

## Waiving in vivo studies for monoclonal antibody biosimilar development: National and global challenges

Kathryn Chapman<sup>a</sup>, Akosua Adjei<sup>b</sup>, Paul Baldrick<sup>c</sup>, Antonio da Silva<sup>d</sup>, Karen De Smet<sup>e</sup>, Richard DiCicco<sup>f</sup>, Seung Suh Hong<sup>g</sup>, David Jones<sup>b</sup>, Michael W. Leach<sup>h</sup>, James McBlane<sup>b</sup>, Ian Ragan<sup>i</sup>, Praveen Reddy<sup>j</sup>, Donald I. H. Stewart<sup>k</sup>, Amanda Sutters<sup>l</sup>, and Jennifer Sims<sup>m</sup>

<sup>a</sup>National Center for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK; <sup>b</sup>Medicines and Healthcare Regulatory Agency (MHRA), UK; <sup>c</sup>Covance Laboratories, Harrogate, UK; <sup>d</sup>Sandoz, Holzkirchen, Germany; <sup>e</sup>Federal Agency for Medical and Health Products (FAMHP), Belgium; <sup>f</sup>Harvest Moon Pharmaceuticals, US; <sup>g</sup>Celltrion, Japan; <sup>h</sup>Pfizer, US; <sup>i</sup>Independent, UK; <sup>j</sup>Biocon, India; <sup>k</sup>Plantform Corp, Canada; <sup>l</sup>Paraxel, UK; <sup>m</sup>Integrated Biologix, Switzerland

### ABSTRACT

Biosimilars are biological medicinal products that contain a version of the active substance of an already authorised original biological medicinal product (the innovator or reference product). The first approved biosimilar medicines were small proteins, and more recently biosimilar versions of innovator monoclonal antibody (mAb) drugs have entered development as patents on these more complex proteins expire. In September 2013, the first biosimilar mAb, infliximab, was authorised in Europe. In March 2015, the first biosimilar (Zarxio™, filgrastim-sndz, Sandoz) was approved by the US Food and Drug Administration; however, to date no mAb biosimilars have been approved in the US. There are currently major differences between how biosimilars are regulated in different parts of the world, leading to substantial variability in the amount of in vivo nonclinical toxicity testing required to support clinical development and marketing of biosimilars. There are approximately 30 national and international guidelines on biosimilar development and this number is growing. The European Union's guidance describes an approach that enables biosimilars to enter clinical trials based on robust in vitro data alone; in contrast, the World Health Organization's guidance is interpreted globally to mean in vivo toxicity studies are mandatory.

We reviewed our own experience working in the global regulatory environment, surveyed current practice, determined drivers for nonclinical in vivo studies with biosimilar mAbs and shared data on practice and study design for 25 marketed and as yet unmarketed biosimilar mAbs that have been in development in the past 5y. These data showed a variety of nonclinical in vivo approaches, and also demonstrated the practical challenges faced in obtaining regulatory approval for clinical trials based on in vitro data alone. The majority of reasons for carrying out nonclinical in vivo studies were not based on scientific rationale, and therefore the authors have made recommendations for a data-driven approach to the toxicological assessment of mAb biosimilars that minimises unnecessary use of animals and can be used across all regions of the world.

**Acronyms:** 3Rs, Replacement, refinement and reduction of animals in research; BIO, Biotechnology Industry Organization; ADCC, antibody-dependent cell-mediated cytotoxicity; CNS, Central nervous system; CRO, Contract research organization; EU, European Union; EMA, European Medicines Agency; Fc, Fragment crystallizable; FcRn, Neonatal Fc receptor; FDA, US Food and Drug Administration; HCP, Host cell protein; ICH, International Committee on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; mAb, monoclonal antibody; MHRA, Medicines and Healthcare products Regulatory Agency; NC3Rs, National Center for the Replacement, Refinement and Reduction of Animals in Research; PD, pharmacodynamic; PK, pharmacokinetic; US, United States of America; WHO, World Health Organization

### ARTICLE HISTORY

Received 6 November 2015  
Revised 8 January 2016  
Accepted 19 January 2016

### KEYWORDS

Biosimilar; development; EMA; FDA; guidelines; in vitro; in vivo; regulatory; study design; WHO; 3Rs

### Introduction

Biosimilars are biological medicinal products that contain a version of the active substance of an already authorised original biological medicinal product (the innovator or reference product). Initial development of a proposed biosimilar follows stringent 'quality by design' principles to engineer a molecule that is essentially undistinguishable from the reference biologic. This approach provides the foundation to support the 'totality of evidence' required to demonstrate that the biosimilar active

substance is highly similar to the reference product, and will have the same biological activity. To be marketed as a biosimilar, the new product must be determined by a regulatory authority to be highly similar to the reference medicinal product developed by the innovator in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise.<sup>1-4</sup> Thus, the active substance of an approved biosimilar should have the same biological activity as the reference medicine. Authorised/approved

**CONTACT** Kathryn Chapman  [kathryn.chapman@nc3rs.org.uk](mailto:kathryn.chapman@nc3rs.org.uk)

Published with license by Taylor & Francis Group, LLC © Kathryn Chapman, Akosua Adjei, Paul Baldrick, Antonio da Silva, Karen De Smet, Richard DiCicco, Seung Suh Hong, David Jones, Michael W. Leach, James McBlane, Ian Ragan, Praveen Reddy, Donald I. H. Stewart, Amanda Sutters, and Jennifer Sims

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

biosimilars are in use in over 50 countries, including in the EU and US, and there are a substantial number in various stages of development throughout the world.

While the initially approved biosimilar medicines were smaller proteins such as somatropin and erythropoietin  $\alpha$ , more recently biosimilar versions of innovator monoclonal antibody (mAb) drugs have entered development as patents on these more complex proteins expire. The entry of mAbs into the biosimilar space is significant to the medical field because various mAbs are safe and effective therapies given to many patients across the globe, in particular in the fields of inflammation and oncology. In September 2013, the first biosimilar mAb, infliximab, was authorised in Europe.<sup>5</sup> In March 2015, the first biosimilar (Zarxio™, filgrastim-sndz, Sandoz) was approved by the United States Food and Drug Administration (FDA); however, there have been no mAb biosimilars approved in the US.

