

# Multidisciplinary approach to evaluating immunogenicity of biosimilars: lessons learnt and open questions based on 10 years' experience of the European Union regulatory pathway

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**Abstract:** Clinical evaluation of comparative immunogenicity represents an important component of the European Union regulatory review process for candidate biosimilar products. The clinical evaluation is part of a multidisciplinary review that cross-refers to product quality attributes as well as preclinical and ongoing risk management considerations. Results from the monitoring of anti-drug antibody formation in relevant populations treated for an adequate period of time are interpreted in relation to clinically relevant endpoints, including pharmacokinetics, pharmacodynamics, efficacy, and safety parameters. The European Union regulatory standard for designation of biosimilarity requires a suitable weight of evidence, determined on a product-specific basis, to demonstrate that the immunogenicity associated with the biosimilar product does not lead to a higher negative impact on clinically relevant outcomes compared with the reference product. The experience gained during the 10-year period following the implementation of the European Union biosimilars pathway indicates that a suitably cautious approach was applied, insofar as no immunogenicity-related issues have emerged for the approved applications of the different biosimilar products. In some cases, product quality-related issues were identified in the preauthorization setting as being potentially relevant for heightened risk of immunogenicity and were duly taken into account for the biosimilarity decision. Some unresolved issues remain, most notably concerning the limitation of noninterventional post-marketing surveillance measures to monitor the potential for changes in immunogenicity over the longer term, eg, following introduction of changes in manufacture, formulation, or primary product container. Lack of standardization of bioanalytical methods precludes comparison of anti-drug antibody formation for different products that are evaluated in noncomparative clinical studies, and correlation with relevant clinical parameters is also lacking.

**Keywords:** immunogenicity, biosimilar, regulatory, clinical, preclinical, bioanalytical

## Introduction

The purpose of this paper is to review the experience gained in the European Union (EU) regulatory system for evaluation of undesirable immunogenicity of candidate biosimilar products, within the context of the overall scientific assessment required for registration on the basis of Article 10(4) of Directive 2001/83/EC (“Similar Biological Medicinal Products”). This legal pathway was established in 2004, enabling the authorization between 2006 and December 2013 of biosimilar versions of five different reference products, ie, human growth hormone, filgrastim, erythropoietin-alfa, infliximab, and follitropin-alfa. In addition to the approved products, applications for three candidate biosimilar insulin products were withdrawn during the review procedure, and one

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negative opinion was given in respect of a candidate biosimilar interferon-alfa product.<sup>1</sup> Table 1 summarizes the candidate and approved biosimilar products considered in this paper.

The effectiveness of the applied regulatory standards for managing the potential unfavorable effects associated with undesirable immunogenicity of these products is reviewed in a multidisciplinary manner, taking into account product quality, bioanalytical, preclinical, clinical, and post-authorization risk management aspects.

The impact of immunogenicity-related risks on overall benefit and risk is also considered in relation to extrapolation to therapeutic indications not studied in the preauthorization phase, as well as interchangeability of biosimilar and innovator versions.

## Regulatory guidance

The most relevant reference points for guiding the design of the immunogenicity evaluation of candidate biosimilar products for registration in the EU are summarized in Table 2. In addition to the primary (often referred to as “overarching”) guidelines for biosimilar products, product-specific guidelines contain additional recommendations for evaluation of immunogenicity; these should be consulted for advice on the extent of the clinical immunogenicity evaluation required for different product types.

It is important to emphasize that these guidelines are not legally binding; an applicant may elect to apply alternative

approaches, subject to adequate scientific justification. This is particularly the case for selection of bioanalytical methodology, ie, the choice of technical format for pharmacokinetic and anti-drug antibody (ADA) assays is the responsibility of the applicant, although the validation of suitability should comply with prevailing regulatory standards. Regulatory guidelines are dynamic, ie, are updated on a regular basis to reflect the experience gained. For example, as discussed later in the section Nonclinical immunogenicity evaluation, the role of immunogenicity evaluation in nonclinical studies has been moderated in the more recent EU biosimilar guidelines.<sup>2</sup>

## Product quality-related issues

### General considerations

Biosimilar candidates for registration in the EU should always have 100% identical amino acid sequence to the reference product.<sup>3</sup> In general, the post-translational modification profile should be within the range demonstrated for the reference product, although there may be exceptions in the case of identified safety risks for the originator (eg, for cetuximab). Designation of biosimilarity is based on a highly rigorous demonstration of comparability of product attributes, taking into account variables that could influence undesirable immunogenicity. From the immunogenicity perspective, the requirement for 100% amino acid sequence identity implies that the biosimilar can-

**Table 1** Biosimilar candidates reviewed for marketing authorization in the European Union to the end of 2013

Biosimilar candidate	MA status	Reference product	Difference in formulation*
Omnitrope®	Approved April 4, 2006	Genotropin®	Different preservative for multi-use presentations (m-cresol for Genotropin; benzyl alcohol for Omnitrope) Genotropin has additional excipient (mannitol)
Valtropin®	Approved April 24, 2006 No longer marketed	Humatrope	Same qualitative composition, but difference in quantitative composition of excipients
Alpheon®	Refused June 28, 2006	Roferon-A	Not disclosed
Binocrit®	Approved June 28, 2007	Erypo®	Same qualitative and quantitative composition
Silapo®	Approved December 18, 2007	Erypo	Different qualitative and quantitative composition
Ratiograstim®	Approved September 15, 2008	Neupogen®	Differ only in pH and concentration of Polysorbate 80
Zarzio®	Approved February 6, 2009	Neupogen®	Only excipient difference is qualitative; Zarzio contains glutamate as a buffer ion whereas Neupogen contains acetate
Nivestim™	Approved June 8, 2010	Neupogen®	Same qualitative and quantitative composition
Solumarv®	Application withdrawn	Humulin R®	Same qualitative composition as corresponding reference product; quantitative amounts of respective excipients not disclosed
Isomarv Medium®	November 15, 2012	Humulin N	
Combimarv®		Humulin 70/30 (M3)	
Remsima®	Approved September 10, 2013	Remicade®	Same qualitative and quantitative composition
Ovaleap®	Approved October 23, 2013	Gonal-F®	Ovaleap contains mannitol (instead of sucrose) and methionine and benzalkonium chloride as additional excipients

**Notes:** \*Information taken from product information and European public assessment reports. Manufacturer details are as follows: Omnitrope®, Binocrit® and Zarzio® (Sandoz GmbH, Kundl, Austria); Valtropin® and Alpheon® (BioPartners GmbH, Reutlingen, Germany); Silapo® (Stada Arzneimittel AG, Bad Vilbel, Germany); Ratiograstim® (Ratiopharm GmbH, Ulm, Germany); Nivestim™ (Hospira UK Ltd., Royal Leamington Spa, United Kingdom); Solumarv®, Isomarv Medium® and Combimarv® (Marvel LifeSciences Ltd., Harrow, United Kingdom); Remsima® (Celltrion Healthcare Hungary Kft, Budapest, Hungary); Ovaleap® (Teva Pharma B.V., Utrecht, the Netherlands).

**Abbreviation:** MA, Marketing Authorization.

**Table 2** European Union regulatory guidelines relevant for immunogenicity risk assessment of candidate biosimilar products\*

CHMP/BMWP reference	Title
14327/2006	Immunogenicity assessment of biotechnology-derived therapeutic proteins
86289/2010	Immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use
42832/2005 rev1	Similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues
247713/2012	Similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues
671292/2010	Similar biological medicinal products containing recombinant follicle-stimulating hormone
562000/2010	Similar biological medicinal products containing interferon beta
403543/2010	Similar biological medicinal products containing monoclonal antibodies: nonclinical and clinical issues
301636/2008	Similar biological medicinal products containing recombinant erythropoietins
118264/2007	Similar biological medicinal products containing low molecular weight heparins
102046/2006	Nonclinical and clinical development of similar biological medicinal products containing recombinant interferon alpha
31329/2005	Similar biological medicinal products containing recombinant granulocyte-colony stimulating factor
94528/2005	Similar biological medicinal products containing somatropin
32775/2005	Similar biological medicinal products containing recombinant human insulin and insulin analogs (draft revision)

**Note:** \*Available at <http://www.ema.europa.eu>.

didates would be expected to contain the same T-cell epitopes as the reference product, but that differences in immunogenic potential could be related to differences in:

- conformational structures, eg, multimers or aggregates of the therapeutic protein that alter recognition by B-cell receptors and/or uptake by antigen-presenting cells, or are able to stimulate immune effector cells directly
- post-translational glycosylation or PEGylation, involving structures that can be recognized by pre-existing antibodies in sensitized individuals or might alter antigen processing, binding to major histocompatibility complex, or recognition by B-lymphocytes or T-lymphocytes
- process-related impurities, eg, *Escherichia coli*-derived proteins or yeast beta-glucans or endotoxins, which could stimulate innate immune cells to modify the adaptive immune response to the therapeutic protein.

## Aggregates and subvisible particles

Recent work has revealed that aggregation of therapeutic proteins influences antigen uptake, processing, and presentation via a mass action mechanism in addition to activating antigen-presenting cells.<sup>4</sup>

The presence of tungsten residues in prefilled syringes has been cited as a risk factor for enhanced immunogenicity of therapeutic proteins, being associated with detection of increased levels of product aggregates.<sup>5</sup> This was implicated as a causal factor for induction of neutralizing antibodies in two patients with chronic kidney disease following subcutaneous administration of a biosimilar erythropoietin product.<sup>6,7</sup>

### Issues for erythropoietin

In preauthorization studies of Binocrit® and Silapo® using intravenous administration, there was no difference detected in clinical immunogenicity of the biosimilar candidates in direct comparison with the reference product. Since it was not possible to perform a directly comparative clinical comparison of subcutaneously administered Binocrit or Silapo versus the reference product, Erypo®, at the time of the initial Phase III nephrology studies (because administration of the reference product by the subcutaneous route had been temporarily contraindicated due to an elevated incidence of antibody-mediated pure red cell aplasia consequent to a change in product formulation), the initial marketing authorizations for Binocrit and Silapo in patients with chronic kidney disease were restricted to intravenous administration.

