunogenic epitopes, if present, are detected. Also, the biosimilar should be used as the excess competing antigen in the confirmatory assay. The advantages and disadvantages of both approaches are described in Table I.

One-Assay Approach

Advantages

The conservative approach is to use biosimilar-based reagents for the assay to ensure that antibodies that are generated against the biosimilar are reliably detected and guarantees that the question whether the biosimilar is more immunogenic than the originator can be answered. The use of one assay eliminates "between-assay" variability, as the method will have one screening and one confirmatory cut-point. Samples generated during a blinded study, where both the biosimilar and the reference product have been dosed, can be analyzed using the same assay. In addition, reduced amount of critical reagent management is needed, as only biosimilar reagents are required for the studies.

Disadvantages

The use of biosimilar based reagents may miss antibodies that are specific for unique epitopes existing only in the reference product, e.g., ADAs against immunogenic structures (e.g., carbohydrates).

Two-Assays Approach

Advantages

A two-assay approach should ensure that ADAs against potential unique structures of both the biosimilar and reference product will be detected independently. Assuming that the two assays are comparable with respect to sensitivity and drug tolerance, antibodies against the originator will not go undetected and a comparison of true immunogenicity rates is possible.

Disadvantages

It is inevitable that two assays will have different assay characteristics (screening and confirmatory cut-points, sensitivity, drug tolerance, target interference, etc.), as different reagents are used. There is complexity regarding comparing results from two different assays used for evaluation of samples from the same study. The expectation of "head-to-head" comparison is not met, and the introduction of additional variability between two independent assays may reduce reliability and may confound immunogenicity comparison. For example, if a bridging format is used for the detection of ADAs, small differences in the labeling of the respective drugs for the assays may impact the ability of each of the assays to detect certain immune responses. For blinded studies, samples have to be unblinded before the assay, or all samples have to be tested in both assays, doubling the work, reagents, and required study sample volumes. Comparison and interpretation of immunogenicity data is more challenging because two different assays are used for study sample analysis, unless the assay cut points, relative sensitivity, drug tolerance, and precision are evaluated to be equivalent. There is an inherent difficulty in assessing equivalence of two assays even when the assay performance is similar. Therefore, to ensure that the two assays are truly comparable, additional statistical analysis is required. The development, validation, and sample analysis workload and resources are significantly increased.

The advantages of using one assay outweigh the advantages of using two assays and allow for the determination of the immunogenicity of a biosimilar candidate compared to the originator. While the one-assay approach using the biosimilar may underestimate immunogenicity for the reference product, it is the preferred option as it minimizes the variability of the two-assay approach and avoids the challenges associated with developing two assays. Also, it is the immunogenicity of the biosimilar which needs to be investigated rather than the reference product. In addition to the reasons highlighted above, in the last few years, several discussions have taken place in the industry and with regulatory agencies which support the use of one assay (37–39), and several biosimilars have been approved using such approach (40,41).

Thus, the one-assay approach (one screening assay and one confirmatory assay) is recommended in this paper, and considerations for its development and validation are described in detail below. If for any reason a sponsor prefers to use two assays (or multiple assays), they should develop and validate two identical assays (one for the

Table I. Advantages and Disadvantages of One-Assay Versus Two-Assays Approaches

Attribute	Advantages	Disadvantages
One-assay approach (using biosimilar)		
General	Minimizes variability whilst determining relative immunogenicity	May not be reflective of true immunogenicity rates or true differences between biosimilar and reference product
ADA detection	ADA against the biosimilar are readily detected	Potential bias of greater sensitivity in detecting ADA against the biosimilar (i.e., ADA against the reference product may be missed)
Assay development and validation	Rigorous development and validation exercise generating acceptance criteria for a single (biosimilar-based) assay	Requires convincing demonstration of antigenic equivalence, drug tolerance etc. for biosimilar and reference product
Assay variability	Minimized. Inter-assay and intra-assay variability of single (biosimilar-based) assay only	
Critical reagents	Only for one assay (biosimilar)	
Sample analysis	Allows blinded assessment	
Data analysis	Single data set by treatment group minimizes discordant results	
Two-assays approach (using biosimilar and reference product)		
General	Assuming very similar sensitivity and drug tolerance, it reflects "true" immunogenicity rates for each product without potential bias	Significant additional development, validation, technical and analytical costs
ADA detection	Detects ADA against biosimilar and reference product including any unique immunogenic structures present	Analytical and biological variables require resolution within an acceptable equivalence range
Assay development and validation	All parameters tested during assay development and validation for both assays. Accommodates slight differences for biosimilar and reference product	Significant additional work generating acceptance criteria that are similar for both assays May be challenging to have identical sensitivity or cut-point for the 2 assays
Assay variability		High; Inter-assay and Intra-assay variability of two assays to be considered
Critical reagents		Two assays: biosimilar and reference product
Sample analysis		Precludes blinded assessment
Data analysis		Dual data set by treatment group complicates data interpretation, e.g., need to resolve results when ADA positive in only one assay

