Contribution of animal studies to evaluate the similarity of biosimilars to reference products

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The European Union (EU) was the first region to establish a regulatory framework for biosimilars, in which animal studies are required to confirm similarity to a reference product. However, animal studies described in European public assessment reports (EPARs) or marketing authorization applications (MAAs) did not identify clinically or toxicologically relevant differences despite differences in quality, suggesting that animal studies lack the sensitivity to confirm biosimilarity. Scientific advice provided learning opportunities to evolve existing guidance. Altogether, the data support a step-wise approach to develop biosimilars that focuses on quality and clinical efficacy of biosimilar. This approach might be more effective and does not necessarily require animal studies, which is also reflected in new EU draft guidance.

Introduction

When small-molecule drugs go off-patent, generic copies are allowed on the market after these copies are shown to be pharmaceutically equivalent to the originator product and their bioavailabilities lie within acceptable predefined limits. For small molecules, pharmaceutical equivalence is established through physicochemical characterization and demonstration of comparable bioavailability, usually in randomized, crossover studies in a limited number of subjects, without the need for animal studies [1]. Medicinal products of recombinant biotechnology are relatively large and complex proteins that are difficult to characterize fully [2]. In addition, biopharmaceuticals are mixtures of closely related molecules, the exact composition of which is dependent on the manufacturing process for the product, resulting in differences in, for example, protein aggregation and glycosylation patterns [3,4]. These differences can influence the pharmacodynamics (PD), pharmacokinetics (PK), or toxicity parameters of the drug [5–8]. Therefore, tailored regulatory requirements have been developed for the authorization of competing versions of biopharmaceuticals, so-called 'biosimilars'. Compared with small-molecule drugs, more studies are required to establish the similarity of the biosimilar in terms of quality, safety, and efficacy, compared with a reference product to obtain marketing authorization [9].

The EU was the first region to adopt legislation that allows the registration of biosimilars based on an abbreviated marketing authorization application (MAA) [10]. Overarching guidelines have been released that lay down quality, nonclinical, and clinical issues for biosimilars [11–13]. In addition, product-specific guidelines have been released [14–22]. In the current overarching guideline on nonclinical and clinical issues of biosimilars, emphasis is placed on performing comparative nonclinical studies that are sensitive enough to detect differences between the biosimilar and the reference product. This case-by-case approach limits the number of animal studies needed compared with a full application (Table 1) [12]. In June 2013, draft guidances relating to the revised biosimilar guidelines were released for consultation in which a risk-based, step-wise approach is suggested, opting for fewer or perhaps even no animal studies [23,24]. However, it remains unclear what the contribution is of animal studies in establishing biosimilarity and how biosimilar guidance has influenced nonclinical studies. Therefore,

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TABLE 1

Animal studies recommended to demonstrate biosimilarity in the current overarching guideline on non-clinical and clinical issues[a]

Type of study	Type of investigation	Necessity of study
PD	PD effect or activity relevant to clinical application	Recommended
Repeat-dose toxicity	Toxicity profile including toxicokinetics and determination of antibody titers, cross-reactivity, and neutralizing capacity	Recommended
Specific concerns	If relevant (e.g. local tolerance), addressed in the same repeat-dose toxicity	Recommended
Routine toxicological studies	Safety pharmacology, reproduction toxicology, mutagenicity, and carcinogenicity	Not recommended unless indicated by results of repeat-dose studies

[a] From [12].

we assessed nonclinical animal study programs of the MAA of all biosimilar products registered in the EU or submitted for authorization until 1 October 2013.

Review inclusion criteria and analysis

EPARs of all biosimilars up to 1 October 2013 were obtained from the website of the European Medicines Agency (EMA, http://www.ema.europa.eu). Data from the EPARs were supplemented with data from MAAs and scientific advice for registered biosimilar products. MAAs and scientific advice were obtained from the database of the Medicines Evaluation Board (CBG-MEB, Utrecht, The Netherlands). Nonclinical studies were tabulated and categorized by type of study and inclusion of a reference group. Categories for study type were ordered by studies recommended in the biosimilar guideline, including PD, repeat-dose toxicity, and local tolerance or studies that were not recommended in the biosimilar guidelines, such as single-dose toxicity, safety pharmacology, repeat-dose PK or toxicokinetics, developmental and reproductive toxicity studies, including embryo–fetal development and peri- and postnatal development, immunogenicity studies, and special toxicity studies. Only scientific advice pertaining to the nonclinical development or animal studies was included. The manufacturer's questions and the responses of the Committee for Medicinal Products for Human Use (CHMP) were first filtered for nonclinical questions. Nonclinical questions were categorized as either pertaining to the sufficiency of the nonclinical program, its design, or other regulatory requirements. The answers of the company were compared with the response of the scientific advice working party.

Overview of biosimilar applications

Seventeen biosimilars were registered in the EU up to 1 October 2013. With the exception of Remsima™ and Inflectra™, these were all copies of endogenous proteins. Several biosimilars are registered under different trade names and, effectively, nine biosimilar applications have been developed (Table 2). Alpheon® (interferon α-2a) was refused market entry and the MAA for Insulin Human Rapid Marvel® (insulin) was withdrawn by the manufacturer [25]. The manufacturer of Epostim® (epoetin) withdrew its biosimilar application following a request from CHMP for additional data [26]. Filgrastim Ratiopharm® (filgrastim) and Valtropin® (somatropin) received marketing authorization, but were withdrawn from the market for commercial reasons in April 2011 and May 2012, respectively. Neither of these products was marketed in any EU country [27,28].

The submission of the MAA of XM-02 was intended as a full application, but was registered in the EU as a biosimilar. For the MAA of LBD-009, the manufacturer referred to the biosimilar guideline for the development of their product [29]. However, the submitted nonclinical package was a full application containing a safety pharmacology study in nonhuman primates, developmental and reproductive toxicity studies, single- and repeat-dose PK and toxicity studies (in a rodent and a nonrodent animal model), and genotoxicity and antigenicity studies. Extensive direct comparisons between Valtropin and its reference product were not done on the basis of comparable pharmacological activity in parallel PD studies. LBD-009 and XM-02 were registered in the USA via a new drug application, which could explain the full package of nonclinical studies. Valtropin was also marketed in Korea. Omnitrope® (somatropin) was registered in the USA via a biological license application. In a pre-investigational new drug (IND) meeting with the US Food and Drug Administration (FDA), an abbreviated *in vivo* package was justified, which was also submitted to support approval for this drug as a biosimilar in the EU. Infliximab and insulin glargine have both been submitted as a biosimilar in the USA [30,31].

In vitro comparability

Most study programs included both *in vivo* and *in vitro* characterization of the biosimilar to support marketing authorization. In addition to the thorough physicochemical characterization of the biosimilar protein required to evaluate the quality of the product, *in vitro* receptor-binding assays and biological activity assays were performed for HX-575, SB309, EP2006, PLD-108, XM-02, and CT-P13 (Table 3). Similarity of the biosimilar to the reference product of both receptor-binding affinity and *in vitro* potency measured by various assays was established for all these products. For the development of the biosimilar CT-P13, extensive *in vitro* testing to establish PD biosimilarity was done exclusively *in vitro*.

Use of animals

In total, 7590 animals were used in 72 studies for nine distinct nonclinical biosimilarity exercises, including studies required according to the European or United States Pharmacopoeia monographs. Pharmacopoeial assays to determine potency or PD were often extensive, including multiple batches over multiple dose ranges and controls or reference standards requiring the use of a large amount of animals. A median of four (interquartile range, IQR 4–9) animal studies was done to support demonstration of