Nonclinical aspects of biosimilar development – practical considerations from an industry perspective

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Abstract
Current regulatory thinking, notably in guidance issued in Europe, indicates that some nonclinical testing is necessary as part of the evaluation to show comparability for a biosimilar drug with the innovator product (see related articles: ‘Approaches to estimating starting doses in first-in-human clinical trials in the UK’, Regulatory Rapporteur, October 2011 and also ‘Review of the new EMA draft guideline setting the Quality requirements for biotechnological IMPDs’, Regulatory Rapporteur, December 2011.)
Examination of publicly available data for the more than a dozen biosimilars approved in Europe showed that nonclinical testing was indeed performed. This paper will discuss the process of determining what testing is needed for new biosimilars in development and practical considerations when performing this work. Considerations include the relevant biological and pharmacological studies and, for toxicolgy testing, the relevant species of use, study design and comparator requirements, all with the aim of showing comparability but with the avoidance of unnecessary animal use in mind.

Introduction
With many blockbusting biological drugs now, or soon to be, off patent there has been great interest in developing follow-on versions, generally termed biosimilars. Indeed, since the initial marketing approval of a biosimilar form of the recombinant human growth somatropin (Omnitrope) in Europe in 2006, there have been 13 further products approved in that region. However, this process is not as straightforward as developing a generic version of a new chemical entity. Indeed in Europe, one regulatory application (for interferon-alpha) has been rejected and three applications (for insulins) have been withdrawn by the sponsoring company. Biological drugs tend to be produced using living production systems (cell culture or fermentation) and this leads to small differences in the drug substance. The formulation of a biological drug can also lead to differences between the innovator and the biosimilar compound. These changes may be clinically relevant, leading to altered pharmacokinetic and/or pharmacodynamic characteristics of the biosimilar drug. Differences may thus occur at the level of the drug substance and/or drug product which leads to the requirement for physicochemical characterisation as well as biological activity characterisation in comparison to the named innovator product. It is likely that two biosimilars of a particular biological drug will also be slightly different from each other, thus there will not be one standard approach to development. Each biosimilar developer will have to critically assess the information generated during the comparable characterisation of its compound and tailor its development plans accordingly. Overall, there are certain critical considerations that need to be evaluated in formulating development plans which are highlighted below, with these considerations put into action as shown by two case studies, one with a recombinant protein and one with a monoclonal antibody (mAb).

Considerations
What regulatory help is available? This is always the first consideration and in Europe there are several biosimilar guidelines (see Table 1). Some guidance documents are general in nature but there are also those dealing with specific product classes.

What is meant by nonclinical data? Nonclinical data refers to comparative in vitro pharmacology work and in vivo studies comprising efficacy testing, pharmacokinetic (PK) assessment and toxicology studies including toxicokinetics (TK), anti-drug antibody (ADA) and local tolerance assessment. Biological characterisation studies which usually form part of the Chemistry Manufacturing and Controls (CMC), or drug quality package, are also included as part of the nonclinical safety data package.

What nonclinical data are available for the innovator drug? Information in regulatory approval documentation is frequently the main source of such data for the innovator drug, such as European Public Assessment Reports (EPARs) in Europe (see Table 2). (Note: For all the biosimilars examined in EPARs, it was concluded that no remarkable/important differences were seen in pharmacological activity, PK behaviour or toxicological/local tolerance profile in comparison with the innovator drug.) Other sources include published literature.

How good are the data? Older biological drugs have been around for at least 25 years but available data can be limited, or not detailed enough to allow identical comparison of study endpoints and thus need to be used as more of a guide. Cell based assays may have been used originally when other methods such as Biacore can now be used for binding information, and there may be little or no information on TK and/or ADA assessment. Specific animal models may have been used that are not commercially available and thus the biosimilar developer may have to consider utilising its own model, which can be a time-consuming process.

Is it generally difficult to develop and market a compound as a biosimilar? Biosimilar versions of only three substances (somatropin, epoetin and filgrastim) have been approved in Europe to date (see Table 2), suggesting developing biosimilars in other product classes may be more difficult.

