Minireview

Preclinical biomarker qualification

John-Michael Sauer and Amy C Porter;
for the Biomarker Programs, Predictive Safety Testing Consortium

Critical Path Institute, 1730 E. River Road, Tucson, AZ 85718, USA
Corresponding author: John-Michael Sauer. Email: jsauer@c-path.org

Impact statement

This minireview provides an overview of the history of preclinical biomarker qualification by summarizing the three examples of this type of qualification with US Food and Drug Administration, European Medicines Agency, and Japanese Pharmaceuticals and Medical Devices Agency. In addition, an overview of the biomarker qualification process is included to educate key stakeholders with links to relevant white papers that provide information on current evidentiary considerations. The manuscript also provides new information on the evolution of the role that preclinical qualification plays in clinical qualification of biomarkers and the novel approaches that are being utilized to improve the process.

Abstract

Biomarkers are ubiquitously used within drug development programs in both nonclinical species and in humans to assess safety and efficacy of novel compounds. To routinely apply such novel biomarkers with certainty, a well-defined data package is necessary for review and endorsement by regulatory agencies including the US Food and Drug Administration, European Medicines Agency, and Japanese Pharmaceuticals and Medical Devices Agency. This type of endorsement is known as regulatory qualification. Novel approaches are being applied to speed the process, lower the resource intensity, and increase the accessibility of biomarker qualification data and it is likely that consortia will continue to play a fundamental role in the qualification process by bringing together like-minded stakeholders focused on specific tools to accelerate drug development. This article will focus on learnings from the previous three nonclinical biomarker qualification projects, as well as discuss the progression of preclinical biomarker projects into the clinical qualification space and the current strategy for the use of nonclinical biomarker data in the translational qualification of clinical biomarkers; much like nonclinical information is used in the approval of drug development candidates.

Keywords: Biomarker, regulatory qualification, preclinical, drug development tool, context of use, safety

Experimental Biology and Medicine **2018; 243: 222–227. DOI: 10.1177/1535370217743949**

Introduction

The objective of this minireview is to discuss the history and future of preclinical biomarker qualification, as well as describe the translational role that preclinical biomarkers play in the qualification process for clinical biomarkers. Over the past several years, we have seen the regulatory qualification process evolve across the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and Japanese Pharmaceuticals and Medical Devices Agency (PMDA). Although the processes and scientific strategies associated with qualification will continue to evolve, there is a significant amount of learning that can be taken from previous projects. This article will focus on learnings from the previous three nonclinical biomarker qualification projects, as well as discuss the progression of preclinical biomarker projects into the clinical qualification space.

Biomarkers are ubiquitously used within drug development programs in both nonclinical species and humans to assess safety and efficacy of novel compounds. In every case, these standard biomarkers are well accepted by the scientific community, including regulators. Innovative biopharmaceutical companies commonly use exploratory or novel biomarkers in Investigational New Drug or New Drug Application/Biologic License Application submissions. The use of novel biomarkers is done without the need for FDA reviewers to extensively evaluate the suitability of the biomarkers as their use is limited to supporting a specific drug development program. However, in some cases, a novel biomarker will be applicable for use across multiple drug development programs. To routinely apply such novel biomarkers with certainty, a well-defined data package is necessary for review and endorsement by regulatory agencies. This type of endorsement is known as regulatory qualification.

Regulatory qualification is the formal regulatory endorsement or acceptance of a drug development tool for a specific context of use (COU). The qualification of a biomarker results in certainty as to how the biomarker can be applied and how the biomarker data generated in drug development programs should be interpreted by both drug developers and regulatory authorities. The qualification process was initiated in response to the Critical Path Initiative which is FDA's strategy to drive innovation in the scientific processes through which medical products are developed, evaluated, and manufactured.[1] Both the EMA and PMDA defined a similar approach to qualification following the initial lead of the FDA. Qualification of novel nonclinical biomarkers for use in animal models provides a regulatory endorsed tool to assess the safety and efficacy of new drugs prior to clinical development with the next step being translation of the preclinical biomarker to a qualified clinical biomarker.