Phase III studies comparing Binocrit or Silapo versus the reference product were then performed at a later date. Therapeutic equivalence and comparable safety and immunogenicity were demonstrated for Silapo in direct comparison with Erypo, resulting in the approval of Silapo for subcutaneous as well as intravenous administration in the chronic kidney disease population. However, the sponsor voluntarily halted the first Phase III study comparing subcutaneous administration of Binocrit versus Erypo following the detection of neutralizing anti-erythropoietin antibodies in two subjects.<sup>6</sup> Pure red cell aplasia was diagnosed in one of these subjects; this subject was an 86-year-old male who had a number of comorbidities, a history of food allergy, and an interrupted treatment pattern in the Phase III study. The sponsor performed a thorough follow-up investigation that revealed a potential risk factor for a heightened immune response to the therapeutic protein, ie, an association with residual tungsten in the prefilled syringes was suggested as a possible causal factor for

induction of the detected neutralizing antibodies.<sup>7</sup> A follow-up Phase III study using low-tungsten prefilled syringes is ongoing.

It is relevant to note that cases of pure red cell aplasia, featuring the development of antibodies that react with, and neutralize the biological activity of, the endogenous cytokine are detected at very low frequency in subjects treated with different recombinant erythropoietin products.<sup>8</sup>

Differences in excipient composition could influence immunogenicity indirectly by modification of the solubility of a therapeutic protein, potentially to increase levels of subvisible particles. For example, trehalose may reduce the propensity of certain therapeutic monoclonal antibodies to form aggregates in solution: replacement of trehalose with an alternative sugar, eg, sucrose, might result in an increased risk of aggregation that enhances immunogenicity, making it advisable to generate comparative stability data using worst-case storage conditions of the drug product formulated with different excipients.<sup>9</sup>

## Post-translational glycosylation

The EU regulatory pathway for biosimilars permitted the use of a different host cell line for expression of a nonglycosylated therapeutic protein, namely growth hormone (Valtropin® [BioPartners GmbH, Reutlingen, Germany]), but acceptability for glycosylated proteins is equivocal.<sup>10</sup> As a general rule, for a glycoprotein biosimilar candidate, the host cell substrate should be as similar as possible to that used for expression of the reference product, reflecting the influence of the host cell on post-translational glycosylation profile. For cetuximab, where the particular nonhuman glycosylation profile associated with the SP2/0 host cell line used for the originator was implicated in the induction of serious systemic hypersensitivity reaction,<sup>11</sup> there would be a justifiable case to use an alternative cell line (eg, CHO) that enables expression of cetuximab bearing negligible levels of the problematic galactose- $\alpha$ -1,3-galactose carbohydrate in the Fab domain.<sup>12</sup>

Even in the case that a similar host cell line were used, it is possible that there could be detectable differences in levels of the potentially nonhuman antigenic glycan, N-glycolyl neuraminic acid (Neu5Gc), that could result in an altered propensity for binding to pre-existing antibodies in human subjects.<sup>13</sup> This issue was raised during the review of the marketing authorization application for a human follitropin- $\alpha$  expressed in CHO cells (Ovaleap® [Teva Pharma B.V., Utrecht, the Netherlands]), which was found to have slightly higher levels of Neu5Gc compared with the reference product.<sup>14</sup> The risk was mitigated by quantitative analysis of Neu5Gc by

high-performance anion-exchange chromatography with pulsed amperometric detection, measurement of pre-existing Neu5Gc-reactive antibodies in the clinical trial population (incidence found to be 18%), and demonstration that baseline status for Neu5Gc-reactive antibodies did not impact treatment outcomes in direct comparison with the reference product.

## Process-related impurities

Elevated levels of host cell-derived protein impurities were associated with detection of treatment-emergent antibodies reactive with these impurities, in association with increased levels of antibodies to the therapeutic protein, in the case of an investigational formulation of Omnitrope® (Sandoz GmbH, Kundl, Austria).<sup>15</sup>

## Issues for growth hormone

A biosimilar growth hormone candidate manufactured using an early version of the manufacturing process induced antibodies that reacted to the *Escherichia coli*-derived protein impurity in association with a reported increase in antibodies reactive with the human growth hormone product.<sup>15</sup> The purification process was modified in addition to improving the performance of the assay used to measure the levels of the contaminating host cell protein. Anti-growth hormone antibody levels were then found to decline in subjects who started treatment on the earlier version of the product but who continued therapy with the drug product from the improved process (Table 3). Most notably, for subjects receiving the commercial grade product (EP2K-02-PhIII-Lyo study), none developed anti-growth hormone antibodies and only one subject had a positive result for anti-*E. coli* protein antibodies at the 12-month treatment time point.

## Clinical evaluation

### General considerations

Regulatory guidelines in the EU require evidence that the immunogenicity of the biosimilar candidate does not have a higher negative clinical impact in direct comparison with the reference product. It is important to note that the arbiter is clinical impact, and not the incidence or magnitude of detected levels of ADA per se. Also, it is acceptable for a biosimilar candidate to be less immunogenic than the reference provided, provided that the two product versions are demonstrated to have equivalent efficacy and posology.<sup>2</sup>

Because safety may not extrapolate across different therapeutic indications in the same manner as efficacy, additional safety data might be required in particular therapeutic indications/patient populations that were not evaluated in

the main therapeutic equivalence studies. This consideration may be relevant from the immunogenicity perspective as discussed below.

The clinical studies should be designed for an adequate duration to demonstrate differences, if these were to occur, in clinically significant immunogenicity. Clearly, the experience gained for the reference product would be most relevant. In general, for products administered in a chronic basis, it is advisable to provide 12-month continuous exposure data to support registration.<sup>2</sup> For products administered in a cyclical manner, samples for ADA testing should be collected prior to the first treatment cycle, then prior to one or more (depending on identified immunogenicity risk level for reference product) subsequent cycles, and finally at 4–6 weeks following the last treatment.

In cases where it may be difficult to detect differences in low incidence adverse effects, heightened monitoring in the post-authorization setting may be required to compensate for the uncertainty at the time of registration.<sup>2</sup> Indeed, as discussed below, immunogenicity has featured in the risk management plan for all of the biosimilar products approved to date in the EU.

## Weight of evidence

The extent of the clinical data submitted in support of approval, as reported in the European public assessment reports<sup>1</sup> and other information in the public domain, is summarized in Table 3. Since the assessment reports do not provide a fully comprehensive picture of the data submitted, Table 3 is not complete with respect to the full weight of evidence actually submitted to the agency.

The biosimilarity assessment for the products reviewed to date by EU regulatory agencies has always included directly comparative clinical evidence of immunogenicity of the biosimilar candidate versus the reference product in at least one therapeutic indication. Notable features for the clinical immunogenicity evaluation of these biosimilar candidates are summarized in the next paragraph.

### Main features of immunogenicity review for biosimilars in the European Union

- Immunogenicity was assessed in a descriptive manner by interpreting results of anti-drug antibody testing relative to clinical outcomes, including pharmacokinetics/pharmacodynamics (filgrastim, epoetin, infliximab), efficacy (all products), and safety (all products)
- The clinical indication used for demonstration of therapeutic equivalence also provided the main evidence for assessment of comparative immunogenicity

- Where the product was to be used in settings with a potentially different immunogenicity risk profile (subcutaneous administration of epoetin in renal disease or oncology subjects), it was necessary to provide additional preauthorization immunogenicity data to rule out heightened risk
- For chronic administration products, immunogenicity monitoring for a 12-month period of continuous treatment (no switching) was required
- Anti-drug antibody sampling enabled characterization of the timing of antibody formation, as well as magnitude and neutralizing capacity of anti-drug antibody
- A study design involving immunogenicity monitoring in subjects switched between candidate biosimilar and reference products was not required in the preauthorization setting
- Follow-up, post-treatment monitoring of subjects treated in pivotal comparative clinical studies was required for products with identified immunogenicity-related risks (epoetin and growth hormone)
- Product quality differences were influential in the interpretation of anti-drug antibody test results (erythropoietin, growth hormone, follitropin-alfa)
- Preauthorization immunogenicity assessment of the candidate biosimilar was based on the drug product formulation/primary container combination intended for commercialization.

In the case of some therapeutic monoclonal antibodies, supportive data on relative immunogenicity may be obtained from a single-dose comparative pharmacokinetic study in healthy volunteers. Accordingly, it is recommended to include ADA monitoring at appropriate time points in the Phase I study.<sup>16</sup>

The agency raised a number of concerns relating to the marketing authorization applications for Alpheon<sup>®</sup> (BioPartner GmbH) and the insulins marketed by Marvel LifeSciences Ltd (Harrow, United Kingdom) (Solumarv<sup>®</sup>, Isomarv Medium<sup>®</sup>, and Combimarv<sup>®</sup>), as summarized in the following two paragraphs.

### Issues for interferon-alfa

The refusal assessment report for Alpheon<sup>®17</sup> referred to major concerns relating to uncertainties about product quality and clinical safety and efficacy. Specific concerns about the extent of immunogenicity evaluation were cited as contributing to the overall negative opinion. These included an incomplete validation of the specificity and dilutional linearity of the double-antigen enzyme-linked immunosorbent assay method used