With the above general points in mind, two case studies are presented showing some of the nonclinical considerations (and associated quality and clinical ones) for developing a biosimilar in Europe. The first is a recombinant protein and the second a mAb. In both cases, regulatory agency interaction occurred to support the considerations made below.
Case study 1 – Recombinant protein

The first consideration here was the presence of regulatory help and in this case specific guidance was available for this compound. This guidance formed the framework for the development strategy. Information on the innovator product was sourced and reviewed. This information included the EPAR as well as published literature on the innovator product. The review guided dose level selection and provided information on kinetics as well as toxicity. Literature on the innovator product was also reviewed to understand any clinical safety issues.

Next, to assist nonclinical development, the manufacturing strategy was considered. This step is a critical aspect of biosimilar development. The cell line used, as well as the production platform, affects the drug substance produced. It is highly unlikely that the biosimilar developer will use the same production technique to produce its biosimilar and thus the biosimilar drug substance may show small differences when compared with the innovator product, as was the case in this example. These small differences need to be characterised because understanding the differences is essential to ensure the differences do not impact on the pharmacokinetics or efficacy of the biosimilar.

As part of the quality characterisation of the biosimilar, appropriate physicochemical and biological characterisation studies were performed. Biological characterisation (which also comprised part of the nonclinical package) included receptor binding studies as well as pharmacological bioassay. This work was carried out on a minimum of three batches of both the biosimilar and the innovator product. Clearly the innovator product needed to be procured and required careful planning. For this work, it is critical to understand that differences with respect to the actual analysed quantity of protein in the innovator product ampoules/vials can occur versus what is actually stated on the product label. Thus, the biosimilar developer needs to determine these differences in the commercially available material.

Before commencing nonclinical testing, the next aspect to consider was the clinical strategy. Perhaps the most critical aspect here was whether the initial clinical study would be performed in healthy volunteers or whether, due to the compound’s characteristics and patient population, the initial clinical study would have to be performed in patients. In this instance, a Phase I study in healthy volunteers was possible. The availability of a robust pharmacodynamic (PD) marker is an exceptionally useful clinical and nonclinical tool for assessing exposure as well as efficacy. In this case, a robust and easily measured PD marker was available. With these steps in place, the nonclinical package was determined and undertaken. In vitro assay work involved using Biacore to assess ligand/receptor binding kinetics and a specific bioassay to demonstrate potency of the compound. These studies were undertaken as a comparability exercise where the innovator product and the biosimilar were run in parallel. It was necessary to use innovator product sourced from multiple regions since the majority of the current requirements state that the comparator product must be registered in that particular territory. In vivo work required for this product included a rodent PD study as well as a separate rodent PK study via two parenteral dose routes. This approach of splitting the studies into separate PK and PD studies was for ethical reasons. These were important studies since the formulation for the biosimilar was different from the innovator product and this may have influenced the efficacy and/or kinetics of the compound. These studies could thus have been an early warning of any formulation issues.

At this point, a decision was made to perform a full GLP toxicology study. The decision to perform this study was based on the total package of information available and the fact that particular study findings were identified in the original work performed by the innovator product developer. If toxicology testing is required this will usually be a short-term study (eg, four weeks duration). In this example, a four-week parenteral toxicology study in the rodent was considered appropriate with TK and local tolerance assessment (one control group, and one high dose level of innovator product and biosimilar were compared – from one region only – and with no non-dose recovery period). Consideration was also given as to whether the non-rodent should be tested, but it was concluded that only a single species was necessary as the rodent was biologically relevant. Dose-range finding work was not performed in this case as the information was available from the literature and from the PK/PD work. Although samples were taken for ADA measurement, these were not assessed because there was no effect on TK nor on the PD marker; a suggested routine practice is therefore to take samples but not to analyse them unless for a specific reason.

Case study 2 – Monoclonal antibody

Many of the considerations for this compound were the same as for the previous case study but there were some differences. Most notable of these was the lack of a finalised regulatory guidance specific to the product class. However, a draft guidance document was available. Once again, a literature review was performed to guide the development strategy. Comparative physicochemical evaluation was undertaken and as before, the clinical Phase I study strategy was defined. 