It is generally recognized that the package of nonclinical in vivo toxicity studies needed to support human clinical trials and approval of biosimilar mAbs can be abbreviated compared with what is outlined in ICH S6 (R1) for novel mAbs, and what was conducted in support of the original innovator product. The main reasons for this are: 1) that the pharmacology and toxicity related to the mechanism of action of the proposed biosimilar is likely to be well understood (from the animal data used to support human clinical trials and approval of the innovator, and more importantly from the extensive human data available from clinical use); and 2) in vitro studies are likely to be more sensitive compared with in vivo studies in detecting differences between the proposed biosimilar and the reference product.<sup>6</sup> Additionally, for mAb products that have been approved and marketed, the value of animal testing to adequately predict adverse human effects has been questioned.<sup>7-9</sup>

While these reasons support an abbreviated nonclinical package, exactly how much abbreviation of the nonclinical program for proposed biosimilars is acceptable and scientifically rational has not been agreed upon. There are currently major differences between how biosimilars are regulated in different parts of the world, leading to substantial variability in the amount of in vivo nonclinical toxicity testing required to support clinical development and marketing of biosimilars. For example, for the same proposed biosimilar product, some regions require extensive animal testing such as that expected for an innovator product, and others, such as the EU, require only limited or no animal testing based on the available data. For companies developing products intended for global development and marketing, studies are often conducted to meet the requirements of the region that requires the most animal testing. With many mAbs, a non-human primate is often the only relevant species for in vivo toxicity studies, and the cynomolgus macaque (*Macaca fascicularis*) is the most frequently used monkey species.<sup>10-13</sup> This brings wide-ranging challenges to the industry, forcing companies to guess what country will require the most significant in vivo toxicity studies; companies then conduct those studies to prevent program delays related to regulatory issues, leading to an increase in animal use, particularly in non-human primates.

Organisations such as the NC3Rs have been working closely with the pharmaceutical, biotechnology and contract research industries and the regulatory agencies for the past 10 y to determine how to minimize the use of animals (particularly non-human primates) in the nonclinical toxicity assessment of mAbs in a scientifically rational manner, while still safely allowing new mAbs to reach patients. This work and the work of other organisations (e.g., BIO preclinical safety committee) was incorporated into the recent regulatory guidance, which includes approaches to reduce animal use.<sup>14</sup> To further extend these efforts to biosimilar mAbs, the NC3Rs created a mAb biosimilar expert working group in 2014 in collaboration with the UK Medicines and Healthcare Products Regulatory Agency (MHRA). The group comprised manufacturers, contract research organisations (CROs) and regulators from Europe, North America and Asia, and members are co-authors of this paper. The objectives of this group were to review the global regulatory environment, survey current practice, determine drivers for nonclinical in vivo studies with biosimilars, and make recommendations for a data-driven approach to toxicological assessment of mAb biosimilars that minimises unnecessary use of animals and can be used across all regions of the world.

### Current global regulatory environment

The EU, in 2005, was the first region to set up a legal framework and regulatory path for biosimilars that involved providing evidence to show that the biosimilar is essentially the same as the reference product. Development of the biosimilar does not involve nonclinical or clinical testing to prove the mechanism of action, pharmacology, or efficacy of the product because this was already performed for the approval of the original product. The EU guidance has evolved over the past 10 y to include more product-specific guidance and minimise the use of animals. In 2009, the World Health Organization's (WHO) guidelines were released and in 2012, the Biosimilar User Fee Act was enacted by the US FDA, which resulted in recent publication (April, 2015) of final guidances that discuss the nonclinical development of biosimilars.<sup>3,15</sup> Currently, there are guidance documents from an additional 26 countries or regions (for a review see Krishnan *et al*, 2015).<sup>16</sup> There is no specific ICH guideline that covers biosimilar products. Examples of global regulatory guidances are shown in Table 1.

### EU and US

The latest guidances on all types of biosimilars from the EU and US describe a 3 step tiered approach to regulatory authorisation and approval.<sup>1-3</sup> The EU has also issued a number of product-specific guidances, including a recent guidance on the development of mAb biosimilars.<sup>2</sup> The stepwise approach suggested by the EU and the US is designed to minimise the use of animals in a scientifically driven manner. Overall, the EU regulatory guidance specifies that 'the conduct of repeat-dose studies in non-human primates is usually not recommended' whereas the US guidance mentions that animal studies can be used to support the degree of similarity and that PK and PD measures can be incorporated into a single animal toxicity study, where

appropriate. Recent publications from EU scientists and regulators specifically question the need for any *in vivo* studies.<sup>17,18</sup>

Step one of the process, which covers the quality of the biosimilar product, includes *in vitro* methods such as a direct comparison of primary and higher order protein structure, biochemical differences associated with post-translational glycosylation and phosphorylation, *in vitro* mechanistic studies such as binding assays for target antigen and Fc receptors, and potency/functional activity assays in appropriate systems using the proposed biosimilar and the reference product. The European Public Assessment Report for Remsima<sup>TM</sup> (biosimilar to the innovator product Remicade<sup>®</sup>) demonstrates the extensive *in vitro* studies that may be carried out.<sup>19</sup>

Step two is the ‘determination of the need for *in vivo* studies’, which provides the opportunity for the sponsor to make a case to develop a biosimilar with *in vitro* data alone. The guidances indicate that, under certain circumstances, biosimilars that meet specific criteria, principally related to quality and a comprehensive *in vitro* demonstration of similarity, can enter the clinic and receive marketing authorisation with no *in vivo* animal data. Step three describes a 3Rs approach to *in vivo* nonclinical studies, when it is determined an *in vivo* study is necessary, and the EU guidance states that ‘the conduct of repeat-dose studies in non-human primates is usually not recommended’.