The FDA's Biomarker Qualification Program (BQP) is designed to provide a mechanism for external stakeholders to work with the Center for Drug Evaluation and Research (CDER) to develop biomarkers for use as tools in the drug development process.[2] The goals of the BQP are to provide a platform to (1) qualify biomarkers and make supporting information publicly available, (2) facilitate uptake of qualified biomarkers in the regulatory review process, and (3) encourage the identification of new biomarkers for use in drug development and regulatory decision-making.[3] Terms used in biomarker qualification have been defined by the FDA-NIH Biomarker Working Group and can be found in the BEST (Biomarkers, EndpointS, and other tools) Resource.[4]

> A biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feels, functions, or survives".[4]

> Qualification is defined as "a conclusion, based on a formal regulatory process, that within the stated context of use (COU), a medical product development tool can be relied upon to have a specific interpretation and application in medical product development and regulatory review."[4]

Once a biomarker is qualified, it can be used for the qualified COU in drug development programs without the need for CDER to re-review the supporting information.

> The Context of Use (COU) is "a statement that fully and clearly describes the way the medical product development tool is to be used and the medical product development-related purpose of the use."[4]

A biomarker's COU is proposed early in the biomarker qualification process, at the Letter of Intent stage,[5] as it is the basis of the level of evidence that needs to be considered for qualification. The COU may be modified, as needed, as new data are acquired. The COU consists of a concise "Use Statement" containing the biomarker's name, identity and proposed use in drug development, as well as the "Conditions for Qualified Use," a comprehensive description of how the biomarker will be used in the qualified setting.[6]

One of the most challenging issues in the process is defining the level of evidence needed to achieve qualification. This has been an ongoing topic of conversation ever since the start of the FDA's BQP, nearly 10 years ago. Recently, several key white papers have been authored defining an approach to align scientific and regulatory expectations around qualification at least with respect to the FDA. In April 2016, key stakeholders including FDA CDER, Critical Path Institute (C-Path), and the Foundation for the National Institutes of Health (FNIH) Biomarkers Consortium held a workshop to develop an evidentiary criteria framework for safety biomarker qualification.[7] The resulting white paper[8] delineates the proposed framework and provides specific examples of its applicability to clinical safety biomarkers. In November 2016, a conference led by C-Path was held at the Duke Margolis Center for Health Policy to bring together key stakeholders to discuss a draft framework outlining key criteria and best practices for biomarker assay performance expectations and validation.[9] A draft white paper[10] was prepared in advance of the public forum and input and feedback was solicited. Currently, the framework is being utilized by biomarker qualification stakeholders to evaluate assay acceptability in ongoing and planned biomarker qualification projects.

Regardless of the species of interest, the approach to qualification is similar. In general, correlation of the biomarker's response with a change in the biological process of interest is demonstrated. Furthermore, it must be demonstrated that the biomarker's response is exclusively related to the change in the biological process. To date, all the nonclinical qualifications accepted by the regulatory agencies (FDA, EMA, and PMDA) have been safety biomarkers. In each of these cases, the preclinical qualification project utilized histopathology as evidence that the biomarker's response was associated with the organ toxicity of interest.

The Critical Path Institute's Predictive Safety Testing Consortium

The most active group in the safety biomarker qualification space has been C-Path's Predictive Safety Testing Consortium (PSTC). This consortium brings together pharmaceutical companies to share and validate innovative safety testing methods under advisement of the FDA, EMA, and PMDA.

The mission of PSTC is to identify new and improved safety testing methods (safety biomarkers) and submit them for formal regulatory qualification by the FDA, EMA, and PMDA. PSTC's members share their data and

expertise as collaborators to achieve this mission. Qualified safety biomarkers provide a clear and easily measurable indication of organ injury, providing all parties involved standardized, reliable tools to aid in drug development and regulatory review of new drugs in order to speed new drugs to patients in need.

PSTC was initially established with the primary aim of qualifying novel nonclinical safety biomarkers for use in nonclinical safety assessment studies to support drug development. As discussed elsewhere, most novel safety biomarkers being evaluated by PSTC have been correlated with histopathological changes in target organs and are designed to substitute for histopathological evaluation in its absence.[11,12] The PSTC nonclinical qualification strategy is to use specific prototypical organ toxicants in rats, dogs, and nonhuman primates to define correlations between novel biomarker response and histopathological changes. Currently, the primary target organs of interest for PSTC are the liver, kidney, testis, pancreas, heart, skeletal muscle, and vascular system.

