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Pharmacokinetic and toxicology comparator testing of biosimilar drugs – Assessing need

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ABSTRACT

A key element in the development of a biosimilar molecule is the comparability of the biological activity/nonclinical similarity to the innovator drug. Although some regulatory guidelines are encouraging little or no *in vivo* testing, currently a common practice is to perform at least one toxicology and/or one pharmacokinetic (PK) study to assess if any different findings occur for in-life, clinical pathology and histopathological parameters or in exposure. An exercise was performed in which the results of such testing were evaluated. It was found that 10 PK comparison studies in the cynomolgus monkey across 4 monoclonal (Mab) classes showed similar exposure in all cases. In 17 toxicology comparison studies with 5 Mab classes performed in the same species and in 7 toxicology comparison studies with non-Mab biosimilars in the rat, no new/unexpected findings were seen and drug exposure measurement gave comparable values in all cases. Overall, although this work does not rule out possible utility of some *in vivo* testing (notably in the form of stand-alone PK testing) to confirm similar exposure between the 2 molecules tested, it is unclear what benefit can be gained from toxicology testing, especially if comparability has been demonstrated from physiochemical and *in vitro* characterisation.

1. Introduction

A variety of regulatory guidelines exist to assist drug companies in developing a biosimilar molecule, whether a Mab or a non-Mab protein. A key consideration is biological activity/nonclinical comparability to the marketed (innovator) drug. Examples (with some interpretation of actual testing needs) comprise:

- European Union (EU): Nonclinical testing using a step-wise approach needs to occur with *in vitro* binding/biological activity studies, then evaluation if *in vivo* testing (pharmacodynamic [PD] and/or PK studies) is deemed necessary but conduct of standard repeated dose toxicity studies in non-human primates is usually not recommended (EMA, 2014). If testing is considered to be needed (and appropriately justified), it can take the form of one dose level of biosimilar and reference product and/or one gender and/or no recovery animals OR an in-life evaluation of safety parameters. The conduct of toxicity studies in a non-relevant species is not recommended.
- United States (US): Pharmacological comparability from *in vitro* and/or *in vivo* assays and some animal safety data is indicated (FDA, 2015). Toxicology testing can range from limited animal toxicity data to the need for a general toxicology study although allowance is given in the guidance to discuss justification for not conducting such work. It is also possible that a single-dose study comparing the proposed product and the reference product using PK and PD measures may contribute to the totality of *in vivo* evidence that supports a demonstration of biosimilarity.
- World Health Organisation (WHO): Demonstration of biological/PD activity and toxicological work in at least one repeat dose toxicity study needed (WHO, 2009). However, a new guideline, which has a focus on Mabs, states that biological activity comparison from *in vitro* and/or *in vivo* studies is still needed but repeated dose toxicity studies in non-human primates is usually not recommended (WHO, 2016).
- India: *In vitro* and/or *in vivo* PD studies and toxicological studies in at least one repeat dose toxicity study using 3 dose levels of biosimilar (India, 2016).
- China: PD and PK evaluation as well as consideration for a repeat dose toxicology study (China, 2015).

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- Japan: *In vitro* and/or *in vivo* PD studies plus nonclinical safety data needed involving repeat dose toxicology testing and/or PK evaluation (Japan, 2009).
- Canada: *In vitro* and/or *in vivo* PD studies and at least one repeat dose toxicity study (Canada, 2010). An update of the 2010 guideline has a changed situation and states that "Where similarity is well established by structural and functional studies, and where extensive *in vitro* mechanistic studies are indicative of similarity, *in vivo* non-clinical studies may not be necessary" (Canada, 2016).

Overall, based on available guidance, companies developing biosimilars globally tend/have tended to follow a "default option" of performing *in vivo* studies that can include PK evaluation and toxicity testing. In order to examine what such testing is actually showing, an exercise was performed by examining the results of recent studies performed within our laboratory (Covance) for both Mab and non-Mab biosimilars. Both PK (Mabs only) and toxicology (Mabs and non-Mab proteins) studies were available. Available published literature for Mab biosimilar PK and/or toxicology comparator studies was also reviewed.