Overall, the EU regulatory guidelines provide a path that allows the development and authorisation of biosimilars with the submission of nonclinical *in vitro* data alone in certain circumstances, and without nonclinical *in vivo* data. While the US guidances also suggest a stepwise approach, they do not specifically state that approval can be granted without *in vivo* studies.

### WHO and the rest of the world

The WHO guidance describes nonclinical evaluation of a similar biotherapeutic with demonstration of biological/PD activity and toxicological work in at least one repeat-dose toxicity study. This is interpreted globally to mean *in vivo* toxicity studies are mandatory, and this requirement is reflected in national guidances. Indeed, many countries have established national guidelines that appear to be based on the WHO guidance (e.g., Brazil, India, Republic of Korea). For example, the Brazilian guidance states ‘the applicant company must submit the reports of the following *in vivo* nonclinical studies: .... cumulative toxicity studies (repeated dose), including characterization of parameters of toxicity kinetics, conducted in relevant species.’ The guidelines on similar biologics from India describe at least one repeat-dose toxicity study, generally not less than 4 weeks, with 3 dose levels and an assessment of recovery. Japanese guidelines for follow-on biologics were published in 2009 and encourage manufacturers to follow the ICH S6 guideline: ‘Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals’, but the Japanese guidelines also state ‘where the similarity of bioactivity between a follow-on biologic and the original biologic is fully evaluated by *in vitro* comparability studies, *in vivo* comparative studies of pharmacodynamics may not be necessary. However, useful information may often be obtained through *in vivo* pharmacological studies conducted at the stage prior to clinical study.’ Rather than following the WHO guidance, other countries have directly adopted the EU guidelines unchanged (e.g., Australia).

Due to the variation and lack of clarity between national and regional regulations, we explored which *in vivo* studies are being carried out by companies developing biosimilars in practice.

**Table 1.** Examples of Regulatory Guidance for Biosimilars Medicinal Product Development from around the World.

Region/Country	Regulatory Guidance
Global	WHO Guideline on evaluation of similar biotherapeutic products <a href="http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf">http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf</a>
European Union	EU Guideline on Similar Biological Medicinal Products (CHMP/437/04) <a href="http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003517.pdf">http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003517.pdf</a> EU Guideline on similar biological medicinal products containing monoclonal antibodies – nonclinical and clinical issues (EMA/CHMP/BMWP/403543/2010) <a href="http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500128686.pdf">http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500128686.pdf</a>
United States of America	Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (Finalised April 2015) <a href="http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf">http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf</a>
China	Guidance for research and evaluation techniques for biosimilars (Finalised February 2015) <a href="http://www.cde.org.cn/zdyz.do?method=largePage&amp;id=212">http://www.cde.org.cn/zdyz.do?method=largePage&amp;id=212</a>
Canada	Guidance For Sponsors: Information and Submission Requirements for Subsequent Entry Biologics (SEBs) <a href="http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/brgtherap/applic-demande/guides/seb-pbu/seb-pbu-2010-eng.pdf">http://www.hc-sc.gc.ca/dhp-mps/alt_formats/pdf/brgtherap/applic-demande/guides/seb-pbu/seb-pbu-2010-eng.pdf</a>
Japan	PMDA Guideline for the Quality, Safety, and Efficacy Assurance of Follow-on Biologics <a href="http://www.pmda.go.jp/files/000153851.pdf">http://www.pmda.go.jp/files/000153851.pdf</a>
India	Similar Biologics: Regulatory requirements for Market Authorization in India (15th Sept, 2012) <a href="http://cdsco.nic.in/writereaddata/Bio%20Similar%20Guideline.pdf">http://cdsco.nic.in/writereaddata/Bio%20Similar%20Guideline.pdf</a>
Cuba	Center for State Control on the Quality of Drugs (CECMED) Requirements for marketing authorization of known biological products <a href="http://www.cecmecd.cu/sites/default/files/adjuntos/Reglamentacion/reg_56-11_requisitos_para_el_registro_de_productos_biologicos_conocidos.pdf">http://www.cecmecd.cu/sites/default/files/adjuntos/Reglamentacion/reg_56-11_requisitos_para_el_registro_de_productos_biologicos_conocidos.pdf</a>
Iran	Guidelines for registration of biologics (recombinant medicines and monoclonal antibodies) in Iran - Iranian Food and Drug Organization (FDO) (15 March 2014) The guideline, which is in the local language (Farsi), is available from the FDO website: <a href="http://fdo.behdasht.gov.ir/">http://fdo.behdasht.gov.ir/</a>
Korea	Korea Evaluation Guidelines for Biosimilars (KFDA, July 2009) <a href="http://www.biosimilars.ca/docs/Evaluation_Guidelines_for_Biosimilars.pdf">http://www.biosimilars.ca/docs/Evaluation_Guidelines_for_Biosimilars.pdf</a>
Australia	Therapeutic Goods Administration: Evaluation of Biosimilars <a href="https://www.tga.gov.au/publication/evaluation-biosimilars">https://www.tga.gov.au/publication/evaluation-biosimilars</a>
Mexico	Guidelines for Bio-comparable Biotechnological Products <a href="http://dof.gob.mx/nota_detalle.php?codigo=5214882&amp;fecha=19/10/2011">http://dof.gob.mx/nota_detalle.php?codigo=5214882&amp;fecha=19/10/2011</a>
Singapore	Guidance on registration of similar biological products in Singapore. <a href="http://www.biosimilars.ca/docs/Guidance_Registration_Similar_Biological_Products_Singapore.pdf">http://www.biosimilars.ca/docs/Guidance_Registration_Similar_Biological_Products_Singapore.pdf</a>
Saudi Arabia	Saudi Food and Drug Authority: Biosimilar Guidelines <a href="http://old.sFDA.gov.sa/en/drug/news/documents/DrugMasterFileRequirementsforRegistrationofBiosimi.pdf">http://old.sFDA.gov.sa/en/drug/news/documents/DrugMasterFileRequirementsforRegistrationofBiosimi.pdf</a>

### **What is happening in practice?**

The working group collected information by questionnaire on practice, rationale and impact for 25 marketed and as yet unmarketed proposed biosimilar mAbs that have been in development in the past 5 y. The majority of products were for inflammatory or oncology indications (36% and 33%, respectively), with other therapeutic areas including respiratory, CNS and infectious disease. A fifth of the biosimilars had gone into Phase 1 clinical studies, mostly in patients, with the remainder entering clinical trials within the next 12 months. An in vivo toxicity study had been carried out for all products. There were a total of 26 in vivo studies carried out for 25 products, with 2 in vivo studies in rats being conducted for one product. For all products, there were no differences detected between innovator and reference products in the in vivo studies.