Table 3 Preauthorization clinical studies providing immunogenicity data\*

Study design	Reported ADA incidence	Other observations
<b>Omnitrope®</b> 48-month treatment over three sequential substudies: EP2K-99-Phill (0–6 months), EP2K-00-PhillIF <sub>0</sub> (6–9 months); open, comparative, somatropin Sandoz powder (API Covance), n=44 versus Genotropin®, n=45 9–15 months, EP2K-00-PhillIAQ, Part A: comparative, somatropin Sandoz liquid (API Sandoz), n=42 versus Omnitrope; somatropin Sandoz powder (API Sandoz), n=44 15–48 months, EP2K-00-PhillIAQ, Part B: noncomparative; somatropin Sandoz liquid (API Sandoz), n=86	ADA samples collected at baseline and at months 3, 6, 9, 12, 15, 18, 24, 30, 36, and 48 Non-neutralizing anti-GH antibodies detected at least once in 26 (59%) subjects treated initially with somatropin Sandoz powder (API Covance) versus one (2%) subject treated for 9 months with Genotropin only Following the switch to drug product (liquid and powder) manufactured with API Sandoz, the level of positive anti-GH antibody subjects then declined to 14% at 48 months. Anti-HCP antibodies detected in all subjects treated with somatropin Sandoz powder (API Covance); no anti-HCP antibodies were detected in subjects treated with Genotropin At the 12-month time point, none of the subjects had developed anti-GH antibodies; only one subject had a positive result for anti-HCP antibodies (final results not published)	Single batch of API Covance used in studies EP2K-99-Phill and EP2K-00-PhillIF <sub>0</sub> had high <i>Escherichia coli</i> HCP Higher incidence of anti-GH antibodies was attributed to enhancement of immunogenicity by <i>E. coli</i> HCP (although data confirming signal specificity have not been published) No effect on any growth efficacy parameters was observed Final study results not published
<b>Valtropin®</b> BP-EU-003: comparative, double-blind; Valtropin (n=98) versus Humatrope (n=49) GH-deficient children treated daily for 12 months, subcutaneous administration BP-EU-003-RO: 135 subjects continued for additional 12 months with Valtropin (including 45 who switched from Humatrope)	During first 12 months of treatment, anti-GH antibodies detected in 3/98 (3.1%) subjects treated with Valtropin versus 1/49 (2.0%) subjects treated with Humatrope During second year of treatment, anti-GH antibodies were detected in the same four subjects	No effect on any growth efficacy parameters was observed Anti-yeast antibodies detected in 2/98 (2%) of patients treated with Valtropin
<b>Binocrit®/Abseamed®/Epoetin-alfa Hexal®</b> Binocrit safety population from five Phase I studies performed in healthy volunteers (INJ-4, INJ-5, INJ-6, INJ-7, and INJ-12); n=98 total 2003-29-INJ-9: comparative, double-blind; Binocrit (n=261) versus Erypo® (n=142) completed; CRF patients on hemodialysis; intravenous administration three times per week for 28 weeks, followed by 28-week open-label extension with all patients receiving Binocrit	ADA samples collected at 2-week intervals No ADA detected by ELISA or RIP measurements ADA samples collected at 4–8-week intervals Six patients (two in Binocrit group, four in the Erypo/Binocrit group) had a transient positive result in RIP screening assay at week 28 or week 42 The two patients in the Binocrit group had antibodies from baseline attributed to prior treatment One patient in the Binocrit group who was positive in the RIP screening assay at baseline had a borderline positive result in the Nab assay; no clinical evidence of PRCA ADA samples collected at 6-week intervals No treatment-emergent ADA detected ADA samples collected at screening, then at weeks 8, 16, 24, 40, and 52 During the treatment period, five subjects in the Binocrit group and two patients in the Erypo group had positive results in RIP screening assay Two subjects on Binocrit had a positive Nab result	No patients showed evidence of PD effects of neutralizing ADA, eg, a sudden decrease in hemoglobin level or reticulocyte count No new safety concerns identified PRCA confirmed in one of the Nab-positive subjects; Study halted voluntarily by sponsor to enable root cause investigation 151 Binocrit and 119 Erypo-treated subjects completed 6-month safety follow-up

<p>SENSE (NCT01576341): open-label, noncomparative; anemic predialysis CKD patients; Binocrit subcutaneous administration, <math>\geq 1</math> per week for 52 weeks; estimated enrollment 360</p> <p>ACCESS (NCT01693029): Comparative, Binocrit versus Epopo/Procrit® double-blind; CKD patients; subcutaneous administration, <math>\geq 1</math> per week for 52 weeks; estimated enrollment 360</p> <p><b>Silapo®/Retacrit®</b></p> <p>Correction phase study, 411-54-04-05-0000: comparative, double-blind; Silapo (n=275) versus Erypo (n=272) completed treatment; CRF patients on hemodialysis; intravenous administration, three times per week for 24 weeks; completed patients enrolled into open-label extension period</p> <p>Maintenance study 411-54-04-04-0000: comparative, double-blind; Silapo (n=135) versus Erypo (n=134) completed treatment; CRF patients on hemodialysis; intravenous administration, 1–3 times per week for 24 weeks, preceded by an open run-in period of 12–16 weeks; completed patients enrolled into open-label extension period</p> <p>Open-label extension safety study 441-54-04-14-0000: uncontrolled; n=532 CRF patients completed further intravenous treatment with Silapo for 52 weeks, 1–3 doses per week; n=123 also completed 108 weeks of treatment</p> <p>Study 441-54-04-46-0000: uncontrolled safety study in chemotherapy-induced anemia; Silapo n=208, 12 weeks, subcutaneous</p> <p>Study 441-54-07-08-0000: comparative, observer-blind; Silapo (n=154) versus Erypo (n=165) completed treatment per protocol; CRF patients on hemodialysis; subcutaneous administration, 1–3 times per week for 28 weeks, preceded by an open run-in period of 12–16 weeks with Silapo</p> <p>Completed (n=346) patients enrolled into open-label extension period to continue treatment for 54 weeks with Silapo®</p> <p><b>Ratiograstim®/Biograstim®/Tevagrastim®</b></p> <p>XM02-02-INT (breast cancer): comparative, Ratiograstim® (n=135) versus Neupogen® (n=130) completed four cycles of chemotherapy; 5 µg/kg daily for at least 5 days/maximum 14 days per cycle; subcutaneous</p>	<p>Study ongoing</p> <p>Study ongoing</p> <p>Positive signal in ADA/RIP assay reported for 11 subjects at baseline; seven subjects in Silapo group versus four subjects in Erypo group.</p> <p>No increase in the number of subjects with positive signals following treatment with either product</p> <p>Positive signal in ADA/RIP assay reported for four subjects at baseline.</p> <p>No increase in the number of subjects with positive signals following treatment with either product</p> <p>No treatment-emergent ADA were detected</p> <p>All patients negative for ADA</p> <p>All patients tested for ADA at least at last available sample (full sample schedule not reported)</p> <p>No subjects were positive for ADA in RIP screening assay</p> <p>Combined results for cancer patient dataset: n=356 received Ratiograstim only, n=134 received Neupogen only</p> <p>At screening (pretreatment):</p> <p>12 positives for Ratiograstim of 356 (3.4%) compared with two positives for Neupogen of 134 (1.5%) at screening</p> <p>At any subsequent visit:</p> <p>Seven positives for Ratiograstim of 356 (2.0%) at any subsequent visit compared with 2 positives for Neupogen of 134 (1.5%)</p> <p>One positive signal detected in neutralizing antibody assay for a subject receiving Ratiograstim only; this was described as a borderline positive sample (corresponding to assay cut-point) at the follow-up visit</p>	<p>Follow-up to 2007-22-INJ-17, to support a future authorization of subcutaneous administration in CKD patients</p> <p>Comparison versus US market reference product</p> <p>No clinical evidence of PRCA in any subjects with baseline positive results in ADA/RIP assay</p> <p>No clinical evidence of PRCA in any subjects with baseline positive results in ADA/RIP assay</p> <p>No clinical evidence of PRCA in any subjects</p> <p>Extension of indication in renal anemia for subcutaneous administration granted on basis of these data</p> <p>No immunogenicity findings of clinical relevance in cancer patient dataset</p>
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(Continued)

Table 3 (Continued)

Study design	Reported ADA incidence	Other observations
<p>XM02-03-INT (lung cancer): comparative efficacy in first cycle; safety of Ratiograstim over six cycles; Ratiograstim (n=148) versus Neupogen (n=77) per protocol; 5 µg/kg daily for at least 5 days/maximum 14 days per cycle (Neupogen first cycle only); subcutaneous</p>	<p>No confirmed positive anti-filgrastim antibodies were detected at any time point using the RIP assay</p>	<p>No relationship between detection of ADA and virological response was detected at 12, 24, 48, or 72 weeks, or with adverse events</p>
<p>XM02-04-INT (NHL): comparative efficacy in first cycle; safety of Ratiograstim over six cycles; Ratiograstim (n=55) versus Neupogen® (n=29) per protocol; 5 µg/kg daily for at least 5 days/max 14 days per cycle (Neupogen first cycle only); subcutaneous</p>	<p>ADA samples collected at baseline or day 1 of first cycle; day 1 of cycle 2; day 21 of cycle 4; day 91 (study termination) No confirmed positive anti-filgrastim antibodies were detected at any time point using the RIP assay</p>	<p>Concerns expressed regarding the validation of the ADA assay to exclude false negatives; in addition, no reporting of ADA titers.</p>
<p><b>Zarzio®/Filgrastim Hexal®/Filgrastim ratiopharm®</b> Three Phase I studies in 106 healthy volunteers: EP06-102, EP06-103 and EP06-105; comparison of Zarzio versus Neupogen, double-blind; two-way cross-over; single or multiple dosing; intravenous or subcutaneous administration, 1–5 µg/kg/day</p>	<p>ADA samples collected at baseline or day 1 of first cycle; day 1 of cycle 2; day 21 of cycle 4; day 91 (study termination) No confirmed positive anti-filgrastim antibodies were detected at any time point using the RIP assay</p>	<p>Negative opinion given by CHMP regarding marketing authorization of Alpheon</p>
<p>EP06-301 (breast cancer): uncontrolled, safety/immunogenicity; 153 patients completed four cycles of chemotherapy, with mean total daily dose of Zarzio of approximately 440 µg</p>	<p>Two subjects gave a positive antibody response following treatment. No attribution of ADA response to particular treatment in this cross-over study</p>	<p>Concerns expressed regarding the validation of the ADA assay to exclude false negatives; in addition, no reporting of ADA titers.</p>
<p><b>Nivestim™</b> Phase I PK/PD study in 48 healthy volunteers, GCF062: comparative, Nivestim™ versus Neupogen, double-blind; two-way cross-over; multiple dosing; subcutaneous administration, 5 or 10 µg/kg/day for 5 consecutive days GCF071 (breast cancer): comparative, double-blind; Nivestim (n=165) versus Neupogen (n=85, cycle 1 per protocol population); daily subcutaneous administration of 5 µg/kg up to 14 days per cycle; up to six cycles of chemotherapy</p>	<p>Three patients treated with Nivestim gave borderline positive result in highly sensitive RIP screening assay; negative in neutralizing antibody assay No patient in the Neupogen treatment group tested positive for anti-filgrastim antibodies</p>	<p>Concerns expressed regarding the validation of the ADA assay to exclude false negatives; in addition, no reporting of ADA titers.</p>
<p><b>Alpheon®</b> BP-IFN-002 Open-label, parallel-group, comparative; chronic hepatitis C; Alpheon (n=114) versus Roferon-A® (n=102) completed 48 weeks of treatment; three subcutaneous doses of 3×10<sup>6</sup> IU/dose; post-treatment monitoring for 6 months ADA samples collected at baseline; 24, 48, and 72 weeks</p>	<p>Week 24 Positive ADA detected in 50 of 121 subjects receiving Alpheon compared with 51 of 115 receiving Roferon-A Week 48 Positive ADA detected in 31 of 114 subjects receiving Alpheon compared with 38 of 102 receiving Roferon-A Week 72 Positive ADA detected in 16 of 106 subjects receiving Alpheon compared with 21 of 97 receiving Roferon-A</p>	<p>No relationship between detection of ADA and virological response was detected at 12, 24, 48, or 72 weeks, or with adverse events Concerns expressed regarding the validation of the ADA assay to exclude false negatives; in addition, no reporting of ADA titers. Negative opinion given by CHMP regarding marketing authorization of Alpheon</p>