2. Material and methods

A snapshot of studies in the Covance database revealed 10 PK and 17 toxicology comparison studies performed in the cynomolgus monkey with Mabs and 7 toxicology comparison studies performed in the rat with non-Mab proteins. From these studies the following information was extracted: Covance site location, biosimilar/innovator identification, year performed, Good Laboratory Practice (GLP) status, rationale around dose level selection, study design/dose levels, study parameters measured and study findings. It was established that studies were performed across 4 different Covance sites, namely Harrogate, (UK), Muenster (Germany), Madison (USA) and Shanghai (China). Obviously for confidentiality reasons, no information on the identity of biosimilar or innovator is presented; testing of biosimilars to the same innovator drug occurred in different studies (ie, different Sponsoring companies) on a number of occasions and are captured as "Mab 1", etc or "Non-Mab protein 1", etc as shown in Tables 1–3.

The 10 PK comparison studies were completed in the cynomolgus monkey across 4 Mab classes from 2009 to 2016. Although not specifically needed for PK studies, more than half were performed to GLP. Dose level selection was usually based around what was known for the innovator drug and was always a single dose. Standard laboratory PK blood sampling with subsequent analysis using a validated method occurred pre-dose and on a number of occasions post-dose. Some studies also included a PD endpoint and/or anti-drug antibody (ADA) response measurement assessed from blood sampling on various days of the study using standard laboratory techniques.

The 17 Mab toxicology comparison studies were performed in the cynomolgus monkey across 5 different Mab classes from 2009 to 2016, while toxicology comparison studies were performed in the rat with 7 non-Mab proteins from 2011 to 2013. All studies were carried out to GLP with dose level selection usually based on prior knowledge of toxicology testing with the innovator. Standard toxicology study designs occurred with assessment of clinical observations, bodyweights, food consumption, ophthalmoscopy, electrocardiography (non-human primate studies only), clinical pathology (haematology, clinical chemistry and urinalysis), organ weights plus macroscopic and histopathological examination. Toxicokinetic evaluation occurred on all occasions on various study days from blood samples using a validated method. Some studies also had evaluation of a PD endpoint (although generally not included in studies with non-Mab proteins) or for ADA response based on blood samples taken on a few occasions and analysed using validated methods and established laboratory techniques. However, although taken, analysis of ADA samples did not always occur, especially for studies with Mabs, presumably under the assumption that even if an antibody response had been induced, it had not interfered with assessment of exposure.

3. Results

Findings are summarised in Tables 1–3 Table 1 shows that for 10 PK comparison studies performed in the cynomolgus monkey across 4 Mab classes, exposure was reported as similar for innovator and biosimilar in all cases, with an overall mean exposure range (lower-to-higher difference between the 2 molecules of 68–150%). A degree of inter-animal variability in exposure was noted. It should be noted that due to the small numbers of animals used, each study was not statistically powered to formally assess biosimilarity. For the Mab class in which a PD marker was included (5 studies), the expected response was measured but with no obvious difference between innovator and biosimilar. Despite the relatively simple nature of this type of study, a range of study designs was noted including small (8) to large (48) animal numbers, use of one or multiple dose levels and actual dose/s used, as well as group size.

Table 1
PK findings for Mab biosimilar comparator studies.

Compound	Animal numbers	Study design	PK[a]	ADA	PD
Mab 1 5 studies (2009–2016)	12-48 monkeys used/study	Single IV dose: variation in control/no control and group size (although 3 studies used 3M + 3F), one/multiple dose levels, actual dose levels used in different studies, duration of study, one or both genders	Exposure range of 82–150% across studies	3 out of 5 studies – all animals positive	Expected PD response
Mab 2 3 studies (2010–2013)	12-28 monkeys used/study	Single IV dose: variation in one/multiple dose levels and group size, actual dose levels used in different studies, duration of study	Exposure range of 78–131% across studies	1 out of 3 studies – one animal positive	–
Mab 3 1 study (2013)	12 monkeys used	Single SC dose: no control, groups of 3M + 3F, one dose level	Exposure of 68–104%	–	–
Mab 4 1 study (2014)	8 monkeys used	Single IV dose: no control, groups of 2M + 2F, one dose level	Exposure of 70–94%	–	–

IV Intravenous, SC Subcutaneous, M Male, F Female, PK Pharmacokinetic, ADA Anti-drug antibody, PD Pharmacodynamic, - Not assessed.
[a] Cmax, AUC, T_{max}, $T_{1/2}$, clearance and volume of distribution measured.