FDA Raises the Bar in Bioanalytical Method Validation

For many years, the FDA guidance on bioanalytical method validation (BMV) issued by the CDER in 2001[1], has been the Holy Grail for laboratories which deal with the pharmacokinetic analysis of drugs and their metabolites in clinical trials. A revised version has been expected at least since the EMA issued its guideline on BMV in 2012[2], which is much more explicit and detailed in its requirements compared to the FDA guidance from 2001. The new FDA draft guidance is summarised in this editorial, and its implications for clinical studies are discussed.

The basic objective of method validation is to assess the performance of an analytical method before clinical trial samples with unknown concentrations of specific analytes are going to be measured. The core parameters of method validation are accuracy, precision, selectivity, sensitivity, reproducibility and sample stability. Further aspects are standard curve/response function, interference by other substances, specificity etc. These performance parameters have to be tested by each laboratory offering a specific method used for bioanalytical assessment of clinical trial samples, and summarised in a validation report. Bioanalysis, in its original sense, is focused on the analysis of drugs or drug candidates and their metabolites in plasma and urine, or other biological matrices. While the FDA guidance from 2001 was primarily written for chromatographic methods (especially LC-MS/MS), the EMA guideline also provided guidance for immunoassays like ELISAs and other ligand binding assays. Interestingly, the EMA has taken a very unambiguous position regarding the validation of biomarker assays for the assessment of pharmacodynamic endpoints as it is defined in the scope of the guideline: *'Methods used for determining quantitative concentrations of biomarkers used in assessing pharmacodynamic endpoints are out of the scope of this guideline.'* Therefore there is still a lack of regulatory guidance regarding the design of validation studies for biomarker assays under European regulation – it is basically not defined how to validate biomarker assays under current EMA guidelines.

However, the new draft guidance on bioanalytical method validation released by the FDA in September 2013[3] is taking a clear position on biomarkers. The FDA states, even in the first paragraph, that the principles of bioanalytical method validation are not only applicable to the methods used in pharmacokinetic studies, but also for the assessment of biomarkers. In the section 'Additional Issues, Biomarkers', the draft guideline explains: *'Biomarkers can be used for a wide variety of purposes during drug development; therefore, a fit-for-purpose approach should be used when evaluating the extent of method validation that is appropriate. When biomarker data will be used to support a regulatory action, such as the pivotal determination of safety and/or effectiveness or to support labeled dosing instructions, the assay should be fully validated.'* This clear statement confers some clarity to sponsors and central laboratories since neither the EMA guideline nor the FDA guidance from 2001 have claimed any applicability beyond the area of pharmacokinetics. With the new draft guidance, it is now clear that basically all methods used for the assessment of safety, efficacy and pharmacokinetics should be validated according to the same standards - provided that this guidance will come into force. For biomarkers that are primarily studied to better understand mode of action or other aspects of supportive information, a fit-for-purpose validation approach is obviously acceptable. However, there are two aspects that need further clarification:-

1. Does this need to fully validate methods used for pivotal biomarkers apply to classical clinical chemistry parameters, such as amino-transferases for monitoring liver toxicity, or creatinine for kidney function, as well? Examples for efficacy parameters might be LDL-cholesterol for monitoring the pharmacodynamic effect of statins, or ostase for controlling the effect of osteoporosis drugs. It remains to be seen whether the FDA also regards such classical clinical chemistry parameters as safety or pharmacodynamic biomarkers. Since these parameters can be of crucial importance for certain drugs and drug candidates, it should be expected that the FDA will recommend to validate these methods according to the new BMV guidance, as soon the revised version supersedes the guidance from 2001. On the contrary, one could argue that such parameters are sufficiently well-covered by proficiency testing and extensive experience with these frequently-assessed parameters, and therefore a partial validation might be sufficient. It has to be seen how the final version of the BMV guidance will deal with this question.
2. Is it appropriate to request the same performance in terms of accuracy and precision for biomarker assays as for bioanalytical assays? The FDA requests a maximal imprecision of 15 % (CV < 15 %) and a relative error of less than 15 % (RE < 15 %) for all chromatographic methods. These limits can both be expanded towards 20 % CV and 20 % RE for ligand binding assays. This is feasible for many assays, but there are situations where – especially at low concentrations – precision and accuracy will only reach CV and RE values of 30 %. In such situations, it is sometimes not possible to choose a different method because these are often parameters for which only a few vendors offer reagents or kits. Like the EMA guideline, the FDA also points out that commercial assays ('kits') need to be validated to the same standards as methods that have been developed by the laboratory which will conduct the analysis of clinical trial samples. Here it would be desirable if the FDA guidance would finally leave an option for using other means to obtain

biomarker data that can be used in clinical studies. One approach is the analysis of all samples of one subject in one analytical campaign to avoid inter-assay variability.

Another important requirement relates to the so-called ISR, incurred sample reanalysis: 5-7 % of the totally analysed samples need to be re-analysed to test reliability of the reported concentrations after storage. This applies not only to pharmacokinetic assays, but also to biomarker assays - a requirement that is not self-evident since the stability of biomarkers in their respective matrix, even after repeated freeze-thaw-cycles, is generally tested during validation studies. It has been requested by the EMA in the 2012 validation guideline as well, but was limited to bioanalytical applications. In combination with the generally relative wide acceptance criteria for ISR, and the fact that biomarkers are generally endogenous compounds, it might be questioned whether the reanalysis of biomarker samples is indeed increasing the reliability of biomarker data in clinical trials.

One difficulty scientists are facing when it comes to the assessment of accuracy and lower limit of quantification (LLOQ) for an endogenous biomolecule used as a biomarker, is the fact that this molecule is endogenously present in the clinical matrix. Therefore, it is not possible to assess the LLOQ by spiking known concentrations of the biomolecule to the analyte-free matrix as for xenobiotic compounds. The FDA does not generally recommend using artificial matrices for endogenous substances, but accepts such matrices if no other options are available. Furthermore, quality control samples can be prepared in clinically-relevant matrices by spiking known concentrations of the endogenous molecule to well-characterised samples of these matrices with known concentration levels of the same molecule. These suggestions are certainly helpful to address this issue.

Comparing the new draft guidance to the FDA guidance on BMV from 2001, the revision has led to a regulatory paper that is similar to the EMA BMV guideline from 2012 in many aspects. A detailed comparison between the FDA draft guidance and the EMA guideline from 2012 has recently been published[4]. The fact that the FDA BMV draft guidance requests to validate biomarker assays according to the same principles as pharmacokinetic methods, will most likely have the biggest impact on the conduct of clinical trials, and will endorse the role of specialised central labs for clinical trials in the assessment of such parameters.

References

1. Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001
2. EMEA/CHMP/EWP/192217/2009, Committee for Medicinal Products for Human Use (CHMP), Guideline on bioanalytical method validation
3. Guidance for Industry, Bioanalytical Method Validation, DRAFT GUIDANCE, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), September 2013
4. Zimmer D, New US FDA draft guidance on bioanalytical method validation versus current FDA and EMA guidelines: chromatographic methods and ISR; Bioanalysis (2014) 6, 13–19

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