<p><b>Ovaleap®</b>            XM17-05: comparative, physician-blinded; Ovaleap (n=152) versus Gonal-F® (n=145); infertile ovulatory women undergoing ART; daily subcutaneous administration            Part A: main study, fixed dose (5 days) followed by adaptive dose (up to 15 days) in single treatment cycle            Part B: follow-up, adaptive dose for up to 20 days in up to two additional cycles</p>	<p>Incidence of detection of anti-FSH antibodies not reported            Narrative of assessment report focuses on evaluation of immunogenicity risk related to pre-existing antibodies reactive with the Neu5Gc post-translational glycosylation; raised as a regulatory issue because of an observed difference in the Neu5Gc content between Ovaleap® and Gonal-F            Pre-existing anti-Neu5Gc antibodies detected in 18% of patients</p>	<p>Pre-existing anti-Neu5Gc did not affect PK or efficacy or safety            Immunogenicity of Ovaleap was considered to be similar to that of Gonal-F</p>
<p><b>Remsima®/Inflectra®</b>            CT-P13 1.1: comparative, Remsima (n=128) versus Remicade® (n=122), double-blind, parallel-group, Phase I PK study in AS patients, multiple single-dose, 5 mg/kg, intravenous infusion; three loading doses and six maintenance doses given over 54-week treatment period. The aim was to compare PKs at steady state (weeks 22–30). ADA samples collected at screening and weeks 14, 30, and 54; then 8 weeks after last dose</p>	<p>At least one ADA positive sample detected for 44 of 128 (34.4%) patients treated with Remsima versus 39/122 (32.0%) patients treated with Remicade            Comparable ADA titers were observed for Remsima-treated versus Remicade-treated groups            Almost all ADAs detected in screening assay were found to be neutralizing in competitive ligand binding assay; highly similar distribution of negative, low, medium and high titer neutralizing antibody titers for Remsima versus Remicade</p>	<p>ADAs significantly reduced systemic exposure to infliximab            Magnitude of impact of ADA on PK parameters was comparable for Remsima versus Remicade            PK parameters were comparable between the two treatment groups</p>
<p>CT-P13 3.1: comparative, Remsima (n=302) versus Remicade (n=300) double-blind, parallel-group, Phase I study in RA patients, multiple single-dose, 3 mg/kg + MTX, intravenous infusion; three loading doses and six maintenance doses given over 54-week treatment period            Aim was to demonstrate therapeutic equivalence; PK parameters included as secondary endpoints            ADA samples collected at screening and weeks 14, 30, and 54; then 8 weeks after last dose</p>	<p>At least one ADA-positive sample detected for 168 of 302 (55.6%) patients treated with Remsima versus 163 of 300 (54.3%) patients treated with Remicade            Highly similar distribution of negative, low, medium, and high neutralizing antibody titers for Remsima versus Remicade</p>	<p>ADAs had a significant negative impact on peak and trough drug concentrations.            Magnitude of impact was comparable for Remsima versus Remicade            A higher incidence (16.6%) of infusion-related reactions in ADA-positive patients was reported for Remicade compared with Remsima (10.8%)            Comparable efficacy was demonstrated</p>
<p><b>Marvel insulins (Solumarv®, Isomaryv Medium®, Combimarv®)</b>            411-BK-03-01-0000: Comparative, double-blind; type 1 (n=243) and type 2 (n=283) diabetes patients (full analysis set); daily subcutaneous flexible (test n=144 versus reference n=142) or fixed (test n=121 versus references n=119) dose combinations for 24 weeks. (Results from 6-month open-label extension were not included in dossier)</p>	<p>Since treatment-naïve patients were excluded, a significant proportion of subjects may have had anti-insulin antibodies at baseline. Thus, the primary output was the percentage of patients who developed new antibodies during the first 24 weeks of treatment, as follows:            Type 1 diabetes: 21.9% for Marvel insulins versus 14.0% for Humulin®            Type 2 diabetes: 10.7% for Marvel insulin versus 12.5% for Humulin</p>	<p>Application was withdrawn during review procedure            The European Medicines Agency assessment report concluded that the immunogenicity of Marvel insulins had not been adequately evaluated            Differences in PD were observed between test and reference products: Marvel insulins showed reduced efficacy in the 12–24-week treatment period, and a higher incidence (24% versus 12%) of adverse events in patients in type 1 diabetes</p>

**Note:** \*Data from EPARs<sup>1</sup>. Manufacturer details are as follows: Omnitrope®, Binocrit® and Zarzio® (Sandoz GmbH, Kundl, Austria); Valtropin® and Alpheon® (BioPartners GmbH, Reutlingen, Germany); Silapo® (Stada Arzneimittel AG, Bad Vilbel, Germany); Ratiograstim® and Filgrastim ratiopharm® (Ratiopharm GmbH, Ulm, Germany); Nivestim™, Retacrit® and Inflectra® (Hospira UK Ltd., Royal Leamington Spa, United Kingdom); Remsima® (Celltrion Healthcare Hungary Kft, Budapest, Hungary); Ovaleap® and Tevagrastim® (Teva Pharma B.V., Utrecht, the Netherlands); Solumarv®, Isomaryv Medium® and Combimarv® (Marvel LifeSciences Ltd., Harrow, United Kingdom); Biograstim® (Merckle Biotec GmbH, Ulm, Germany); Epogen® (Amgen Inc., Thousand Oaks, CA, USA); Procrit® (Centocor Ortho Biotech, Inc, Horsham, PA, USA); Humulin® (Eli Lilly and Company, Indianapolis, IN, USA); Epoetin-alfa Hexal® and Filgrastim Hexal® (Hexal AG, Holzkirchen, Germany); Genotropin® (Pfizer, Inc., New York, NY, USA); Abseamed® (MEDICE Arzneimittel Pütter GmbH & Co. KG, Iserlohn, Germany).  
**Abbreviations:** ADA, anti-drug antibody; AS, ankylosing spondylitis; NAB, neutralizing antibodies; ELISA, enzyme-linked immunosorbent assay; RIP, radioimmuno-precipitation; CKD, chronic kidney disease; CRF, chronic renal failure; PKCA, antibody-mediated pure red cell aplasia; Neu5Gc, N-glycolyl neuraminic acid; MTX, methotrexate; RA, rheumatoid arthritis; ART, assisted reproductive therapy; FSH, follicle-stimulating hormone; GH, growth hormone; HCP, host cell protein; PK, pharmacokinetics; PD, pharmacodynamics; NHL, non-Hodgkin lymphoma.

to monitor anti-drug antibodies to Alpheon compared with Roferon-A<sup>®</sup>. The commercial kit method, whilst acceptable in principle, had not been fully validated prior to the clinical sample analysis; and the retrospective validation was considered incomplete. The agency noted that false negatives were not adequately excluded, there was no reporting of antibody titers and there was no assay of neutralizing capacity of the detected anti-drug antibody. Thus, although the incidence of anti-drug antibody formation in the respective treatment groups was similar, the agency considered that bioanalytical limitations precluded a definitive conclusion on relative immunogenicity of Alpheon compared with Roferon-A.

### Issues for insulin

Following an initial application in 2007, which was subsequently withdrawn, Marvel Life Sciences resubmitted Marketing Authorisation Applications for three biosimilar candidate insulin products in December 2011: Solumarv<sup>®</sup> (soluble rapid-acting insulin); Isomav Medium<sup>®</sup> (intermediate-acting isophane insulin); Combimarv<sup>®</sup> (a mixture of the other two products, 70% long-acting isophane insulin + 30% soluble rapid-acting insulin). As a result of major concerns expressed by the agency, the applicant withdrew these applications in November 2012. The respective withdrawal assessment reports many observations and deficiencies for the three Marvel insulins<sup>18</sup> catalog, including lack of demonstration of comparability of product attributes and pharmacodynamics. The analysis of clinical safety revealed a higher incidence of hypersensitivity reactions in subjects receiving the biosimilar insulin candidates; in addition, the Marvel insulins had lower efficacy during the 12–24-week treatment period compared with the reference product versions. Unfortunately, insufficient data were available to exclude a role for heightened immunogenicity in these clinical safety and efficacy observations.

Although the anti-drug antibody incidence was similar for the treatment groups, patients treated with Marvel insulins had higher mean anti-insulin antibody titer at week 28. The agency noted that differences in the product process could have contributed to higher levels of potentially immunogenic impurities. However, the anti-drug antibody assay specificity had not been validated for detection of the specific impurity. Furthermore, clinical samples were not tested for antibodies reactive with host-cell derived proteins or for product-specific immunoglobulin E antibodies. Although a neutralizing antibody assay was applied, the agency questioned the suitability of this method, on the basis of equivocal relevance to measure inhibition of the biological activity of insulin.

The submission did not include immunogenicity results from the 6-month open-label extension period of the main safety study. The risk management plan was not adequately specific regarding the proposed post-authorization activities to address the uncertain impact of immunogenicity on treatment outcome.