In vitro biological assay work was performed which included a comparative assessment of the mAb’s effector function (Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC)) as well as antigen binding studies and a target neutralisation assay. No in vivo efficacy studies were performed. In vivo studies undertaken included a single dose PK study in the rabbit. A PK study in the rat was considered but, as both species were not biologically relevant, individual animal data in the rabbit allowed more robust PK comparison between the innovator product and the biosimilar. This study was performed to check that the different formulation of the biosimilar did not significantly alter the single dose PK of the biosimilar in a healthy animal.

Careful consideration was given on whether a toxicology study was required because the primate was the only relevant species for repeat dosing. In this case, such a study was considered necessary partly for assurance that similar kinetics to the innovator product on repeat dosing in a relevant species occurred before moving into human studies, but also to satisfy an international development approach (the stance of regulators on the need for such toxicology work outside Europe is still not clear). One four-week parenteral study in the non-human primate (NHP) with TK and local tolerance assessment was performed (one control group, and one high dose level of innovator and originator – from one region only – and no non-dose recovery period). Dose-range finding work was not included due to information being available on the innovator product and also a lack of toxicity known for the innovator product. Safety pharmacology endpoints of blood pressure and electrocardiograms were also included. Once again, although samples were taken for ADA measurement, these were not assessed because there was no effect on TK.
Table 1: Key European guidelines for biosimilars

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Nonclinical data</th>
</tr>
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<tbody>
<tr>
<td>In vitro pharmacology</td>
<td>In vivo pharmacology</td>
</tr>
<tr>
<td>Similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues¹</td>
<td>Yes (eg, receptor-binding studies)</td>
</tr>
<tr>
<td>Annex to above guideline for products containing recombinant human soluble insulin³</td>
<td>Yes (appropriate animal work). [Not normally required for recombinant human soluble insulin and may be part of toxicology study for low molecular weight heparins]</td>
</tr>
<tr>
<td>Annex to above guideline for products containing somatropin²</td>
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<tr>
<td>Annex to above guideline for products containing recombinant granulocyte-colony stimulating factor⁴</td>
<td></td>
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<tr>
<td>Annex to above guideline for products containing low molecular weight heparins⁵</td>
<td></td>
</tr>
<tr>
<td>Nonclinical and clinical development of similar medicinal products containing recombinant interferon alfa⁶</td>
<td></td>
</tr>
<tr>
<td>Nonclinical and clinical development of similar biological medical products containing recombinant erythropoietins⁷</td>
<td></td>
</tr>
<tr>
<td>Similar biological medicinal products containing monoclonal antibodies (draft stage)¹⁰</td>
<td>STEP 1: In vitro studies</td>
</tr>
<tr>
<td></td>
<td>STEP 3: If in vivo testing is needed due to “concerns”, possibility of performing comparative PK and PD and/or toxicology work on a case-by-case basis. Immunogenicity and local tolerance assessment may be needed in this work.</td>
</tr>
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</table>

Note 1: The Dutch Medicines Evaluation Board issued a paper in 2010 on use of NHPs for the development of biosimilar drugs (with emphasis on mAbs) which indicated that use of NHPs for nonclinical comparability trials should be discouraged.¹¹

Note 2: The World Health Organization issued Guidelines on Evaluation of Similar Biotherapeutic Products (SBPs) in 2009 and indicated the need for nonclinical data for comparability.¹²

Note 3: Health Canada issued Information and Submission Requirements for Subsequent Entry Biologics (SEBs) guidance in 2010, indicating the need for appropriate in vitro and in vivo nonclinical studies to assess for comparability.¹³


Note 5: The European Medicines Agency has issued a Concept Paper on Similar Biological Medical Products containing Follicle Stimulation Hormone¹⁴ and a Concept Paper on Similar Biological Medical Products containing Recombinant Interferon Beta.¹⁵ Both documents state that recommendations will be given on what pharmacodynamic and toxicological work will be needed.
**Table 2: Nonclinical studies with marketed biosimilars**