It is important to note that more recently the mission of PSTC has evolved beyond nonclinical qualification and is now focused on the qualification of biomarkers for use in clinical trials. PSTC has derived a translational qualification strategy for gaining the acceptance of novel clinical biomarkers that leverages nonclinical characterization of the biomarker and the correlative relationship of the biomarker's response to histopathology in preclinical species as part of the clinical qualification process. This approach is described in more detail later in this article.

Qualified preclinical biomarker projects

Below is a synopsis of three nonclinical biomarker projects that have led to the qualification of 10 novel preclinical biomarkers. At this point, across all requestors, only safety biomarkers have been qualified in the preclinical space. The FDA has qualified eight novel urinary kidney safety biomarkers for use in the rat and two novel cardiac safety biomarkers in the rat and dog. The EMA has also qualified eight urinary kidney safety biomarkers for use in the rat, while the PMDA has qualified seven urinary kidney safety biomarkers for use in the rat.

Qualification of seven biomarkers of drug-induced nephrotoxicity in rats

The first nonclinical biomarkers were qualified by C-Path's PSTC in 2008. The qualified urinary kidney safety biomarkers include kidney injury molecule 1 (KIM-1), albumin, total protein, β2-microglobulin, cystatin C, clusterin, and trefoil factor-3. In April 2008, the FDA stated that these biomarkers are "acceptable biomarkers for the detection of acute drug-induced nephrotoxicity in rats and can be included along with traditional clinical chemistry markers and histopathology in toxicology studies."[13] In January 2009, the EMA published their "Final Conclusions on the Pilot Joint EMEA/FDA XVDS Experience on qualification of the Nephrotoxicity Biomarkers" in support of the qualification of the same seven nonclinical safety biomarkers.[14] In May 2010, the Japanese PMDA also qualified these seven novel kidney safety biomarkers for use in nonclinical studies.[15]

In 2008, the FDA accepted these biomarkers for voluntary use along with blood urea nitrogen (BUN) and serum creatinine (sCr) in rat kidney safety assessment studies that use histopathology as the gold standard. The data presented by PSTC in support of the biomarkers demonstrated that in most cases, the novel biomarkers were more sensitive and specific for kidney injury when compared to BUN and creatinine. KIM-1, albumin, clusterin, and trefoil factor-3 were approved by FDA for use as biomarkers of drug-induced acute kidney tubule alterations in Good Laboratory Practice (GLP) rat studies used to support clinical trials. Total protein, β2 microglobulin, and cystatin C were approved by FDA for use as biomarkers of drug-induced acute glomerular alterations, damage and/or impairment of kidney tubular reabsorption in GLP rat studies used to support clinical trials.

In addition to the outline of the conditions for the qualified use of these biomarkers, FDA also provided information necessary to support potential future clinical use of these nonclinical biomarkers. They suggested that human qualification studies demonstrating the "pattern of elevation and the degree and timeframe of reversibility of elevation of these markers after human exposure to known nephrotoxicants such as aminoglycosides, may be helpful in moving these markers into clinical use."

These kidney safety biomarkers were also qualified by EMA in a joint process with FDA. Like FDA's statement, EMA considered the biomarkers "acceptable in the context of non-clinical drug development for the detection of acute drug-induced nephrotoxicity, either tubular or glomerular with associated tubular involvement." EMA went on to say that the biomarkers provide "additional and complementary information to BUN and serum creatinine to correlate with histopathological alterations considered to be the gold standard." EMA requested additional data on the correlation between the biomarkers and the evolution and reversibility of acute kidney injury, and information on species-specificity.