### Data correlation

The example of infliximab represents a case in which there was a relatively high, but comparable, incidence of subjects with rheumatoid arthritis and ADA during treatment with Remsima<sup>®</sup> (Celltrion Healthcare Hungary Kft, Budapest, Hungary) or Remicade<sup>®</sup> (Janssen Biologics B.V., Leiden, the Netherlands). There was a high level of concordance both in timing and relative magnitude of neutralizing antibodies to the two products.<sup>19</sup> Although detection of ADA was associated with reduced peak and trough drug concentrations, the magnitude of this impact was the same for Remsima when compared with Remicade. In addition, the incidence of infusion-related reactions was similar for the two treatment groups. This case illustrates the critical importance of being able to correlate ADA signals with relevant clinical parameters in a temporal manner to assess whether there could be a heightened risk of clinically significant immunogenicity associated with a candidate biosimilar product.

### Predefined acceptance criteria

To date, EU regulators have not required application of predefined acceptance margins of detected ADA incidence for the biosimilar candidate versus the reference product. This reflects the rather different sensitivities of the bioanalytical assays used, allied to lack of knowledge of what level of difference in detected ADA might have a negative influence on clinical parameters. Thus, it is clinical outcome, rather than detected ADA per se, that is the main arbiter for assessing relative immunogenicity for the biosimilarity decision.

### Extrapolation of therapeutic indications

The approval of biosimilar versions of filgrastim, growth hormone, and infliximab represent examples in which the biosimilar product was granted all of the therapeutic indications that had been authorized for the reference product. The extrapolation was based on extensive evidence of commonality of the principal mechanisms of action of the product across the different indications, allied to lack of evidence that there could be an increased risk of clinically relevant immunogenicity in the therapeutic indications that were not

studied in preauthorization studies. Residual uncertainty at the time of approval was addressed by provisions included in the risk management plan for monitoring of immunogenicity in the post-marketing setting.

For filgrastim, a potential concern could be administration to healthy volunteers for stem cell transplantation, since these subjects would not derive any benefit from the procedure. In regard to the biosimilar filgrastim products approved to date, the post-approval experience of use in hematopoietic stem cell mobilization, comprising a total of 904 subjects, including 156 sibling or unrelated volunteer donors, has not revealed a different safety profile from that of the reference product, Neupogen® (Amgen Europe B.V.).<sup>20</sup> However, data on ADA formation are lacking.

The approval of Omnitrope was based on demonstration of equivalent efficacy and safety, including immunogenicity, in the most sensitive population, namely treatment-naïve growth hormone-deficient children. This enabled a valid extrapolation to all of the indications approved for the reference product.<sup>21</sup> In accordance with the provisions of the risk management plan, the sponsor has initiated noninterventional post-marketing studies in children and in adults that will include monitoring for anti-growth hormone antibodies and the potential clinical effects of such antibodies.<sup>22,23</sup>

Given the known association between anti-infliximab antibodies, reduced efficacy, and increased incidence of infusion-related reactions, it is pertinent to question whether there could be a differential risk for a biosimilar product relative to the reference product in alternative therapeutic settings. For example, controlled clinical studies performed using patients who receive less immunosuppressive medication might reveal differences in immunogenicity that are not apparent in a patient population receiving a more intensive concomitant immunosuppressive regimen.<sup>24</sup>

However, selection of the most sensitive population for an objective comparison of the anti-infliximab antibody response must take account of the potential interference of residual drug in different bioanalytical assay formats.<sup>25,26</sup> This represents a critical variable because a lower dose level (3 mg/kg) is used in patients with rheumatoid arthritis than in those with other indications (5 mg/kg in Crohn's disease, psoriasis, ulcerative colitis, and ankylosing spondylitis), thereby potentially enhancing sensitivity to detect differences in patients with rheumatoid arthritis that could be obscured by higher circulating drug levels in, for example, patients with ankylosing spondylitis.<sup>27</sup> Higher drug interference is a possible reason why the reported incidence of anti-infliximab

antibodies was lower in the ankylosing spondylitis population (not receiving methotrexate, but possibly receiving other immunosuppressive medications) compared with rheumatoid arthritis patients receiving methotrexate in the studies comparing Remsima with Remicade. Most importantly, the preauthorization studies for Remsima demonstrated that there was no difference in clinically impactful immunogenicity across the rheumatoid and ankylosing spondylitis populations when directly compared with the reference product. This evidence supports the extrapolation of a conclusion of no difference in immunogenic potential from a study performed in the rheumatoid arthritis population to the other authorized indications.

## Nonclinical immunogenicity evaluation EU experience

The hierarchical nature of the biosimilar approach applied in the EU requires a substantial weight of evidence of similarity at the product quality level.<sup>3</sup> Accordingly, the role of nonclinical studies is to assess the impact of any detected differences, or of residual uncertainties, for the benefit-risk to proceed into comparative clinical studies.<sup>2</sup>

Product-specific guidelines (see Table 2) in the EU for candidate biosimilars containing recombinant growth hormone, erythropoietin, filgrastim, insulins, or interferon- $\alpha$  recommend that immunogenicity should be evaluated during nonclinical, repeat-dose toxicology studies. Three (for recombinant growth hormone, filgrastim and insulins) of five of these guidelines advise that "special emphasis" should be placed on evaluation of the immune response, while the other two (recombinant erythropoietin and interferon- $\alpha$ ) simply recommend that antibody determination should be performed. In addition, the currently effective version of the overarching guideline on nonclinical and clinical requirements for biosimilar products (CHMP/BMWP/42832/20005) endorses a role for nonclinical evaluation of immunogenicity in recommending the inclusion of antibody measurements, encompassing both cross-reactivity and neutralizing capacity, as part of the comparative toxicokinetics data package.

As a consequence of this regulatory guidance, the initial wave of biosimilar submissions in the EU incorporated data on the comparative immunogenicity of the respective candidate biosimilar versus reference products as measured during nonclinical toxicology/toxicokinetics studies. Table 4 provides a summary of the extent of the nonclinical

**Table 4** Summary of nonclinical studies contributing to immunogenicity assessment for biosimilar candidates reviewed in the European Union

Product	Species	Study design	Relative immunogenicity
Omnitrope®	Rat	Noncomparative, repeat-dose toxicology/TK 0, 2, or 8 mg/kg/day, subcutaneously, 14-day TK analysis on days 1, 7, and 14 for hGH concentration	Not measured Absence of sharp decline in C <sub>max</sub> /AUC at day 14 provided indirect evidence of absence of significant antibody formation
Valtropin®	Rat	Comparative (versus US Humatrope®) repeat-dose toxicology, 28-day, with daily administration; bridging to earlier studies using mice, rats, and rabbits	No ADA data were reported Adequate exposure to hGH was achieved
Binocrit®	Dog	Comparative (versus Eprex®/Erypo®), repeat-dose toxicology, daily dosing for 13 weeks via intravenous administration	ADA detected in 2–3 animals per group; no obvious difference
Silapo®	Rat	Comparative (versus Erypo), repeat-dose toxicology, three doses per week for 13-week duration, subcutaneous	No meaningful difference
	Dog	Comparative (versus Erypo), repeat-dose toxicology, 13-week duration, intravenous	Higher incidence of non-neutralizing ADAs detected for Silapo (8/16 dogs) relative to Erypo (1/8); not considered instructive for clinical immunogenicity due to foreign nature/expected immunogenicity of human rhEPO
Ratiograstim®	Rat	Noncomparative, 26-week, repeat-dose toxicology	No ADA results reported
	Monkey	Noncomparative, 26-week, repeat-dose toxicology	No reduction in drug exposure between day 1 and day 42 in either species
	Rat	28-day comparative (versus Neupogen®) immunogenicity: 2 weeks of treatment followed by a 2-week treatment-free period, followed by a second 2-week treatment period; subcutaneous daily administration of three dose levels of each product (5, 25, and 125 µg/kg/day)	Median ADA titer at day 56 was higher (1.3–3.2-fold depending on dose level) for Neupogen compared with Ratiograstim at all three dose levels
Zarzio®	Rat	Comparative (versus Neupogen), repeat-dose toxicology; 28 days dosing + 42-day recovery; three dose levels of Zarzio (20, 100, or 500 µg/kg/day) versus two dose levels of Neupogen (20 or 500 µg/kg/day) versus placebo; daily subcutaneous administration	Based on results at day 70 (end-of-recovery), n=5 animals per group, signal levels in anti-G-CSF ELISA were indistinguishable between the respective treatment groups; specificity of signals uncertain because preincubation of serum samples in confirmatory step did not result in reduction of signal
	Rat	Comparative (versus Neupogen), repeat-dose TK; 14 days dosing; three dose levels of Zarzio (20, 100, or 500 µg/kg/day) versus two dose levels of Neupogen (20 or 500 µg/kg/day) versus placebo; daily subcutaneous administration	No ADA results reported No reduction in drug exposure between day 1 and day 13 for any of the treatment groups
Nivestim™	Rat	Comparative (versus Neupogen), repeat-dose toxicology with TK; 28-day, subcutaneous + 14-day recovery; three dose levels of each product, 20, 80, or 320 µg/kg/day versus vehicle	Low incidence of anti-filgrastim antibodies in both Nivestim and Neupogen-treated groups; no significant difference in neutralizing ADA between products Detection of neutralizing ADA associated with substantially reduced drug plasma concentration at day 28 (two animals treated with low-dose Nivestim and two animals treated with low-dose Neupogen)
Alpheon®	Rhesus monkeys	Comparative (versus Roferon-A®), repeat-dose toxicology, TK and immunogenicity evaluation; 4-week + 2-week recovery; dosing every other day; three animals per group; 6 or 12 million IU/kg/dose Alpheon versus 6 IU/kg/dose Roferon-A or vehicle	No conclusion was obtained
Ovaleap®	Rat	Comparative (versus Gonal-F®), repeat-dose toxicology + TK; 28-day, daily subcutaneous administration	Some differences reported in serum FSH levels at day 28, possibly related to higher anti-FSH antibody titers; not considered to be relevant for humans
	Rat	Noncomparative, repeat-dose toxicology; 14-day; 10, 50, or 100 IU/kg/day	In both species, a dose-related increase in incidence and titer of anti-rhFSH antibodies was observed
	Dog	Noncomparative, repeat-dose toxicology; 14-day	
Remsima®	Rat	Comparative (versus Remicade®), repeat-dose toxicology; 0, 10, or 40 mg/kg/dose intravenously, two doses, one week apart	No anti-infliximab antibodies detected
Insulin Marvel		No nonclinical studies of repeat-dose toxicology, TK, or immunogenicity were submitted. Major objections were raised by the agency in this respect.	

