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 Gonadotropin, 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 (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 one, 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 and USP<1033>. 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 considers 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-
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.

References

3. USP<1033> Biological Assay Validation.
4. USP<126> Somatropin Biodentity test.