biosimilar and one for the originator) and demonstrate similar performance in all validation parameters (cut-point, drug tolerance, sensitivity). This paper provides collective industry and regulatory recommendations for the assay design, development, and validation of ADA assays in support of biosimilar programs, using the one-assay approach.

SELECTION OF ASSAY PLATFORM AND METHODOLOGY

As a first step, the assay platform and method should be selected, followed by a feasibility phase to ensure that the assay can detect the positive control at appropriate concentrations. As for any ADA assay, properties such as drug product modality (i.e., Mab vs. cytokine), target interference, matrix interference, circulating concentrations of the drug and required tolerance, sensitivity, presence of pre-existing antibodies to the drug, and endogenous counterparts should be considered for the selection of the assay platform. Published information on immunogenicity methods, assay sensitivity, and known immunogenicity profile of the originator molecule should be reviewed when selecting an appropriate assay platform for a biosimilar. However, state-of-the-art technology/methods should be used, as the evolution in immunoassay technology platforms may have rendered obsolete the assay platform employed by the originator sponsor. Importantly, regulators will expect the most discriminating assays to be utilized in a biosimilar program. Developers should not be concerned by the increased sensitivity and specificity of new different assay platforms and/or methodology, since the key issue is to demonstrate similar ADA rates for the biosimilar and the originator in the same study.

SELECTION OF POSITIVE CONTROL

The positive control antibody for assessing the performance of ADA assays is typically generated by hyper-immunizing an animal with the therapeutic drug. In a biosimilar program, suitable positive control antibodies may be generated against the biosimilar drug or against the originator. While use of a positive control against the biosimilar is preferred for assay validation and subsequent clinical assessment, commercial antibodies against the reference product have been accepted in rare instances, subject to demonstrating comparable ability to bind to the biosimilar and to the originator. A positive control antibody generated against the reference product could be appropriate for a biosimilar program for several reasons: (1) Where the assay detects binding to the biosimilar, showing that the assay can also detect antibodies against the originator supports the one-assay approach; (2) positive control antibodies against the same molecule can differ in binding site, affinity, and avidity when generated in different animals or different immunization campaigns; and (3) separate positive control antibodies against the biosimilar and reference product would be generated by the hyper-immunization of different animals, which may again result in different antibody characteristics. The recommendation is to use a single positive control during sample analysis, ideally generated using the biosimilar. Whichever positive control antibody is used, equivalent binding to the biosimilar and the reference product should be demonstrated during assay development and validation (see below). A panel of multiple positive control antibodies (against the biosimilar and the originator molecule), if available, can be useful for demonstrating equivalence during assay development or validation, comparing competitive inhibition with the biosimilar and the reference product. Considering the surrogate nature of the positive control antibodies in immunogenicity assays, potential differences in the immunogenicity response in actual clinical studies are unlikely to be predicted by the positive control used to evaluate the assay. However, when ADA detection rates are significantly lower for the biosimilar than for the reference product in the clinical studies, this may be justified as a "true" observation based on the characteristics of the biosimilar and supported by a comparison of several positive control antibodies during the assay development and validation phases.

ASSAY DEVELOPMENT

Once the assay technology and the positive control are selected, the development of an ADA assay used for a biosimilar program should include all the assessments recommended for an innovator program (10-16,29). In addition, specific parameters also need to be evaluated, to gain confidence that the assay can detect antibodies against both the biosimilar and the originator. Specifically, the biosimilar and the originator should be compared in antigenic equivalence (defined as the ability of the biosimilar and the originator to bind in a similar manner to the positive control), confirmatory assay, and drug tolerance experiments. For these assessments, it is important to verify that the amount/concentration of biosimilar and reference product used in the assay is the same. Thus, it is recommended that the actual measured concentration is used instead of the nominal concentration for preparation of the drug stocks. If a difference between the behavior of the biosimilar and originator is observed during assay development, the assay should not move into validation. Rather, assay parameters such as assay platform, MRD, positive controls and other reagents, and assay methodology should be re-evaluated. Testing of additional control antibodies may help to elucidate any observed differences. If a difference remains, a discussion with the regulators is advisable before moving into assay validation, as it may reflect previously un-discovered analytical differences between the biosimilar and the originator.