<table>
<thead>
<tr>
<th>Biosimilar vs innovator name/identity/action/ authorisation date/reference*</th>
<th>Biological activity/ pharmacology</th>
<th>Kinetics</th>
<th>Toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth factor</strong></td>
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<tr>
<td>Omnitrope vs Genotropin/Recombinant human growth somatropin/Growth disorders/2006</td>
<td>Yes (in vivo)</td>
<td>TK from toxicology work</td>
<td>Repeat dose rat toxicity study + rabbit local tolerance studies</td>
</tr>
<tr>
<td>Valtropin vs Humatrope/Recombinant human growth somatropin/Growth disorders/2006</td>
<td>Yes (in vivo)</td>
<td>PK in rabbit + TK from toxicology work</td>
<td>Repeat dose rat toxicity study</td>
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<tr>
<td><strong>Epoetin</strong></td>
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<tr>
<td>Retacrit and Silapo vs Eprex-Eryo/Epoetin alfa/Anaemia/2007</td>
<td>Yes (in vitro and in vivo)</td>
<td>TK from toxicology work</td>
<td>Repeat dose rat and dog toxicity study + rabbit local tolerance studies</td>
</tr>
<tr>
<td>Brinocrit, Epoetin alfa Hexal and Abseamed vs Eprex-Eryo/Epoetin alfa/Anaemia/2007</td>
<td>Yes (in vitro and in vivo)</td>
<td>PK in dog + TK from toxicology work</td>
<td>Repeat dose dog toxicity study + rabbit local tolerance studies</td>
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<tr>
<td><strong>Granulocyte colony-stimulating factor</strong></td>
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<tr>
<td>Biograftist, Ratiograftist, Tevagraftist and Filgrastim ratiopharm vs Neutrogen/Human G-CSF/Neutropenia/2008</td>
<td>Yes (in vitro and in vivo)</td>
<td>PK in rat and monkey + TK from toxicology work</td>
<td>Repeat dose rat and dog toxicity study + rabbit local tolerance studies</td>
</tr>
<tr>
<td>Filgrastim Hexal and Zarzio vs Neupogen/Human G-CSF/Neutropenia/2009</td>
<td>Yes (in vitro and in vivo)</td>
<td>TK from toxicology work</td>
<td>Repeat dose rat toxicity study + rabbit local tolerance studies</td>
</tr>
</tbody>
</table>

*Data extracted from European Public Assessment Report for each biosimilar (EPAR, 2011).

**Conclusions**

**Recombinant protein.** A case study has been presented in which both in vitro and in vivo efficacy, PK and toxicology studies were described to support clinical work and potential marketing of a biosimilar recombinant protein. Lessons learned indicate that there is the potential (on a case-by-case basis) to combine in vivo efficacy and PK work or even move such evaluation to the toxicology study. Toxicology work in non-rodents should be avoided, which may often be the case as the rodent is a biologically relevant species.

**Monoclonal antibody.** A case study has been presented in which in vitro efficacy, PK and toxicology studies were described. Although used in this retrospective example, recommendation is made for little or no NHP use in biosimilar mAb development. However, it should be acknowledged that no NHP work (eg, not even a simple pharmacokinetic comparison in a reduced number of animals) and sole reliance on pharmaceutical quality and in vitro biological tests may be a “step too far” for some companies and also other regulatory agencies. A real industry concern is the current lack of “global” regulatory agency agreement (which could range from little or no NHP work to extensive testing including multiple dose groups). A company could have difficulty if it is following a minimal approach and is then delayed by the need to perform NHP work, as demanded by another agency, to allow rapid progression into the clinic and eventual product licence approval.

Overall, it can be seen that a range of nonclinical (and related quality and clinical) considerations should take place to develop a biosimilar drug. Such considerations include the relevant biological and pharmacological studies and, for toxicity testing, the relevant species of use, study design and comparator requirements, all with the aim of showing comparability but with the avoidance of unnecessary animal use in mind. As a final overall conclusion for any biosimilar development, it is recommended that regulatory agency interaction occurs, preferably before any toxicology or clinical studies have been performed.

**References**


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