**Note:** Manufacturer details are as follows: Omnitrope®, Binocrit® and Zarzio® (Sandoz GmbH, Kundl, Austria); Valtropin® and Alpheon® (BioPartners GmbH, Reutlingen, Germany); Silapo® (Stada Arzneimittel AG, Bad Vilbel, Germany); Ratiograstim® (Ratiopharm GmbH, Ulm, Germany); Nivestim™ (Hospira UK Ltd., Royal Leamington Spa, United Kingdom); Remsima® (Celltrion Healthcare Hungary Kft, Budapest, Hungary); Ovaleap® (Teva Pharma B.V., Utrecht, the Netherlands); Eprex® (Janssen-Cilag BV, Tilburg, the Netherlands); Erypo® (Janssen-Cilag GmbH, Neuss, Germany).

**Abbreviations:** ADA, anti-drug antibody; TK, toxicokinetic; hGH, human growth hormone; rhEPO, recombinant human erythropoietin; FSH, follicle-stimulating hormone; C<sub>max</sub>, maximum drug concentration; AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; G-CSF, granulocyte-colony stimulating factor.

immunogenicity evaluations and the results reported in the various assessment reports available on the European Medicines Agency website.<sup>1</sup>

Table 4 indicates that the EU regulatory agencies applied a cautious approach in seeking to maximize the potential value of nonclinical data to inform the benefit-risk decision to proceed into clinical studies of these biosimilar candidates. Although some differences were detected in the apparent magnitude of the treatment-emergent antibody response to particular products, overall the results from the nonclinical immunogenicity evaluation were deemed not to have been instructive for the biosimilarity assessment: aside from the equivocal validity of extrapolating from an irrelevant immunological context to humans, imprecision associated with high biological variability and small group sizes makes data interpretation extremely difficult; in addition, differences in ADA assay sensitivity preclude a predefinition of meaningful differences based on historical data for the product class.

Bearing in mind the limitations of these studies with regard to providing a reliably instructive index of relative immunogenicity for the biosimilarity assessment, and building on this initial experience, the more recently issued EU regulatory guidelines reflect a transition from an “obligatory” to an “as needed” approach. Thus, both the draft revision to the overarching guidance on nonclinical and clinical considerations for biosimilar products<sup>2</sup> and the product-specific guidance on biosimilar monoclonal antibodies<sup>13</sup> recommend: “blood samples should be taken and stored for future evaluations if then needed.” This less extensive approach, if confirmed in the final effective version of the revised overarching guideline, would then supersede the recommendations in the earlier product-specific guidelines, enabling “as needed” ADA evaluation.

The trigger for performing ADA evaluation of samples collected from nonclinical *in vivo* studies of biosimilar candidates is not specifically described in the draft revision to the overarching guideline. However, it would be logical to cross-refer to the recommendations given in the International Conference on Harmonisation S6R1 guideline,<sup>28</sup> whereby ADA testing samples from comparative (biosimilar versus reference product) *in vivo* nonclinical studies should be triggered in three situations: differences in pharmacodynamic activity between the biosimilar and reference groups; increase or decrease in exposure in the absence of a pharmacodynamic marker; or increase in incidence or severity of immune-mediated reactions. Accordingly, a difference in area under the curve for the biosimilar candidate relative to the reference product could be a trigger for ADA testing if there were no

pharmacodynamic marker available to assess impact. If a nonclinical immunogenicity evaluation were performed, the primary purpose would be to enable a valid interpretation of the nonclinical findings, rather than to predict immunogenic potential in humans. The clinical immunogenicity data, not the nonclinical data, will always be the most relevant arbiter for the biosimilarity assessment regarding the decision to grant approval for marketing.

## Supportive preclinical evidence for risk mitigation of identified differences

Although not an obligatory or standard part of the biosimilarity exercise, regulatory guidance<sup>29</sup> continues to acknowledge the potential of nonclinical data to provide, in the case of molecules having substantial primary amino sequence homology between species, supportive data to detect differences in immunogenic potential associated with identified differences in product-related factors. This reflects the availability of data from comparative studies performed in wild-type animals<sup>30,31</sup> and transgenic animal models<sup>32–34</sup> to detect differences in the ADA response between structurally related molecules. Accordingly, it would be unwise to dismiss a role for comparative *in vivo* immunogenicity evaluation, eg, as part of the preclinical risk minimization exercise for a biosimilar candidate formulated differently to the reference product. Such studies have been used to contribute supportive data to justify suitability of a new formulation of human interferon-beta.<sup>35,36</sup>

Likewise, in principle, there might be a potential role for *in vitro* technologies to contribute to the immunogenicity risk mitigation exercise for a biosimilar development program, in the case that there are identified differences in product properties that have a potential or an uncertain impact on treatment-emergent immune responses in humans. In this respect, *in vitro* evaluation of stimulation of CD86 expression on human dendritic cells was useful in moderating the potential risk associated with degradation products of polysorbate in a formulation of recombinant human erythropoietin.<sup>37</sup> In addition, a combination of *in vitro* and *ex vivo* analyses was used to assess the potential of aggregates of a human monoclonal antibody to influence the interaction between innate and adaptive immune responses.<sup>38</sup>

Presently, there is a lack of published data to demonstrate the discriminatory sensitivity of comparative measures of endocytosis, antigen presentation, and CD4<sup>+</sup> T-helper cell stimulation for biosimilar candidates that meet the EU standard of structural comparability, ie, 100% identical primary amino acid sequence and a highly similar post-translational

modification profile. While differences in post-translational glycosylation could affect uptake by and endocytotic processing in antigen-presenting cells,<sup>39,40</sup> bioinformatics approaches suggest a rather low level (less than 4%) of glycosylation of T-cell epitopes in general.<sup>41</sup> The latter could explain the limited sensitivity of in vitro major histocompatibility complex binding and T-cell activation assays to reveal differences between highly glycosylated factor VIII variants.<sup>42</sup>

Overall, it is still unclear whether in vitro immunogenicity risk mitigation methods could contribute incremental value for early screening of biosimilar candidates, ie, to detect differences that are not within the sensitivity range of state-of-the-art physicochemical methods. Nevertheless, it could be interesting to compare the relative in vitro activation of CD4<sup>+</sup> T-cells in response to biosimilar candidates versus the reference product using blood from subjects who have been previously treated with the reference product, since this would be expected to increase sensitivity to detect differences.

## Bioanalytical considerations

### Assay sensitivity versus clinical relevance

EU regulatory practice has been to review the bioanalytical signals detected in comparative clinical studies of biosimilar candidates versus reference products relative to clinically relevant endpoints to assess whether a detected difference in the incidence or magnitude of ADA could have a negative influence on overall clinical benefit and risk. The evidence is discussed in more detail below.

From the perspective of the design of the bioanalytical strategy, the implication is that the methodology to detect ADA should be sufficiently sensitive, taking into account confounding factors such as interference by residual drug, which can be a particularly influential factor for therapeutic monoclonal antibodies,<sup>43</sup> to detect ADA that could have a negative clinical impact. Since there may be limited historical data to guide the decision, the most pragmatic solution would be to apply methods that are least confounded by drug interference and to correlate ADA assay results with clinically relevant endpoints.

This conundrum is best illustrated by the work of van Schouwenberg et al,<sup>44</sup> who applied alternative ADA assay methods to measure treatment-emergent ADA to adalimumab. Although the two methods had widely differing sensitivities, the additional sensitivity to detect ADA gained by the pH-shift anti-idiotypic method compared with the antigen-binding test did not reveal an incremental negative impact on the measured drug trough concentration. This is

explained by the apparent need to induce relatively high levels of ADA in order to reduce the circulating level of functional adalimumab to an extent that has an impact on the therapeutic response. This observation underlines the importance of interpreting measures of ADA in relation to relevant clinical parameters.

### Hierarchical testing scheme

Samples from clinical studies should be evaluated in a blinded manner using a hierarchical testing scheme, following the recommendations applicable in the regulatory guidance for biopharmaceutical products.<sup>29,45</sup> Thus, positive samples detected in the screening assay should be subjected to a confirmatory test in the presence of an excess of competing antigen (unlabeled biosimilar candidate versus unlabeled reference product). Samples confirmed as positive should then be further qualified for antibody titer and neutralizing capacity.

Testing for ADA of immunoglobulin (Ig)E class is necessary only where there are clinical observations of potential immunoglobulin IgE-mediated hypersensitivity in clinical subjects.<sup>46</sup> Ideally, the screening assay(s) should be designed to detect IgG and IgM class antibodies. Testing for antibodies reactive with host cell proteins is not usually required, but might be merited for proteins manufactured using an *E. coli* cell substrate.

### Screening and confirmatory assay cut-points

Assay cut-points should be established using samples from treatment-naïve subjects, according to recommendations for ADA testing for biopharmaceutical products.<sup>29,45</sup> In the author's experience, application of a confirmatory cut-point with a false-positive rate of 0.1% was effective in classifying a number of "gray zone" signals as confirmed negatives for different product classes associated with a relatively low incidence of treatment-emergent ADA.

### Choice of assay format

The sponsor has the flexibility to select from a wide range of different assay formats, and then to validate the suitability of the methodology for the intended purpose, ie, to detect ADA that could impact on clinically relevant parameters. Review of the assessment reports for the biosimilar products reviewed to date in the EU indicates the diversity of methods applied (summarized in Table 5).