The assessments recommended for assay development and validation are summarized in Table II and described below. All experiments should be performed using the biosimilar, supported by comparison with the reference product where indicated.

This paper also focuses on the specific additional assessments recommended for an ADA assay used in a biosimilar program.

Antigenic Equivalence (Drug Competition Curves)

After selection of the MRD and a preliminary assessment of sensitivity, it is advisable to test antigenic equivalence, or the ability of the biosimilar and the originator to bind in a similar manner to the positive control(s). This is achieved by spiking the matrix with a known concentration of positive control, with and without increasing concentrations of either drug product. The positive control should be spiked at different concentrations (generating a high and a medium/low signal). The concentrations of drug should be selected to generate a concentration-response curve of the competitive inhibition. The curves generated with the biosimilar and the originator should be visually overlapping or comparable, confirming that excess concentrations of both the biosimilar and the originator inhibit the assay signal of the positive control to a similar extent. Figure 1 represents an illustrative example of a successful demonstration of antigenic equivalence using a concentration of positive control that generates a high signal.

In addition to the visual evaluation, a comparison of the signal values at each concentration can also be helpful to confirm that any observed difference between the signal in the presence of each drug product (originator and biosimilar) is within the precision of the assay. For example, demonstrating that the percent CV of the mean signal obtained from both drug products (originator and biosimilar) is $< 20\%$ at the majority of drug concentrations would be indicative of antigenic equivalence.

Confirmatory Assays

The specificity/confirmatory assay is typically a competitive inhibition test. The data for this assay is evaluated for a change in assay signal of a sample (or positive control) with or without pre-incubation with the study drug.

The concentration of drug to use can be selected from the drug competition curve and "should be optimized to

Table II. Assay Development and Validation for Anti-drug Antibodies: Standard Approach Versus Biosimilarity Assessment

Attribute	Standard ADA assays	ADA assays for biosimilarity assessment
Guiding principle	Optimize detection of ADA to test product	Optimize detection of ADA to Biosimilar & Reference product[a]
Minimum required dilution (MRD) (assay development)	Optimize signal to noise ratio from at least 10 individual samples	
Sensitivity (screening and confirmatory assays)	Required	
Antigenic equivalence (assay development)	Not applicable	Essential characteristic of ADA assays for Biosimilar & Reference product
Subject-to-subject variability (screening assay cut point)	Required	
Subject-to-subject variability (confirmatory assay cut point)	Required	Establish comparable confirmatory cut-points for Biosimilar & Reference product[a]
Determination of LPC (for screening and confirmatory assays)	Required	
Selectivity/matrix interference (in relevant disease state samples): test in screening and confirmatory assays	Required	
Drug tolerance (*to inform ON-drug & OFF-drug sampling*)	Required	Demonstrate comparable drug tolerance for Biosimilar & Reference product
Target interference (*if applicable to inform sample preparation*)	Required	
Robustness (establishment of assay parameters ranges)	Required	
Short-term and freeze-thaw sample stability	Required	

LPC = low positive control
[a] It is generally regarded as acceptable by regulators to resolve any bias in favor of detecting ADA against the biosimilar

confirm the presence of antibodies throughout and above the range of the assay" (10). Thus, a concentration of drug product which produces substantial inhibition of the HPC can be used. In a biosimilar program, it is recommended to assess the performance of the confirmatory assay by spiking excess biosimilar or excess originator as one of the means to ensure similar ability to detect antibodies against the biosimilar and against the reference product. For this preliminary assessment, it is sufficient to test 10–15 samples once, to have an idea of subject-to-subject variability and to compare the effect of the biosimilar and the originator on the assay signal. The percent inhibition of the assay signal of the drug-spiked samples relative to the unspiked samples should then be determined separately and compared between the two (or three) drugs.

It is expected that the inhibition obtained by spiking with biosimilar is comparable to the inhibition obtained by spiking with the originator.