The majority (75%) of the in vivo studies were in cynomolgus monkeys, with the remaining studies done in rodents. The length of study varied from a single dose (2 examples) up to 26 weeks (one example), with the majority (56%) being 4 weeks in duration. The group sizes for cynomolgus monkey studies were generally 3 males and 3 females (75%), but ranged from one male plus one female to 5 of each sex. The group sizes for rodents varied from 3 males plus 3 females to 15 of each sex. The number of dose groups ranged from 2 test article-dosed groups (one high-dose group for the biosimilar and one high-dose group for the reference product) to 5 dose groups (low-, intermediate- and high-dose groups for the biosimilar and low- and high-dose groups for the reference product). The most common design was 2 high-dose groups (48%), with the second most common being 2 low- and 2 high-dose groups (biosimilar and reference product (24%)). Recovery animals were only used in one non-human primate study.

Overall, the number of cynomolgus monkeys used per study ranged from 10 to 36, the number of rats from 32 to 96, and the number of mice from 36 to 138. For the overall development program of a biosimilar, the most common study (42%) was 3 male and 3 female cynomolgus monkeys per group, 2 dose groups (biosimilar and reference product) and 4-weeks duration. These studies used 12 cynomolgus monkeys in total. There were examples where the scientific arguments to waive the in vivo study in cynomolgus monkeys were not accepted by regulators, and the minimised study design of 12 animals was successfully used. All companies agreed that this minimized study design was sufficient to provide the “comfort factor” requested by regulatory authorities, clinical investigators or ethical committees. For 25% of products, an in vivo study in rodent provided sufficient data and a non-human primate study was not required.

In addition to the survey, we shared and discussed our experiences, which included aspects about additional programs that could not be submitted to the survey due to lack of internal legal approval. Although the regulations in the EU and the US pave the way for biosimilars to be developed with in vitro data alone, the experience of the working group is that in vivo studies are still currently required in these regions, as well as throughout the rest of the world.

While the EU currently appears to be actively promoting the initiation of clinical trials based on in vitro data only where

appropriate (see current global regulatory environment), the responsibility of implementing EU regulation lies with individual countries within the EU. Experiences from the working group show that some member states within the EU do not necessarily follow the general EU guidances. Some national competent authorities, as well as some ethics committees, are requesting in vivo studies when the in vitro package is considered appropriate by the manufacturer and by other countries within the EU. Additionally, acceptance by the physicians participating in biosimilar clinical trials in the absence of in vivo data (particularly safety data) remains challenging until the concept becomes more widely accepted. However, the most recent experiences of the working group suggest the expectations for in vivo studies with proposed biosimilar mAbs in the EU are decreasing.

Beyond the EU, the experience of the working group is that the US generally requires at least one in vivo study, although it does not necessarily need to be in non-human primates and does not need to include both sexes. Japan requires at least a 2-week repeat-dose in vivo study (not necessarily in a pharmacologically-relevant species) and the experience of the working group has been that both sexes are required. Such studies have been requested by Japan even when there is significant human data available from other regions in the world. Experience in many countries in the rest of the world (e.g., India, China) is that in vivo studies are interpreted as mandatory following the current requirement in the WHO guideline. National guidelines that are based on the WHO guideline often require in vivo studies. There is currently an ongoing regulatory discussion at an international level to determine whether the WHO guideline on biosimilars will be reopened to potentially add clarity around whether animal studies are mandatory or whether this is a misinterpretation of the guidelines by national authorities.<sup>20</sup>

Overall, the experience of the working group is that the EU requires the least amount of in vivo data, the US requires an intermediate amount, and the rest of the world requires the most in vivo work. In some cases, it is known that companies have been asked to follow a biosimilar development pathway that is as extensive as for an innovator product.

### **Rationale for conducting in vivo studies**

When the working group shared their experiences and practice on development of proposed biosimilar mAbs, the rationale for conducting nonclinical in vivo studies fell into several categories.

#### **i. Anticipated or actual regulatory or institutional ethical committee request**

Based on the working group’s experiences, the main reason for conducting nonclinical in vivo studies is the anticipation of a subsequent regulatory request for more data in a specific country or geographic region. This may be based on previous experience with that country or region or to satisfy global regulations. For instance, a manufacturer may make the decision to conduct the longest and most extensive in vivo study that it anticipates will be required anywhere in the world

to reduce the risk of program delays. Anticipated and real requests from institutional ethical committees were also noted for some programs.

### **ii. Meetings with regulators that are not timely**

Obtaining regulatory feedback from the country or region in which clinical trials are initially planned prior to conducting nonclinical in vivo studies can be extremely useful in determining the need for these studies. However, it may not be possible to obtain such feedback in a manner that maintains project timelines. Using the stepwise approach advocated by regulatory guidance takes a substantial amount of time to consecutively generate sufficient in vitro data, schedule and hold meetings with regulators, and then conduct and prepare reports for any nonclinical in vivo studies that may be requested. Short company timelines often do not allow for all these steps to occur consecutively. Thus, in vivo studies may be conducted to prevent potential program delays.

Following the initial clinical studies, most companies continue development globally. It is challenging to get feedback from a large number of countries or regions, especially in a timely manner. Because a manufacturer cannot usually obtain feedback from all countries and regions in which submissions are planned, they may default to conducting in vivo toxicity studies that ultimately might not have been necessary.