For qualification of the seven kidney safety biomarkers with PMDA, three consultation items were part of the discussion. First PSTC's nonclinical results obtained for the seven biomarkers were discussed. Next, PSTC sought to reach agreement with PMDA that the data supported qualification of the biomarkers, and finally PSTC asked to present the strategy for additional studies to gain broader acceptance and to better understand the utility of the biomarkers. PMDA considered the data presented acceptable for use of the seven biomarkers to detect drug-induced acute urinary tubular changes or acute glomerular changes/injury in rat GLP studies, when used in combination with existing biomarkers (e.g., sCr and BUN).

Qualification of three proposed urinary biomarkers of drug-induced nephrotoxicity in rats

In September 2010, the International Life Sciences Institute's Health and Environmental Sciences Institute

(HESI) qualified two biomarkers of drug-induced nephrotoxicity in rats, urinary clusterin and renal papillary antigen (RPA-1), with FDA.[16] The COU for clusterin is the "detection of acute drug-induced renal tubule alterations, particularly when regeneration is present, in male rats when used in conjunction with traditional clinical chemistry markers and histopathology in GLP toxicology studies for drugs for which there is previous preclinical evidence of drug induced nephrotoxicity or where it is likely given the experience with other members of the pharmacologic class." FDA acknowledged that biomarker qualification is considered to be an "incremental process" and that there is support for "submission of additional animal and human data to support further application contexts for biomarkers." In the letter to HESI to qualify clusterin, the FDA mentioned the 2008 qualification of clusterin[13] and stated that the 2010 HESI submission supported the previous (2008) conclusion and clarified the COU for clusterin.

Also in this HESI submission, RPA-1 was qualified by FDA for "voluntary use in detecting acute drug induced renal tubule alterations, particularly in the collecting duct, in male rats when used in conjunction with traditional clinical chemistry markers and histopathology in GLP toxicology studies for drugs for which there is previous preclinical evidence of drug induced nephrotoxicity or where it is likely given the experience with other members of the pharmacologic class." Alpha-glutathione S-transferase (α-GST) was also submitted to FDA by HESI, for qualification along with clusterin and RPA-1. But α-GST was not qualified by FDA at that time because it was found to either increase or decrease depending on the location of the renal injury, which might confound data interpretation. Given the limited data available on α-GST, there was a recommendation from FDA to address the α-GST assay to determine if "interfering substances, dilutional effects or cross reactivity of other GST isoforms" might explain the apparent decrease in α-GST seen with collecting duct injury.

As in 2008, the FDA was very specific that these biomarkers were not qualified to monitor for drug-induced nephrotoxicity in the clinical setting. In addition, these biomarkers were tested only in male rats and FDA suggested that the experiments should be repeated in female rats and other species when assays are available. It was also recommended that studies be done to determine if these biomarkers could detect drug-induced renal injury earlier than histophathological lesions, and whether they could be used to follow reversibility or recovery from injury.

Similar to FDA, EMA pointed to their previous qualification of clusterin in 2008[14] and stated that HESI data increased the evidence for clusterin's use as a nonclinical biomarker of drug-induced nephrotoxicity. EMA also made some recommendations for methodological considerations for future qualification experiments around more robust replication evidence, normalization practices, and blinding of histopathology reading.

Qualification of circulating cardiac troponins

In 2012, representatives from University College, Dublin, Ireland; Pfizer Inc, Groton, CT, USA; GlaxoSmithKline, Ware, Herts, UK; and AstraZeneca, Macclesfield, UK, qualified circulating cardiac troponins T (cTnT) and I (cTnI) for use in nonclinical drug development studies in rats, and dogs for three different COUs.[17] The first COU for instances when there is a "previous indication of cardiac structural damage with a particular drug" states that "cardiac troponin testing can help estimate a lowest toxic dose or a highest non-toxic dose to help choose doses for human testing." The second COU, for instances when "there is known cardiac structural damage with a particular pharmacologic class of a drug and histopathologic analyses do not reveal structural damage" states that "circulating cardiac troponins may be used to support or refute the inference of low cardiotoxic potential." The third COU for instances when "unexpected cardiac structural toxicity is found in a nonclinical study" states that the "retroactive ('reflex') examination of serum or plasma from that study for cardiac troponins can be used to help determine a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL)." This retroactive study may then support utilization of this biomarker in future safety testing.