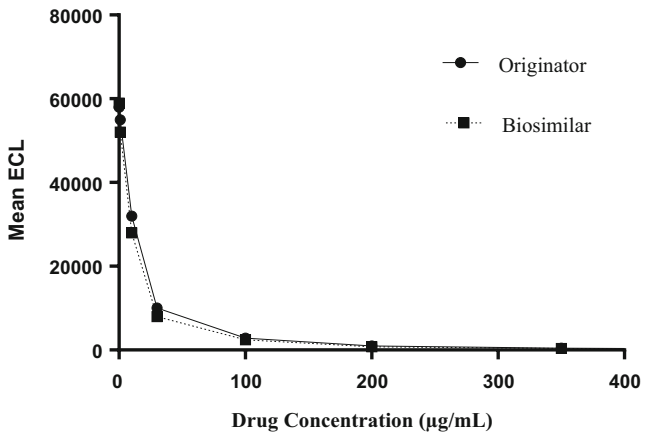

Fig. 1. Example of acceptable results from the antigenic equivalence experiment

Drug Tolerance

Drug product present in the matrix (serum, plasma, etc.) will interfere with the sensitivity of an ADA assay. The assessment of assay sensitivity in the presence of interfering therapeutic drug product is known as the assay's drug tolerance and is expected to be evaluated in all ADA assays (10). At least a concentration of 100 ng/mL of positive control should be tested, based on the recommended assay sensitivity (10). Drug inhibition curves in the presence of PC should be generated by performing serial dilutions (twofold or threefold) of the drug, testing drug concentrations that are expected to be present in the clinical study samples.

The highest drug level at which the signal generated by the positive control exceeds the screening cut point is considered the assay tolerance to the drug. Ideally, the assay should be able to detect at least 100 ng/mL of positive control in the presence of the drug concentrations expected to be present at the time of ADA assessment in the clinical trials. Based on the results of the drug tolerance, assay format improvements may be needed, for example the inclusion of sample pretreatment steps such as acid dissociation (26,27,32). Alternatively, or in addition, clinical sampling can be adapted to accommodate drug tolerance and drug half-life to ensure that suitable off-drug samples are collected.

In a biosimilar program, tolerance to both the biosimilar and originator(s) should be assessed. Drug tolerance is expected to be comparable for the biosimilar and the originator (within ± one dilution factor). Also, the curves should be visually comparable. If a difference is observed, different control antibodies could be tested to understand the extent of the difference.

ASSAY VALIDATION

After assay development has been completed to satisfaction and the assay appears to be fit for purpose, assay validation

should be initiated. Validation parameters for ADA assays have been clearly defined in the FDA and EMA guidance documents (10–12). Additional tests should be conducted as part of assay validation to ensure that the assay is appropriate to detect ADA against both the biosimilar and originator product(s).

As for assay development, all the experiments should be performed using the biosimilar as antigen/reagent; however, the originator should be used in selected experiments during assay validation to ensure that the assay is able to detect ADA against both biosimilar and originator.

It is important to verify that the amount/concentration of biosimilar and originator drug used in the assay is the same. Thus, it is recommended that the actual measured concentration is used instead of the nominal concentration for preparation of the drug stocks. If the results of any validation experiment are different between the biosimilar and the originator, this should be considered in the context of the totality of the data, and a discussion with the regulatory authorities may be advisable. The specific experiments where the originator product is to be used are discussed below.

Confirmatory Cut Points

In the one-assay approach, one confirmatory assay (using the biosimilar) should be used for sample analysis. The purpose of this validation experiment is to establish the cut point to use for the confirmatory assay, and to compare the confirmatory cut points obtained with the biosimilar and the originator. The procedure to determine the confirmatory cut point is the same as for all ADA assays. In brief, at least 50 individual samples from drug naïve subjects should be tested on at least three different days by at least two analysts in the absence and in the presence of an excess amount of drug (in this case either the biosimilar or the originator). It is recommended that the same samples are tested in the presence of excess biosimilar or excess originator on the same plate, to limit variability of the comparison of distributions. The percent inhibition of the assay signal of the drug-spiked samples relative to the unspiked samples should then be determined separately for the two drugs. Statistically valid approaches (10) should be used to calculate the confirmatory cut points for both drugs. The confirmatory cut points for the biosimilar and the originator(s) are expected to be comparable. For example, the distributions of percent inhibition in the presence of the biosimilar and the originator can be evaluated by comparing the means (by ANOVA) and the variances (by Levene's test) (42). If the means and variances are not significantly different, this would support the use of the one-assay approach. If there is a significant difference, the key point is to demonstrate that the assay is not less likely to detect antibodies against the biosimilar than against the originator.