For a PEGylated therapeutic protein, it is important to compare the sensitivity of different assay formats, particularly

**Table 5** Anti-drug antibody assay formats reported in assessment reports for biosimilar product candidates reviewed in the European Union\*

Product	ADA assay formats
Omnitrope®	Anti-GH antibodies: RIA Anti-HCP: Western blot
Valtropin®	Not specified for either the anti-GH or anti-HCP assays
Binocrit®	Anti-EPO binding: RIP Anti-EPO neutralizing antibodies: format not specified
Silapo®	Anti-EPO binding: RIP Anti-EPO neutralizing antibodies: inhibition of EPO-induced stimulation of an erythroleukemic cell line in vitro
Ratiograstim®	Anti-G-CSF IgG ELISA Anti-G-CSF IgG-IgM Luminex assay IgG and IgM-specific Western blot confirmation assays Neutralizing anti-G-CSF antibodies using G-CSF-dependent NFS-60 cell-based assay Total binding antibodies by surface plasmon resonance
Zarzio®	Anti-G-CSF antibody: RIP (clinical samples) Anti-G-CSF antibody: ELISA (nonclinical samples)
Nivestim®	Anti-G-CSF antibody: assay format not specified In vitro cell-based assay for neutralizing antibodies
Alpheon®	Anti-interferon-alfa antibodies: commercially sourced ELISA kit (double antigen ELISA)
Ovaleap®	Anti-FSH antibodies: ECL bridging assay (clinical samples); included specificity test for anti-Neu5Gc antibodies Anti-FSH antibodies: ELISA (nonclinical samples)
Remsima®	Anti-infliximab total antibodies: ECL assay (clinical samples) Neutralizing anti-infliximab antibodies: competitive ligand binding assay Anti-infliximab antibodies: ELISA (nonclinical samples)
Insulin Marvel	Not specified

**Notes:** \*Based on European public assessment reports.† Manufacturer details are as follows: Omnitrope®, Binocrit® and Zarzio® (Sandoz GmbH, Kundl, Austria); Valtropin® and Alpheon® (BioPartners GmbH, Reutlingen, Germany); Silapo® (Stada Arzneimittel AG, Bad Vilbel, Germany); Ratiograstim® (Ratiopharm GmbH, Ulm, Germany); Nivestim™ (Hospira UK Ltd., Royal Leamington Spa, United Kingdom); Remsima® (Celltrion Healthcare Hungary Kft, Budapest, Hungary); Ovaleap® (Teva Pharma B.V., Utrecht, the Netherlands).

**Abbreviations:** GH, growth hormone; RIA, radioimmunoassay; HCP, host cell protein; EPO, erythropoietin; RIP, radioimmunoprecipitation; G-CSF, granulocyte colony stimulating factor; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; ECL, electrochemiluminescence; Ig, immunoglobulin; Neu5Gc, N-glycolyl neuraminic acid.

direct binding versus bridging assay formats, because the relatively large hydrodynamic volume of the poly(ethylene glycol) moiety may alter antigen labeling efficiency and/or capacity for bivalent binding of the ADA (author's observations).

Generally, sponsors should develop a neutralizing assay format to provide an additional level of analysis of all confirmed positive samples detected in the screening assay. Depending on the product, this may be an in vitro cell-based assay or a competitive ligand-binding assay. Thus, in the case of Remsima, a competitive ligand-binding assay was used, reflecting the direct blocking effect of anti-infliximab

antibodies on binding of the target antigen by infliximab. On the other hand, cell-based assays were used for measuring the neutralizing capacity of anti-erythropoietin or anti-filgrastim antibodies.

## One versus two screening assays

The draft revision to the overarching guideline on nonclinical and clinical considerations for biosimilar products<sup>2</sup> recommends that: "Assays should be performed with both the reference and biosimilar molecule in parallel (in a blinded fashion) to measure the immune response against the product that was received by each patient."

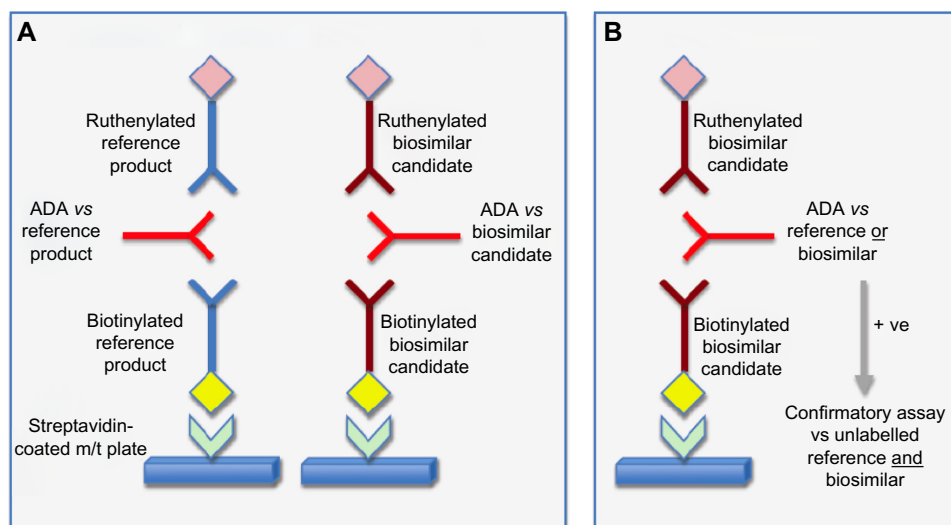
However, even for a highly complex and relatively immunogenic molecule such as infliximab, duplicative testing in parallel assays (one assay using labeled versions of the biosimilar candidate and the other assay using labeled versions of the reference product) did not reveal a difference.<sup>19</sup> Moreover, the risk that labeling of the respective test products could modify antigenicity to an unpredictable extent<sup>47</sup> implies a higher burden for reagent quality control. The use of separate assays/reagents might be expected to decrease assay precision; gain in specificity by using separate assays is equivocal, given the possibility that biotinylation and/or ruthenylation could alter affinity for binding to epitopes proximal to labeling sites.

Therefore, to minimize any bioanalytical bias associated with assays that use different labeled antigens, it would be preferable to use a single assay; this should measure binding of ADAs to the candidate biosimilar in the screening assay, with a test of inhibition of the binding in the confirmatory step using both the unlabeled candidate biosimilar and reference products as competing antigens. The operator should be blinded to the identity of clinical samples. This approach should detect ADAs that recognize novel epitopes on the candidate biosimilar, whilst avoiding the additional level of assay variability associated with use of two different assays/labeled antigens. The alternative approaches are illustrated schematically in Figure 1.

In the case of PEGylated proteins, it would still be acceptable to use a single screening assay, allied to confirmatory testing of signal specificity with the PEGylated and non-PEGylated versions to distinguish between antibodies reactive with the respective [protein versus PEGylated protein versus poly(ethylene glycol)] moieties. The same approach would be applicable in the case of fusion proteins.

## Positive control

Based on the author's experience gained during the EU regulatory approval of the biosimilar candidates reviewed to date, it



**Figure 1** Comparison of one-assay versus two separate screening and confirmatory assays.

**Notes:** (A) In the two-assay approach, each clinical sample is tested in a blinded manner using two parallel assays, one to detect binding to labeled versions of the biosimilar candidate and the other using labeled versions of the reference product. The specificity of any detected positive signals would then be confirmed by adding the unlabeled antigen (biosimilar candidate or reference product) to compete with ADA for binding to the labeled antigens. (B) In the single-assay approach, samples are tested only for binding to the labeled biosimilar candidate, followed by a confirmatory assay step using addition of unlabeled biosimilar or reference product as competing antigens to displace the binding of ADA.

**Abbreviations:** ADA, anti-drug antibody; m/t, microtiter; vs, versus; +ve, positive.

is not essential for each sponsor to prepare in-house positive control reagents using either the biosimilar candidate or the reference product as an immunogen. A commercially sourced antibody reagent specific for the given protein is perfectly acceptable, if qualified appropriately. This reflects the role of the positive control antibody to provide a benchmark of relative assay sensitivity in a nonquantitative assay to detect a heterogeneous population of ADA, rather than as a calibrator for accurate measurement of the same molecule present in a test sample.

Suitability of a candidate positive control reagent should be demonstrated by confirming equivalent reactivity in a ligand-binding assay with the biosimilar and reference products: this could be achieved by titrating the positive control antibody signal in the screening assay format with increasing amounts of competing antigen (biosimilar versus reference product); the inhibition curves for the biosimilar and reference product competing antigens should be essentially overlapping. If not, the reason for the apparent difference in antigenicity would require further investigation.

## Drug interference

The drug tolerance limit should be validated using the selected positive control antibody reagent according to the recommendations given in United States Pharmacopeia Chapter 1106.<sup>45</sup> Drug interference is a particularly important limiting factor for detection of ADAs to monoclonal antibodies.<sup>43</sup> Inclusion of acid-dissociation and partitioning steps provides an opportunity to overcome this interference.<sup>48</sup>

## Baseline positives

In the case of some products, eg, insulin, it may not be feasible to recruit treatment-naïve subjects for comparative clinical studies of biosimilarity. Since a relatively high proportion (up to 80%) of subjects develop antibodies to insulin,<sup>49</sup> it is preferable to establish assay cut-points using samples from treatment-naïve subjects, to avoid a confounding influence of earlier treatment.

## Relationship to historical data

In the author's experience, application of state-of-the-art bioanalytical methods has often revealed a substantially higher incidence of ADA compared with the results reported for the originator products. This situation is analogous to the markedly different results obtained using ADA assays of differing sensitivities for detecting anti-adalimumab antibodies in clinical samples.<sup>50</sup>

Therefore, historical data obtained using different ADA assays are not relevant for comparative purposes, or for setting predefined margins for acceptable differences in ADA incidence or median titer within the statistical analysis plan for bioequivalence or therapeutic equivalence studies of a biosimilar product versus a reference product.

## Reporting of results

ADA (neutralizing and non-neutralizing) results for respective treatment groups at each sample time point should be reported in terms of percent confirmed ADA positives/con-



firmed ADA positive + confirmed ADA negatives and median antibody titer. These data outputs should then be correlated with relevant clinical endpoints.

## Risk management plan

The immunogenicity-related provisions associated with the EU approvals of biosimilar human growth hormone, erythropoietin, and filgrastim have been clearly summarized in a separate review.<sup>51</sup> Therefore, only the provisions relating to the more recently approved biosimilar infliximab, Remsima, are summarized in Figure 2.

Items 3 and 4 are the most significant items on this list in terms of potential for providing data on immunogenicity in a different population, ie, Crohn's disease, and on the potential influence of immunogenicity on longer-term treatment outcomes in the rheumatoid arthritis population. Although no specific reference is given to immunogenicity monitoring, it would seem implausible for these studies to omit monitoring of ADA and drug levels relative to efficacy and safety indices.

