Renewing potency assays: Moving forward from traditional methods

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Abstract

Merck is global market leader in the fertility and growth hormone deficiency treatment. The quality control analytical panels for each new produced batch envisage the potency quantification that is estimated using a dedicated *in vivo* assay. Indeed, no *in vitro* methods for gonadotropin potency quantification are available in any pharmacopoeia. Merck Ivrea started a project to replace the *in vivo* assays with *in vitro* assays able to mimic the physiological mechanism of action of each gonadotropin and growth hormone.

Introduction

Merck is global market leader in the fertility and growth hormone deficiency treatment. The company releases on the market different drug products for the fertility treatments, such as Gonal-F (Follicle Stimulating Hormone, FSH), Ovidrel (Chorionic Gonadotripin, CG), Luveris (Luteinizing Hormone, LH), Pergoveris (Follicle Stimulating Hormone plus Luteinizing Hormone), and Saizen, for the growth hormone deficiency. The quality control analytical panels for each new produced batch envisage the potency quantification that is estimated using a dedicated *in vivo* assay.

As regards to gonadotropin hormones the *in vivo* potency methods are described in the European Pharmacopoeia 2285 and 9002 (FSH and CG *in vivo* bioassay) and in the British Pharmacopoeia 0498 (LH). On the other hand, no *in vitro* methods for gonadotropin potency quantification are available in any pharmacopoeia. The Company strives to substitute animal use whenever possible with equivalent *in vitro* assays in line with 3Rs (Replacement, Reduction, Refinement) mandated by Directive 2010/63/EU[1] (Protection of animals used for scientific purposes, European Union) and US Animal Welfare Act.

In vitro bioassays for fertility treatment products

Years ago, Merck Ivrea started a project to replace the three *in vivo* assay with three *in vitro* assays able to mimic the physiological mechanism of action of each gonadotropin. After the experimental development phase, the strategy for the first bioassay replacement was defined selecting the FSH bioassay as frontrunner by a global team made up by scientists with *in vitro* and *in vivo* expertise, regulatory affair experts and biostatisticians.

The formulated strategy foresees firstly the method robustness evaluation during the set-up phase, designed according to the principles of quality by design. The robustness evaluation starts with a risk analysis performed on each step of the *in vitro* bioassay. Only the most relevant ones, associated with a medium/high risk, are chosen to set-up a Design of Experiment in order to test the experimental space around variation of these critical factors. Once robustness is verified the second step is the formal method validation according to the ICH Q2 R1[2] and USP<1033>.[3] Only after the method is validated a comparability assessment can be performed. The aim of this study is to compare the *in vitro* and *in vivo* bioassay by testing the same samples in parallel to determine method equivalence. The comparability study is a fundamental step of the replacement of an *in vivo* bioassay to demonstrate that the product that will be released to the patients using the new bioassay will not show any differences in comparison with the product released by using the *in vivo* bioassay. For this reason, samples tested during the comparability must represent each different formulation present on the market, both as release and stability samples, with separate statistical evaluations. Moreover, FSH variants are produced in order to verify the ability of the method to detect out of specification samples such as oxidized forms, glycosylation variants, acidic and basic forms, dissociated subunits *etc*. The final step of this strategy is the submission of the change to all the relevant health authorities. In the case of FSH at Merck, this means to perform submission in more then 100 countries and maintain both *in vivo* and *in vitro* method up and running for different years until the last country approval.

In order to mitigate the risk related to the submission, Merck asked a scientific advice to FDA. A Type-C Meeting was called to ask the Agency's agreement on the *in vitro* method mechanism of action and overall design, the validation approach and the comparability strategy. The final outcome was positive, and FDA showed to appreciate the effort of Merck to find an alternative *in vitro* bioassay (the first ever proposed in the US market). In order to increase the chances of a positive submission, the Agency gave to Merck some hints related to the test suitability, the need for a continued supply, the strategy for standard calibration.

Saizen *in vitro* bioassay

The bio-identity test for growth hormone, also called somatropin, is performed in accordance with the *in vivo* method described in the "Somatropin bioidentity tests" USP<126>. The method, which consider somatropin-induced weight gain, requires hypophysectomized rats. Also in this case, Merck is conducting a project to replace the *in vivo* method by the *in vitro* one, recently included as alternative in the General Chapter USP<126>. After preliminary evaluation of the compendial procedure, a few modifications were introduced to improve performance of the method together with an assessment of its robustness.

An overall strategy for the submission of these changes to Health Authorities was designed, bringing together analytical and regulatory experts, and a Type C meeting with FDA was conducted to discuss the replacement of the *in vivo* bio-identity test with an *in vitro* one. The agency agreed on Merck approach for validation and compa-

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rability studies, therefore the method was successfully validated and afterwards, the comparability study will be performed.

Conclusions

Merck is strongly committed in the reduction of animal testing due to ethical reasons. Indeed, Merck is developing four *in vitro* assays to replace four *in vivo* assays currently used for market batch release of four drug products. Nevertheless, this is not a path free of obstacles in particular in a context of GMP Quality Control for market batch release.

In vivo methods are robust, precise and consolidated methods and *in vitro* methods must, at least, maintain the same performances. In this frame the development of *in vitro* methods able to mimic the molecule mechanism of action, precise, robust and easy to apply in a routine environment is crucial.

The *in vivo* methods analyse the sample potency in a complex system (the animal) providing a response belonging to absorption, distribution, metabolism and the activity at the biological target. Since *in vitro* methods are able to monitor only the biological activity at the target, the molecular critical quality attributes impacting the other phases must be monitored with additional orthogonal methods.

Finally, in order to completely replace an assay, envisaged in the release analytical panel of a marketed product, the change must be submitted in each country where the drug product is released. In the case of Gonal-F, for example, it means to perform the submission in more than 100 countries maintaining both *in vivo* and *in vitro* method up and running for different years until the last country approval.

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