The lack of data was cited by FDA as the reason that these biomarkers were not qualified in nonhuman primates (NHP) and a recommendation was made to add to the NHP data. To gain confidence that the lack of increase in cTnT or cTnI is correlated with the absence of cardiac damage, FDA suggested further assay validation, knowledge of the time course of the damage done by the drug, and information regarding the relevance of the nonclinical metabolite profile to humans.

Translating nonclinical qualifications into clinically useful biomarkers

The current strategy for the use of nonclinical biomarker data is in the translational qualification of clinical biomarkers; similar to the way nonclinical information is used in the approval of drug development candidates. For safety biomarkers, this approach uses nonclinical data to develop the relationship between the tissue injury as assessed by histopathology (the truth) and the response of the safety biomarker. This allows for a direct comparison of the novel biomarker to the current standard, and for an evaluation of the superiority of the novel biomarker's sensitivity and specificity. Likewise, characterizing the stability of the biomarker's baseline or control reference range is important to understanding the biomarker's utility. Although this approach is nearly identical to that used for preclinical qualification, preclinical qualification does not require the same level of evidence and only serves to provide the underpinning for the clinical qualification efforts.

The next step in the clinical qualification of translational safety biomarkers is the comparison of the performance of the novel biomarker and current standard biomarker(s) in humans with and without drug-induced tissue injury. As in nonclinical studies, the characterization of the control or healthy subject reference range, definition of medically important cut-points, and the specificity of the biomarker are all required for clinical qualification of safety

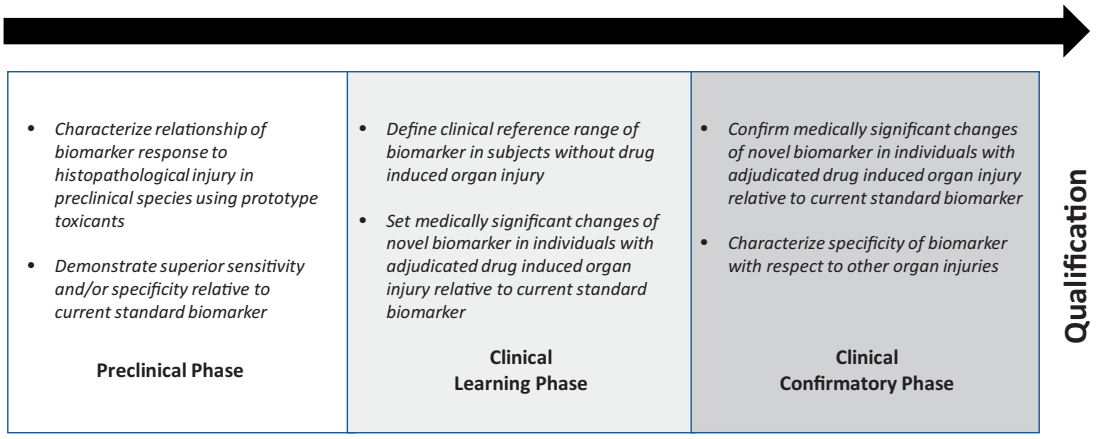

Figure 1. Stages of qualification for novel translational safety biomarkers for use in clinical trials.

biomarkers. A generalized roadmap to the qualification of translational safety biomarkers for use in clinical trials is shown in Figure 1.

Conclusion

Although there are several examples of the qualification of preclinical safety biomarkers with regulatory authorities, current thinking is that qualification is not necessary for preclinical biomarkers because the gold standard of histopathology can be routinely utilized. Therefore, the current strategy for the use of nonclinical biomarker data is in the translational qualification of clinical biomarkers; much like nonclinical information is used in the approval of drug development candidates.

The regulatory qualification of biomarkers results in certainty in how the biomarkers can be used and interpreted in drug development studies. Although the qualification process has evolved significantly over the last 10 years, efforts over the past two years have better defined the scientific and regulatory expectations for the successful qualification of biomarkers across all stakeholders. In the further progression of biomarker qualification, novel approaches are being applied to speed the process, lower the resource intensity, and increase the accessibility of biomarker qualification data. Finally, it is likely that consortia will continue to play a fundamental role in the qualification process by bringing together like-minded stakeholders focused on specific tools to accelerate drug development.