## Limitations of post-marketing data

The Achilles heel for the argument that there is no higher immunogenicity-related risk for biosimilar products approved according to the EU regulatory standards is the limited availability of directly comparative post-marketing data from monitoring of ADA levels relative to clinically relevant parameters of efficacy and safety.

Even if preauthorization studies were sufficiently sensitive and of adequate duration to detect clinically relevant differences in immunogenicity that might manifest in the longer term, the regulatory process does not impose an

obligation on biosimilar product manufacturers to reconfirm equivalent efficacy, safety, and immunogenicity by direct comparison with the reference product to support implementation of changes in the post-marketing lifecycle. In this respect, it is important to remember that manufacturing changes, changes in the formulation or primary container, or even new therapeutic indications, could be authorized in the post-marketing phase for either the reference or biosimilar products. Authorization of such changes would be based on a demonstration of comparability of the pre-change versus post-change material,<sup>10,52</sup> ie, a direct comparison of the post-change biosimilar versus the reference product (or other biosimilar products) is not mandatory. Theoretically, this creates a risk for manufacturing drift to manifest as changes in clinically relevant immunogenicity.

The absence of standardized ADA assays, or common positive control antibody reagents to benchmark relative assay sensitivity, precludes reliance on noncomparative clinical data to monitor the longer-term immunogenic potential of the different product versions.

One potential solution would be for patient registries to include ADA monitoring, using a standardized assay, for both reference and biosimilar products. This might require prospectively planned, interventional, observational cohort studies, rather than noninterventional studies, if additional blood sampling were required above the level recognized as normal clinical practice. Measurement of drug levels and recording of potential immune-mediated adverse events (eg, infusion reactions) should also be included.

## Immunogenicity risk associated with switching medications

It is evident from Table 3 that the preauthorization clinical experience for different biosimilar candidates included open-label extension periods in which patients who had been treated with the reference product were then switched to receive treatment with the biosimilar candidate. Immunogenicity was monitored during open-label periods, thereby providing some evidence of the lack of potential for induction of ADA by the biosimilar candidate in the same patients who had been treated earlier with the reference product.

As discussed in a separate review,<sup>53</sup> there are limited additional data from other studies that have evaluated switching from the reference product to the biosimilar product and, less commonly, from the biosimilar to the reference product.

In the author's experience, scientific advice from the US Food and Drug Administration has encouraged the design of pivotal studies for certain biosimilar candidates to include

1. Completion of reporting for follow-up of AS and RA patients from phase 1 and phase 3 pre-registration studies
2. Reporting of data from Japanese comparative study in RA
3. Phase 1/3 RCT in Crohn's disease: PK/efficacy/safety
4. Registry: observational cohort/RA/safety and efficacy
  - n=>3,000 subjects (final report March 2026)
5. Registry: Crohn's/UC
6. PMS, German RA cohort
7. Risk minimisation:
  - Routine = labelling
  - Additional = patient alert card (serious infections, CHF etc)
  - Additional = educational material for HCP's (eg, serum sickness)

**Figure 2** Immunogenicity-related provisions in the European Union's risk management plan for Remsima® (Celltrion Healthcare Hungary Kft, Budapest, Hungary).

**Note:** Data from European public assessment reports.<sup>19</sup>

**Abbreviations:** AS, ankylosing spondylitis; RA, rheumatoid arthritis; RCT, randomized controlled trial; PK, pharmacokinetics; UC, ulcerative colitis; PMS, post-marketing surveillance; CHF, congestive heart failure; HCP, health care provider.

re-randomization at the end of the controlled double-blind study period to continue subjects on either the biosimilar candidate or the reference product during an open-label extension period that also includes immunogenicity monitoring. This strategy would provide comparative data for a single switch in either direction.

However, one issue that is not addressed is the longer-term impact of switching between multiple products. Since there could be important ethical and feasibility challenges for designing a multiple-switching study, it would again impose upon the patient registry/observational cohort scenario to assume the role for longer-term monitoring of treatment outcomes using relevant measures, including ADA formation, maintenance of drug levels and efficacy, and the incidence/severity of immune-mediated adverse events.

## Interchangeability and substitution

Since the EU regulatory decision to grant a marketing authorization for a biosimilar product has no implication for the substitutability of medicines at the pharmacy level, which is the jurisdiction of individual member states, there are no additional regulatory provisions in the EU governing “interchangeability”.

On the other hand, it is conceivable that national decisions on pricing and reimbursement or product selection, and/or acceptance by clinicians, could be influenced by the relative immunogenicity of the authorized products. Although it is too early to comment on how actual or perceived differences in immunogenicity might play out in the longer term, there would seem to be an essential role for post-marketing immunogenicity data that are comparable in nature, ie, using standardized methodology for monitoring patient populations exposed to different product versions.

Such data might provide objective information regarding the potential for manufacturing drift to modify relative immunogenicity of different product versions. Possibly, organizations such as the World Health Organization could provide standardized assays and reagents for ADA assays, and the risk management plans for approved biosimilars would be linked to appropriate patient registries/observational cohort studies that include different product versions.

Arguably, an open-ended designation of “interchangeability” (in a regulatory jurisdiction such as the USA where this implied “substitutability”) would carry an unsustainably heavy burden to confirm continuing biosimilar status,<sup>54</sup> including no difference in clinically relevant immunogenicity

following implementation of changes (eg, manufacturing process, formulation, primary container). It would then be logical to consider “switchability”, guided by the appropriately informed judgment of the prescribing physician, as the major factor to address.

## Lessons learnt

To date, the only immunogenicity-related issues that have been identified for the biosimilar candidates reviewed in the EU registration process have all occurred in the preapproval setting (summarized in Boxes 1, 2, 4, and 5). A common theme across the four cited examples was the detection of differences in product quality attributes that might have contributed to a heightened risk of clinically relevant immunogenicity.

In the case of growth hormone, an elevated level of a process-related impurity that was not detected by the original analytical method was associated with an increased level of treatment-emergent antibodies in clinical samples from patients treated with an investigational formulation; process improvements implemented in the pre-authorization setting resolved this issue, such that the authorized product (Omnitrope<sup>®</sup>) had an immunogenicity profile that was indistinguishable from that of the reference product.<sup>15</sup>

In the case of erythropoietin, use of prefilled syringes containing a tungsten impurity is the most likely explanation for the detection of neutralizing ADA in two subjects with chronic kidney disease receiving subcutaneous administration of Binocrit<sup>®</sup>.<sup>7</sup>

Differences in product-related substances and/or process-related impurities between other biosimilar candidates (Alpheon<sup>®</sup> and the insulins marketed by Marvel LifeSciences Ltd) and their respective reference products were cited as reasons for noncomparability.<sup>18,19</sup> Directly related to these differences in product quality attributes, inadequate validation of the specificity and sensitivity of ADA assays allied to clinical signals of reduced efficacy and/or increased incidence/severity of immune-mediated adverse events, contributed to the failure of these candidates to achieve designation as biosimilar.

Comparative immunogenicity evaluation in nonclinical toxicology studies was not instructive for the biosimilarity decision to enable marketing authorization. Nevertheless, non-clinical studies might provide helpful data to justify decisions on formulation of biosimilar candidates, or for investigation of unexpected findings arising from changes in the manufacturing process or in primary container or storage conditions.

Overall, based on the experience gained during a 10-year period since implementation of the EU regulatory pathway for biosimilar medicinal products, the regulatory standard for approval has been validated as suitably cautious by the absence of observed differences in clinically relevant immunogenicity between the approved biosimilar and originator products following authorization. Although, as in the case of registration of any medicinal product, some degree of uncertainty has been accepted at the time of marketing authorization, a combination of post-marketing data and a heightened level of pharmacovigilance has not revealed any incremental immunogenicity-related risks in respect of the authorized use of biosimilars in the EU.

## Open questions

### How to optimize post-marketing monitoring to detect longer-term differences in clinically relevant immunogenicity?

The absence of standardized assays and control reagents, allied to the lack of correlation of results of ADA assays to clinically relevant measures of efficacy and safety, is a major impediment to our understanding of whether there is any unrecognized incremental risk of immunogenicity for biosimilar products that have been approved to the EU regulatory standard.

As a result, the clinical uptake of EU-approved biosimilar products could be compromised by an unfounded perception that immunogenicity-related risks had not been adequately evaluated or controlled.

One solution could be for patient registries comprising patients treated with different product versions to incorporate longer-term periodic monitoring of clinically relevant parameters. This might still not be a feasible solution for detection of very rare events such as erythropoietin-induced antibody-mediated pure red cell aplasia, but could yield an objective basis for understanding whether there is a potential for post-approval manufacturing drift to affect other immunogenicity-influenced endpoints, including drug concentration, efficacy, and tolerability.

### Is there a negative impact of switching between different product versions?

Inclusion of two-way switching, eg, by re-randomization of patients treated in the blinded treatment phase of a Phase III study, could provide some assurance that switching between the biosimilar and reference versions is not associated with

clinically relevant differences in immunogenicity. Duration of exposure to the different product versions would need to reflect the dynamics of the treatment-emergent immune response in addition to effective washout of the effects of the previous treatment.

On the other hand, it would not be feasible to compare switching between different biosimilar product versions in preapproval studies. More realistically, adequate monitoring of patients treated with different product versions within a patient registry or observational cohort study, with access to a centralized laboratory able to apply standardized assays, could represent the most practical option for comparing longer-term outcomes associated with elective switching between the respective versions of the product.

### What is an acceptable threshold for differences?

The lack of standardized bioanalytical assays or control reagents for ADA assays will continue to impose a waste of resources, because results using different assays can never be validly compared. For the regulator, the substantially different results obtained by assays with lower or higher sensitivity forces a reliance on clinical evidence of lack of impact of differences detected (or not detected) in bioanalytical assays. Most importantly, it means that it is rarely possible to interpret the clinical relevance of bioanalytical indices of ADA formation; equally, it is not possible to define an equivalence margin for incidence or titer of ADA formation that is relevant for designation of biosimilarity.

Even more challenging for the physician is the questionable validity of reporting arbitrary measures of ADA formation in the prescribing information of the reference and the biosimilar products – what should count is the comparative impact on clinically relevant parameters, such as drug concentration, efficacy and tolerability.

## Disclosure

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