# Pharmacology of Biosimilars

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Testing in animals is an old routine used for new drugs to avoid serious toxicity to humans. However, biological drugs may not always show a pharmacologic response in animal species; thus, the toxicity is an extension of the pharmacological response for biological drugs. Clinical pharmacology comparisons comprise the most relevant testing to support the biosimilarity of a biosimilar candidate. When a novel drug is developed, PK/PD testing is carried out on many volunteers to understand the diversity of disposition in terms of gender, age, and genetic distribution.

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## 1. Nonclinical Pharmacology

Testing in animals is an old routine used for new drugs [1] to avoid serious toxicity to humans. However, biological drugs may not always show a pharmacologic response in animal species; thus, the toxicity is an extension of the pharmacological response for biological drugs. The primary mechanism of action of biological drugs involves receptor binding. Suppose an animal species does not carry these receptors. Then, a pharmacological or toxicological response is not expected, unlike in chemical drugs, where both can be caused by multiple mechanisms and interactions with body tissues [2].

Another reason animal toxicology data are less relevant is how the testing is conducted. Generally, animal testing protocols require administering a higher dose to induce a toxic response; however, within this dose range, the responses are not expected to be linear, making it impossible to differentiate between compared products that are supposed to be the same. Animal testing is extensively conducted for biosimilars despite this knowledge and expertise, evidenced by the recent FDA and EMA filings. As an example, MVASI (bevacizumab) [3] reported five non-clinical investigations; the product Trazimera (trastuzumab) [4] was tested in mice to justify the receptor binding discrepancies, even though trastuzumab does not recognize the neu receptor; and another similar product submitted by Herzuma included many more animal studies [5], which the FDA did not review as irrelevant. Similar submissions were made for Renflexis and Inflectra, two biosimilars of infliximab [6][7], and the etanercept biosimilars Eticovo and Erelzi [8][9]. The highest number of animal studies, 15, was recorded for the epoetin biosimilar Retacrit [10]. The non-clinical development of the two pegfilgrastim biosimilars, Udenyca and Fulphilia [11][12], is notable for the different animal models; Udenyca reported a toxicity investigation in Cynomolgus monkeys, whereas Fulphilia reported a toxicity study in rats. The trend shown here for the US biosimilar filings extends to Europe, where, despite the availability of waivers from animal testing, the developers continued the testing [13].

Another controversial issue in animal studies is the use of non-human primates, which are the only species that may have relevant receptors; it is frequently recommended to conduct PK studies in a small number of animals, especially for monoclonal antibodies, as a measure of their molecular structure rather than toxicity. According to the WHO [14], “based on regulatory experience gained to date in marketing authorization applications for biosimilars, the need for additional in vivo animal studies would be expected to represent a rare scenario”. However, the guidelines in India take a very different view, stating, “Regarding the animal models to be used, the applicant should provide the scientific justification for the choice of animal model(s) based on the data available in scientific literature. However, if the pharmacologically relevant animal species are not available and appropriately justified, toxicity studies need to be undertaken either in rodent or non-rodent species” [15]. This requirement was put in place because India requires at least one animal toxicology study, and no studies are allowed on monkeys for religious reasons.

Stronger support for waiving animal pharmacology or toxicology testing comes from the recent advice by regulatory bodies suggesting that it is unnecessary to test new biological therapies in animals, even if an animal species can show a response unless there is carcinogenicity potential [16].

Generally, it is now believed that testing new drugs in animal species may lead to misguidance if the safety is based on animal testing, resulting in serious threats [17]. In addition, humanized or genetically modified animal species are generally considered to be less sensitive in demonstrating differences in the tested products [18]. With this evolving background, the testing of biosimilars seems redundant, as stated in the European Union legislation on the protection of animals used for scientific purposes [19] and the FDA/CDER advocacy to use new approach methodologies (NAMs) [20] in place of animal testing.

Human and animal cells, organoids, organs-on-chips, and in silico modeling are alternatives to animal testing models, enabling us to create better and more predictive scientific methods. In addition, to reflect changes in animal protection legislation, nonclinical in vivo testing has been substituted by in vitro assays in the previous 10 years [21]. These measures can help to reduce the use of animals. They also align with the EMA's Regulatory Science Strategy for 2025, aiming to create a more adaptive regulatory framework that promotes human and veterinary health [22].

Animal toxicological studies can be misleading if they rationalize discrepancies in impurities, posttranslational modifications, or antibody responses, since an animal model can justify these differences. For example, animal data were submitted in biosimilar applications [5] to substantiate such variability, but the FDA refused to accept the animal data.

More than 100 products have been approved by the EMA and FDA, and none of them have failed animal toxicological testing because they cannot, being least sensitive in detecting any difference between a biosimilar candidate and its reference product. These observations and conclusions are widely accepted as scientifically sound arguments [23][24], but among sponsors, there is always fear that study results will be rejected eventually. This would cause a delay in market access at a high cost, and therefore sponsors like to stay on the safe side by overpowering their studies.

## 2. Clinical Pharmacology

Clinical pharmacology comparisons comprise the most relevant testing to support the biosimilarity of a biosimilar candidate. When a novel drug is developed, PK/PD testing is carried out on many volunteers to understand the diversity of disposition in terms of gender, age, and genetic distribution. However, such a population is not needed for establishing biosimilarity; the purpose of clinical pharmacology studies for a new drug is to characterize its profile. In the case of biosimilars, it compares the profile. A smaller number of subjects can be enrolled in these studies by narrowing down the acceptance criteria that will be acceptable since agencies also recommend that there be no unnecessary exposure for humans [25]. This suggestion reduces the risk of study failure without compromising the purpose of these studies—to compare how the body sees the molecule and how the molecule sees the body.

Besides reducing the size of the study, as described above, this study model can combine antidrug antibody measurements in a parallel design that should be presented to regulatory agencies earlier in meetings with them [26].

The FDA Biosimilar Action Plan [27] also recommends employing in silico methodologies to compare biosimilars, including immunogenicity assessments. Since immunogenicity is entirely structure-dependent, better analytical assessment techniques give greater confidence in reducing or eliminating antidrug antibody testing. In addition, impurities and aggregates induce extrinsic immunogenicity, which may be easily measured and compared to a reference product as part of the analytical evaluation.

The immunogenicity of biological products is caused by the activation of B cells, which generate T cells to express antibodies. However, anti-drug antibodies can be harmful to healthy subjects in future studies. As a result, the FDA is researching new methods for determining immunogenic potential using tiny fragments of DNA-like molecules called aptamers to test proteins and establish their exact structures to avoid the exorbitant costs of forecasting which particular portions of such proteins will stimulate antibody production [26].

Finally, if the immunogenicity profile differs but cannot impact the disposition profile, the differences will be meaningless, as the FDA has acknowledged in its new guidance on insulins [28][29].

Following the idea that humans should not be subjected to unnecessary testing, the FDA has agreed to allow non-US reference products as long as they are approved using “essentially” the same dossier [30] and if an analytical bridging study is also conducted. However, many developers have instead chosen to conduct three-way studies using US and non-US reference standards; such studies are unnecessary.

PK/PD studies are essentially bioequivalence testing using the same statistical limits of 80–125% bioequivalence, a guideline that arose in the era of generic chemical drugs. Intravenously administered drugs were exempt from

bioequivalence testing because, by definition, they are 100% bioequivalent. However, in the case of the evaluation of biosimilars, this testing is intended as an additional assurance of structural similarity, which relates to how the body sees the molecule and how the molecule sees the body. It can be anticipated that above analytical assessment will become more convincing over time.

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