### **iii. Inconsistent approaches between geographic regions or within the same geographic region**

As discussed above, different countries follow different guidelines and have different expectations regarding the need for in vivo animal studies in support of the development of proposed biosimilars. In addition, the experience of the working group is that regulatory advice and requests for nonclinical in vivo studies can differ within the same country or region, even when advice is received in the same timeframe and for similar indications. The variable responses often lead companies to conduct the same nonclinical in vivo studies as those that had been requested on a recent program with a different molecule to minimise the risk of program delays. In addition, regulatory advice is not binding and there is no guarantee that there will not be a later regulatory request for in vivo studies prior to initiation of clinical studies or authorisation/approval; such requests would likely result in program delays.

### **iv. Default practice to carry out nonclinical in vivo studies providing a comfort factor for companies, regulatory agencies, or institutional ethical committees**

There is a general discomfort among many companies, regulators, and ethical committees with dosing humans without having first dosed. This need for a “comfort factor” is not driven by any specific scientific rationale, but is often related to the concern for unexpected off-target toxicity from the test article or unidentified impurities. However, the toxicity of biotherapeutics observed in nonclinical in vivo studies is usually related to on-target effects,<sup>10,21</sup> which should already have been well characterized by the innovator. Further, the relevance of any findings related to on-target effects should also be known based on the extensive clinical experience from the innovator. Another reason given for conducting a “comfort factor” in vivo

toxicity study is to detect effects related to unidentified impurities. However, impurities should be detected during the extensive analytical testing done on biosimilars, and if any impurities are detected, they should follow the general ICH guidances for testing impurities (see next section). Thus, it does not make rational scientific sense to conduct in vivo studies to detect effects related to unidentified impurities. In cases where in vivo clinical data are available and confirm similarity between the innovator and the proposed biosimilar, the working group consensus was that additional animal studies are not needed to provide a comfort factor.

### **v. Assessment of identified impurities**

Impurities may be identified in the proposed biosimilar, and these may include variants of the intended biosimilar protein that may have pharmacologic activity. We believe that in almost all cases, the impurity profile of the proposed biosimilar should be well understood prior to dosing humans based on analytical assessments, and a literature assessment of potential risk of any impurities will usually be sufficient to obviate the need for in vivo studies. In the case of protein variants that may have pharmacologic activity, an assessment of this activity is usually best done using sensitive in vitro methods rather than in vivo studies. It was noted that in some cases interactions with regulatory authorities may occur prior to availability of the full Quality dossier, and, when manufacturers ask whether in vivo studies are necessary under these conditions, regulators may express concerns about potential impurities. This can lead to a request for in vivo studies to assess potential impurities.

One additional concern related to impurities are host cell proteins (HCP). Due to differences in the manufacturing process between the innovator and the proposed biosimilar product (for example, different clones, growth media or pH may be used), it is likely that different HCP contaminants will be present. Major qualitative differences should be evaluated. It is not a regulatory requirement for in vivo studies to evaluate or qualify HCP. The view of the majority of the working group is that it would be very uncommon that in vivo assessment of HCP would add value or be necessary if the manufacturer is able to minimise the levels of HCP.

### **vi. To address a lack of in vitro data**

In most cases, in vitro data is generated throughout the development timeline, with data being limited early in programs and more extensive later on. Nonclinical in vivo studies may be initiated prior to obtaining detailed in vitro data to maintain timelines in the event that the in vitro data suggest some uncertainty regarding similarity. However, the working group consensus is that residual uncertainty in biosimilarity is not best addressed by nonclinical in vivo studies under most circumstances, but rather by in vitro studies. These in vitro studies should focus on relevant mechanisms that are operative in humans, and on addressing any residual uncertainty. For example, if ADCC plays a role in the mechanism of action, in vitro assays evaluating ADCC activity for the innovator and proposed biosimilar would likely be highly relevant.

### **vii. To allow human trials at the intended clinical dose rather than requiring dose escalation**

The working group discussed the potential concern that the absence of animal studies at pharmacologically-relevant doses could lead regulatory agencies or clinical investigators to mandate that dosing in humans begin at lower levels, and then escalate to the clinical dose. There was experience in the working group with a situation where *in vitro* data alone was accepted for a pharmacokinetic (PK) clinical bioequivalence study in cancer patients; however, feedback from the European Medicines Agency and specific national EU health authorities was to amend the clinical trial design to include dose escalation in a subset of patients to verify safety prior to full enrolment. The working group considered this to be ethically unacceptable because an effective treatment (the reference product) already existed. The consensus of the working group is that dose escalation for biosimilars is not needed in the clinic when *in vitro* data indicate biosimilarity, and *in vivo* nonclinical studies should not be needed or required to eliminate the need for clinical dose escalation.

### **viii. To assess differences in the *in vitro* data between the innovator and the proposed biosimilar**

There are always differences between the innovator and the proposed biosimilar in the various *in vitro* assays that are conducted, and often the effect of those differences on *in vivo* safety and efficacy in humans is not known. An example of how this can be addressed has been presented by Schiestl et al.<sup>22</sup> Based on an analysis of a number of batches of reference product across its shelf life, the paper defines goalposts and recommendations of acceptable changes in quality attributes such as glycosylation and biochemical and functional attributes that can be determined *in vitro*. If the results from these studies for the proposed biosimilar falls within these goalposts, we suggest this proposed biosimilar can be considered highly similar to the reference product; we further suggest that there is the potential to waive *in vivo* studies in these cases. In most cases, data from nonclinical *in vivo* studies is unlikely to provide a definitive answer on whether these differences affect clinical safety or efficacy. Instead, additional *in vitro* assays (which are usually more sensitive compared with *in vivo* studies), or an assessment of human clinical data, provide a more clear assessment.

### **ix. Alternative formulations, novel excipients or higher concentrations of known excipients**

Occasionally, the developer of a biosimilar may have to use a different formulation from that of the reference drug. For instance, the original, innovator product is typically developed many years previously, and the innovator may have used formulations that are no longer considered optimal. In addition, biosimilar developers may have a preference for certain formulations at their company. The working group believes that *in vivo* studies are not generally necessary to demonstrate the safety of known excipients when used at concentrations that have already been used in humans, either in the innovator product or in other products, thus negating the need for *in vivo* studies in these cases.