Author contributions: All authors participated in the drafting, review, and finalization of this manuscript.

DECLARATION OF CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

There was no funding to support this minireview.

REFERENCES

1. US Food and Drug Administration. *FDA's Critical Path Initiative*. Critical Path Initiative, www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/ucm076689.htm (2004, accessed 27 July 2016)
2. Biomarker Qualification Program, www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm20086360.htm (accessed 21 February 2017)
3. Amur SG, Sanyal S, Chakravarty AG, Noone MH, Kaiser J, McCune S, Buckman-Garner SY. Building a roadmap to biomarker qualification: challenges and opportunities. *Biomarkers Med* 2015;**9**:1095–105
4. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource, www.ncbi.nlm.nih.gov/books/NBK338448/ (2016, accessed 4 May 2016)
5. US Food and Drug Administration. *Submission resources by stage*. Biomarker Qualification Program, www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm535890.htm#1 (accessed 12 September 2017)
6. US Food and Drug Administration. *Context of use for biomarker qualification*. Biomarker Qualification Program, www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm535395.htm (accessed 12 September 2017)
7. FNIH/FDA Evidentiary Criteria Writing Group. *Framework for defining evidentiary criteria for biomarker qualification*, https://fnih.org/what-we-do/biomarkers-consortium/programs/framework-for-safety-biomarkers (2016, accessed 12 September 2017)
8. Evidentiary Criteria Writing Group. *Framework for defining evidentiary criteria for biomarker qualification*, https://fnih.org/sites/default/files/final/pdf/Evidentiary%20Criteria%20Framework%20Final%20Version%20Oct%2020%202016.pdf (2016, accessed 8 November 2017)
9. Biomarker Assay Collaborative Evidentiary Considerations, Writing Group, Critical Path Institute (C-Path). *Scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices*, https://healthpolicy.duke.edu/events/scientific-and-regulatory-considerations-analytical-validation-assays-used-qualification (2016, accessed 12 September 2017)
10. Biomarker Assay Collaborative Evidentiary Considerations Writing Group, Critical Path Institute (C-Path). *Points to consider document: scientific and regulatory considerations for the analytical validation of assays used in the qualification of biomarkers in biological matrices*, https://healthpolicy.duke.edu/sites/default/files/atoms/files/white_paper_draft_11_4_16.pdf (2016, accessed 8 November 2017)
11. Dieterle F, Perentes E, Cordier A, Roth DR, Verdes P, Grenet O, Pantano S, Moulin P, Wahl D, Mahl A, End P, Staedtler F, Legay F, Carl K, Laurie D, Chibout SD, Vonderscher J, Maurer G. Urinary clusterin, cystatin C,

[beta]2-microglobulin and total protein as markers to detect drug-induced kidney injury. *Nat Biotechnol* 2010;**28**:463–9

12. Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, Muniappa N, Thudium D, Gerhold D, Holder DJ, Bobadilla NA, Marrer E, Perentes E, Cordier A, Vonderscher J, Maurer G, Goering PL, Sistare FD, Bonventre JV. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nat Biotechnol* 2010;**28**:478–85
13. US Food and Drug Administration. *FDA qualification of seven biomarkers of drug-induced nephrotoxicity in rats*, www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/UCM285031.pdf (2008, accessed 19 May 2017)
14. European Medicines Agency. *EMA final conclusions on the pilot joint EMEA/FDA VXDS experience on qualification of nephrotoxicity biomarkers*, www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004205.pdf (2009, accessed 19 May 2017)
15. Japan Pharmaceuticals and Medical Devices Agency. *PMDA record of the consultation on pharmacogenomics/biomarkers*, www.pmda.go.jp/files/000160006.pdf (2010, accessed 19 May 2017)
16. US Food and Drug Administration. *HESI biomarker qualification decision*, www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/UCM285010.pdf (2010, accessed 12 September 2017)
17. US Food and Drug Administration. *Biomarker qualification decision: preclinical cTnT and CTnI*, www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/UCM294644.pdf (2012, accessed 12 September 2017)