Drug Tolerance

A drug tolerance test should assess the effect of both originator and biosimilar drugs on the detection of ADA in the screening assay. Drug tolerance should be examined by spiking different known amounts of positive control antibody. At a minimum, inclusion of a high positive control level (HPC) and low positive control level (LPC) into matrix in the absence or presence of increasing concentrations of the originator or the biosimilar is expected. Drug should be titrated into the antibody-spiked samples by performing serial dilutions (twofold or threefold) and include testing of drug concentrations that are expected to be present in the clinical study samples. The highest drug level at which the signal generated by the positive control exceeds the screening cut point is considered the drug tolerance of the assay. Results obtained in the absence and presence of different quantities of the originator and biosimilar should be compared. The assay tolerance to the originator and to the biosimilar should be similar (within ± one-dilution factor) (Fig. 2).

SAMPLE ANALYSIS

After a successful assay validation, all study samples (regardless of treatment group) should be analyzed using one screening assay and one confirmatory assay (using the biosimilar for both screening and confirmatory assays).

Due to many biosimilar drug candidates being tested in clinical trials on patients or healthy volunteers, it is possible to enroll subjects who have already been exposed to a form of the drug (the originator or a biosimilar candidate) and may have developed ADAs. Thus, the ADA prevalence (rate of ADA predose) may be higher than reported in the literature from originator clinical studies. Also, due to the evolution of assay technologies and methodologies, ADA incidence for the originator may be higher than observed in historical trials. Thus, a comparison with the originally reported ADA rates of the originator is not relevant. However, if ADA rates for the originator are lower than the reported literature, a reason may have to be provided and discussed with the regulatory agencies. Also, if the ADA incidence is much lower for the biosimilar, a root cause analysis is also required (28).

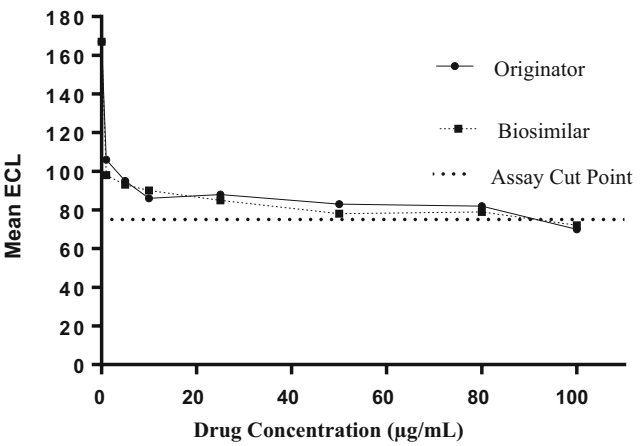

Fig. 2. Example of acceptable results from the drug tolerance experiments (LPC level)

CONCLUSIONS

The demonstration of similar immunogenicity is a critical requirement for the demonstration of similarity between a biosimilar candidate and the respective reference product. The clinical development of a biosimilar includes head-to-head clinical studies to assess potential differences in immunogenicity between the two products. To provide an appropriate comparison of the immunogenicity of the biosimilar and the reference product, it is recommended that a single, biosimilar-based assay be used, subject to rigorous cross-validation of the biosimilar and originator for antigenic equivalence, drug tolerance, and performance in the confirmatory assay. The development and validation of ADA assays to assess the immunogenicity of a biosimilar should follow the same guidance set by the FDA and EMA for any therapeutic protein. However, during assay development and subsequently the assay validation, the originator should also be tested in select experiments to ensure the assay's similar ability to detect antibodies against biosimilar and originator, and similar reactivity of the two drug products with the positive control. As a result, development and validation of ADA assays to be used for biosimilar programs are more complex and require more assessments than ADA assays used for originator programs. The major advantages of the one-assay approach, underpinned by rigorous cross-validation, are uniformity, eliminations of assay bias, streamlining of validation, and operational simplicity in supporting the unequivocal clinical determination of the similar immunogenicity of a candidate biosimilar compared to the originator.

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