Use of novel excipients or excipients used at a higher or lower concentration than previously can have a significant

impact on the biodistribution of mAb biosimilars related to interference with the osmotic gradient and pressure within the interstitial space of the subcutaneous application site due to molarity and pH factors. In cases where the proposed biosimilar has excipients that differ qualitatively or quantitatively from the innovator product, the manufacturer may want to test different formulations *in vivo* in animals to ensure that they are likely to provide a similar clinical PK profile. It is important to note that PK profiling of formulations is distinct from obtaining information on bioequivalence.

Currently, modeling without *in vivo* data is not likely to be adequate to test these variations in formulation, and, therefore, *in vivo* studies may be useful to support selection of the formulation that yields the closest absorption (i.e., affecting  $T_{max}$  and  $C_{max}$ ) to that of the originator. These studies can be carried out in a non-pharmacologically-relevant species, including rodents because target binding is irrelevant and the impact that different excipients have in the interstitial space between humans and rodents is similar enough to make a decision about a formulation. For example, rat and mouse FcRn recognize the Fc fragment of human immunoglobulin G,<sup>23-26</sup> which enables the assessment of non-target related PK associated with FcRn binding and antibody recycling.

### **Experience with *in vitro* data alone to support biosimilar development**

The working group also shared data on their experiences with *in vitro* data packages for first-in-human clinical trials of mAb biosimilars. There were no examples submitted (0/25) where clinical trial entry was on the basis of *in vitro* data alone. Three companies with 8 products had presented a first-in-human package containing only *in vitro* data to regulators or ethical review committees, and all were requested to perform nonclinical *in vivo* studies. In some cases the *in vitro* data showed identical glycosylation patterns between the innovator and the proposed biosimilar using standard, validated assays used to monitor process changes with the reference product. For seven of the 8 products, the National Competent Authority considered the *in vitro* data to be insufficient, and 3 of the 7 also had refusal from the ethical committee. The remaining product refusal was from an ethical committee that required *in vivo* data prior to the first-in-human dosing. In situations where the scientific arguments to waive the *in vivo* study in cynomolgus monkeys were not accepted, a minimised study design of 12–18 animals has usually been successful.

### **Taking the science-led approach**

The main goal of a nonclinical biosimilar development program is to assess whether the proposed biosimilar product is highly similar to the innovator in terms of quality, safety, and efficacy. It is not to assess *de novo* nonclinical or clinical safety or efficacy because such knowledge from the approved product already exists. In particular, there should be extensive human data available to address the pharmacology and toxicity of the mechanism of action, and this human data generally should supersede animal data. There has been much debate within the scientific community regarding which nonclinical *in vivo*

studies (if any) are needed to reach this goal. It is recognized that non-human primate studies are not, and should not be, powered to detect small differences between the proposed biosimilar and the innovator product, and therefore rarely provide useful information if the proposed biosimilar has already shown a high degree of in vitro similarity. In our opinion, the majority of decisions on similarity should be based on in vitro studies of quality attributes, and a lack of similarity or inadequate in vitro data should not justify the conduct of in vivo studies. If, for example, the in vitro data do not show comparable glycosylation patterns, the question still remains as to whether the molecule is indeed a biosimilar, and this question cannot usually be addressed through in vivo studies in animals. Instead, similarity might better be addressed through selected in vitro testing to determine whether there are any functional effects related to the differences in glycosylation.

The working group also was not able to identify an instance where nonclinical in vivo data provided useful information to a proposed biosimilar program that had a strong in vitro data package showing a high degree of similarity. It is the opinion of the working group that nonclinical in vivo studies do not add value in assessing the similarity of proposed biosimilars in most cases, primarily because of the variability and general insensitivity of in vivo studies relative to in vitro assays.

There was unanimous agreement in the working group that fully powered PK bioequivalence studies should not be required in any animal species to demonstrate the highly similar nature of a biosimilar and reference product. Such studies would require very large numbers of animals, could potentially be confounded by immunogenicity that is not necessarily relevant to humans, and in the end the real determination of PK bioequivalence should come from clinical trial data in humans.

### Study design and species selection for biosimilars

The working group does not generally recommend nonclinical in vivo studies to support biosimilar development. If such studies are requested by regulators or institutional ethics committees or to address scientific questions that can only be answered *in vivo*, the minimum numbers of animals possible should be used. The working group recognizes that it will take time for full global consensus on this issue, and therefore nonclinical in vivo studies are still likely to be conducted in support of proposed biosimilar programs in the near future. Based on this, the working group has made recommendations for the conduct and design of these studies:

- In vivo studies should only be carried out if the scientific question cannot be answered in vitro, and when the in vivo studies will add value.
- The exact purpose of the study and the design of the study should be carefully considered and designed to minimize the number of animals rather than taking a tick-box approach.
- Mice or rats, rather than a non-human primate species, are often suitable to assess the PK properties of mAbs. Even when there is no pharmacologic activity present in rodents, the interaction of the mAbs with FcRn can be assessed – see discussion above on alternative

formulations, novel excipients or higher concentrations of known excipients.

- Testing in one sex should be considered wherever possible.
- Assessment of recovery is generally not necessary.
- Testing at one dose level is usually sufficient. It is recommended that this dose match one of those used by the innovator, and not saturate the dose response so that differences between the proposed biosimilar and innovator can be more readily detected. From a practical standpoint, this can be a dose that provides exposure in the clinically relevant range, and is not necessarily the highest dose tested by the innovator which is more likely to be saturating.
- The relevant control for a biosimilar (for analytical, in vitro and in vivo comparisons to support high similarity) is the reference material, and therefore a vehicle control group is not necessarily needed.
- Testing of a single reference product is sufficient. Where there is a regulatory request to test a different version of the reference product, this can be achieved by CMC characterization and a clinical PK/PD bridging study.
- In cases where the innovator used a surrogate (usually because of a lack of pharmacologic activity of the innovator molecule in standard nonclinical species), it is not recommended that any new studies be conducted with a surrogate, as there is already extensive data in humans from the reference product on impacting the intended target.

### Discussion

The findings and conclusions presented in this paper are based on a survey that included 25 biosimilar mAb programs in development over the past 5 years, as well as the output from in-depth working group discussions with representation from sponsoring companies, CROs and regulators from the US and EU. Nonclinical in vivo studies are currently required to progress a global biosimilar mAb program. In contrast to this requirement, the view of the majority of the working group is that the in vivo animal studies that are currently conducted for biosimilar mAbs are adding minimal scientific insight to that already gained from in vitro studies and the innovator data. This conclusion is in agreement with published data on non-clinical development of proposed biosimilar mAbs.<sup>27-32</sup>

The conduct of nonclinical in vivo studies is often driven by anticipated or real requests from regulatory agencies or institutional ethical committees. Accurately anticipating these requests is not possible. Guidances are generally not binding, and authorities can choose to request more data even if such a request is not in agreement with regulatory guidances. Authorities in a given country or region may not agree with the guidances, or with one another, leading to inconsistent requests from the same country or region. Additionally, the same data may trigger different regulatory responses. For example, in countries that follow the EU guidances, an observed difference between the proposed biosimilar mAb and the innovator product in an in vitro quality test does not necessarily trigger a need for in vivo studies. It appears that this argument may be less likely to

be accepted in the US and other regions. The difficulty of predicting the human impact of a difference between the reference and the proposed biosimilar would likely lead to *in vivo* animal testing. However, in the experience of the authors, in virtually all cases the *in vivo* study provides little value, and it is human data that is important. Other reasons for conducting nonclinical *in vivo* studies include an inability to meet with regulators in a timely manner, inconsistent approaches between geographic regions or within the same geographic region, default practice to carry out nonclinical *in vivo* studies within the sponsoring company to provide a comfort factor for the sponsoring company, regulatory agencies or institutional ethics committee, to assess identified impurities including HCP, to address a lack of *in vitro* data, to allow starting human trials at the clinical dose (vs requiring dose escalation), to further assess the impact of differences in the *in vitro* data, to assess novel excipients or higher concentrations of known excipients, and to select alternative formulations. Most of these are not driven by strong scientific rationale.

The working group has written this paper to provide an impetus to change practice of regulatory agencies and institutional ethical committees, and some regulatory guidances, to reduce animal use in the development of biosimilar mAbs. To accelerate this, the working group aims to continue its work and to extend its reach to regulators globally, to support global harmonisation, to work with relevant organisations to review the WHO guidance, and to inform practice on biosimilar development worldwide.

### Disclosure of potential conflicts of interest

Some of the authors (Antonio da Silva, Richard DiCicco, Seung Suh Hong, Michael W Leach, Praveen Reddy, Donald I H Stewart) work for companies who are developing Biosimilar products.

### References

- EU Guideline on Similar Biological Medicinal Products (CHMP/437/04) ([http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2009/09/WC500003517.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003517.pdf))
- EU Guideline on similar biological medicinal products containing monoclonal antibodies – nonclinical and clinical issues (EMA/CHMP/BMWP/403543/2010) ([http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2012/06/WC500128686.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500128686.pdf))
- Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291128.pdf>)
- Bui LA, Hurst S, Finch GL, Ingram B, Jacobs IA, Kirchoff CF, Ng CK, Ryan AM. Key considerations in the preclinical development of biosimilars. *Drug Discovery Today* 2015; 20(1): 3-15; PMID:25912284; <http://dx.doi.org/10.1016/j.drudis.2015.03.011>
- [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Press\\_release/2013/06/WC500144941.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2013/06/WC500144941.pdf)
- EU Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: nonclinical and clinical issues (EMA/CHMP/BMWP/42832/2005 Rev1). [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2015/01/WC500180219.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/01/WC500180219.pdf)
- Martin PL, Bugelski PJ. Concordance of preclinical and clinical pharmacology and toxicology of monoclonal antibodies and fusion proteins: soluble targets. *British Journal of Pharmacology* 2012 Jun;166 (3):806-22; PMID:22168335; <http://dx.doi.org/10.1111/j.1476-5381.2011.01812.x>
- Bugelski PJ, Martin PL. Concordance of preclinical and clinical pharmacology and toxicology of therapeutic monoclonal antibodies and fusion proteins: cell surface targets. *British Journal of Pharmacology* 2012 Jun;166 (3):823-46; PMID:22168282; <http://dx.doi.org/10.1111/j.1476-5381.2011.01811.x>
- van Meer PJ, Kooijman M, van der Laan JW, Moors EH, Schellekens H. The value of non-human primates in the development of monoclonal antibodies. *Nature Biotechnology* 2013 Oct;31 (10):882-3; PMID:24104750; <http://dx.doi.org/10.1038/nbt.2709>
- Bussiere JL. Species selection considerations for preclinical toxicology studies for biotherapeutics. *Expert Opinion in Drug Metabolism and Toxicology* 2008; 4(7):871-7; PMID:18624676; <http://dx.doi.org/10.1517/17425255.4.7.871>
- Chapman K, Pullen N, Graham M, Ragan I. Preclinical safety testing of monoclonal antibodies: the significance of species relevance. *Nature Reviews Drug Discovery* 2007; 6: 120-126; PMID:17268483; <http://dx.doi.org/10.1038/nrd2242>
- Chapman K, Andrews L, Bajramovic JJ, Baldrick P, Black LE, Bowman CJ, Buckley LA, Coney LA, Couch J, Dempster M, et al. The design of chronic toxicology studies of monoclonal antibodies: implications for the reduction in the use of non-human primates. *Regulatory Toxicology and Pharmacology* 2012; 62 (2): 347-54; PMID:22100994; <http://dx.doi.org/10.1016/j.yrtph.2011.10.016>
- Chapman K, Pullen N, Coney L, Dempster M, Andrews L, Bajramovic J, Baldrick P, Buckley L, Jacobs A, Hale G, et al. Preclinical development of monoclonal antibodies. Considerations for the use of non-human primates. *mAbs* 2009; 1:5, 505-516; PMID:20065651; <http://dx.doi.org/10.4161/mabs.1.5.9676>
- ICH S6 (R1). Preclinical safety evaluation of biotechnology-derived pharmaceuticals. In: International Conference on Harmonisation (ICH). Topic S6 (R1). June 2011.
- Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product (<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM291134.pdf>)
- Krishnan A, Mody R, Malhotra, H. Global regulatory landscape of biosimilars: emerging and established market perspectives. *Biosimilars* 2015; 5: 19-32; <http://dx.doi.org/10.2147/BS.S44052>
- van Aerts LAGJM, Smet K, Reichmann G, van der Laan JW, Schneider CK. Biosimilars entering the clinic without animal studies: A paradigm shift in the European Union. *mAbs* 2014; 6:5, 1155-1162; PMID:25517301; <http://dx.doi.org/10.4161/mabs.29848>
- Van Meer PJK, Ebberts HC, Kooijman M, Gispen-de Wied CC, Silva-Lima B, Moors EHM, Schellekens H. Contribution of animal studies to evaluate the similarity of biosimilars to reference products. *Drug Discovery Today* 2014; 120(4): 483-490; PMID:25463036; <http://dx.doi.org/10.1016/j.drudis>
- European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP) EMA/CHMP/589317/2013. European Public Assessment Report Remsima International non-proprietary name: Infliximab
- Heim HK. Geneva, April 27 (2015): Session 3: Review of the current WHO SBPs Guidelines: Regulatory perspectives, Federal Institute for Drugs and Medical Devices (BfArM)
- Leach MW. Regulatory forum opinion piece: differences between protein-based biologic products (biotherapeutics) and chemical entities (small molecules) of relevance to the toxicologic pathologist. *Toxicological Pathology* 2013; 41(1):128-36; PMID:22744226; <http://dx.doi.org/10.1177/0192623312451371>
- Schiestl M, Stangler T, Torella C, Čepeljnik T, Toll H, Grau R. Acceptable Changes in Quality Attributes of Glycosylated Biopharmaceuticals. *Nature Biotechnology* 2011; 29: 310-312; PMID:21478841; <http://dx.doi.org/10.1038/nbt.1839>
- Abdiche YN, Yeung YA, Chaparro-Riggers J, Barman I, Strop P, Chin SM, Pham A, Bolton G, McDonough D, Lindquist K, et al. The neonatal Fc receptor (FcRn) binds independently to both sites of the IgG homodimer with identical affinity. *mAbs* 2015; 7(2):331-43; PMID:25658443; <http://dx.doi.org/10.1080/19420862.2015.1008353>
- Kliwinski C, Cooper PR, Perkinson R, Mabus JR, Tam SH, Wilkinson TM, Giles-Komar J, Scallon B, Powers GD, Hornby PJ. Contribution



- of FcRn binding to intestinal uptake of IgG in suckling rat pups and human FcRn-transgenic mice. *Am J Physiol Gastrointest Liver Physiol* 2013; 304(3):262-70; PMID:23220220; <http://dx.doi.org/10.1152/ajpgi.00340.2012>
25. Ober RJ, Radu CG, Ghetie V, Ward ES. Differences in promiscuity for antibody-FcRn interactions across species: implications for therapeutic antibodies. *Int Immunol* 2001; 13(12):1551-9; PMID:11717196; <http://dx.doi.org/10.1093/intimm/13.12.1551>
26. Andersen JT, Daba MB, Berntzen G, Michaelsen TE, Sandlie I. Cross-species binding analyses of mouse and human neonatal Fc receptor show dramatic differences in immunoglobulin G and albumin binding. *J Biol Chem*. 2010; 285(7):4826-36; PMID:20018855; <http://dx.doi.org/10.1074/jbc.M109.081828>
27. Ryan AM, Sokolowski SA, Ng C-K, Shirai N, Collinge M, Shen AC, Arrington J, Radi Z, Cummings TR, Ploch SA, et al. Comparative non-clinical assessments of the proposed biosimilar PF-05280586 and rituximab (MabThera®). *Toxicological Pathology* 2014; 42:1069-1081; PMID:24604381; <http://dx.doi.org/10.1177/0192623313520351>
28. Hurst S, Ryan AM, Ng C-K, McNally JM, Lorello LG, Finch GL, Leach MW, Ploch SA, Fohey JA, Smolarek TA. Comparative nonclinical assessments of the proposed biosimilar PF05280014 and trastuzumab (Herceptin®). *BioDrugs* 2014; 28:451-459; PMID:25001079; <http://dx.doi.org/10.1007/s40259-014-0103-4>
29. Derzi M, Ripp SL, Ng C-K, Shoieb AM, Finch GL, Lorello L, Leach MW. Comparative nonclinical assessments of the potential biosimilar PF-06410293 and adalimumab. SOT Annual Meeting, Phoenix, Arizona, March 24–27 2014
30. Johnson TR, Derzi M, Bolt M, Ng C-K, Kirchoff CF, Lorello L, McNally J, Leach MW, Smolarek TA. Comparative Nonclinical Assessments of Marketed Infliximab Presentations (Remicade®) and the Potential Biosimilar PF06438179. 14<sup>th</sup> Annual American Association of Pharmaceutical Scientists (AAPS) National Biotechnology Conference, San Diego, California, May 19–21. 2014
31. Ripp SL, Bolt M, Derzi M, Finch G, Hurst S, Johnson TR, Leach MW, Smolarek TA. A fit-for-purpose approach to nonclinical assessments for adalimumab and infliximab biosimilar development. 19th North American Regional ISSX Meeting and 29th JSSX Annual Meeting, San Francisco, California, October 19–23 2014.
32. Peraza M, Shiue M, Phenix S, Rule K, Finch G, Thibault S, Leach MW. Comparative nonclinical assessment of the potential biosimilar PF-06439535 and bevacizumab. In: 2015 Annual Meeting Abstract Supplement, Society of Toxicology, 2015, Abstract no. 603.