



Process map proposal for the validation of genomic biomarkers

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How can we encourage the application of novel genomic biomarkers in drug development? A major step in this direction would be a consensus on how to interpret results from measurements of these biomarkers in regulatory submissions. A transparent process for genomic biomarker validation would be of value both for the pharmaceutical industry as well as for regulatory agencies associated with it. A discussion on process map proposals for genomic biomarker validation can help with drafting of guidance documents for this process.

A key to the transformation of the drug development paradigm has been described as a progressive reduction of uncertainty about effects or/and an increasing level of confidence about outcomes using biomarkers [1]. Biomarkers contributing to this transformation often contain multidimensional and not binary information. New biomarkers can revolutionize both the development and use of therapeutics, but may require commercial development of novel biomarker technologies and regulatory pathways for the efficient development of therapeutic/biomarker pairs.

Processes for the submission and review of pharmacogenomic and toxicogenomic data to the US FDA (FDA) are being established from work within the agency leading to documents summarizing the science, the regulatory expectations, and new approaches to the interaction between sponsors and the FDA. The framework for this work was outlined in the document *Guidance for Industry: Pharmacogenomic Data Submissions* [101] published on March 22, 2005. The guidance:

- Introduces a classification for genomic biomarkers
- Clarifies what type of genomic data needs to be submitted to the FDA and when this data should be submitted
- Introduces a new data submission pathway to share information with the FDA on a voluntary basis
- Encourages the voluntary submission of exploratory genomic data
- Introduces new agency-wide interdisciplinary pharmacogenomic review group (IPRG)
- Clarifies how the FDA will review genomic data submissions

This guidance introduces the concept of, and definition for, exploratory and valid biomarkers. It further defines two categories for valid

biomarkers: 'probable' and 'known'. Known valid biomarkers are defined as biomarkers that are measured in an analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiological, toxicological, pharmacological, or clinical significance of the results. Probable valid biomarkers are defined as biomarkers that are measured in an analytical test system with well-established performance characteristics, and for which there is a scientific framework or body of evidence that appears to elucidate the physiological, toxicological, pharmacological, or clinical significance of the test results. These markers are not categorized as 'known valid' because:

- Data elucidating its significance may have been generated within a single company and may not be available for public scientific scrutiny
- Data elucidating its significance, although highly suggestive, may not be conclusive
- Independent verification of the results may not have occurred

Exploratory biomarkers are potential precursors for probable or known valid biomarkers. As hypothesis-generating agents, exploratory biomarkers can be used to fill in gaps of knowledge about disease targets and variability in drug response, bridge the results of animal model studies to what may be expected in the clinic, or can be used for the selection of new compounds and the improvement of anticipated cost-benefit ratios for future drug development programs.

The guidance next defines what type of reports should be submitted to the FDA depending on the classification of the biomarker included in the submission (Table 1). It is in these reports that a particularly compelling argument may be made for the validity of genomic biomarkers in a

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Medicine

Table 1. Biomarker classification and pharmacogenomic submissions*.

Data to be submitted	IND	New (unapproved) NDA, BLA, or supplement	Previously approved NDA or BLA
Known valid biomarker	Must be submitted, pursuant to 21 CFR 312.23 (a) (8), (9), (10) (iv) or (11)	Must be submitted, pursuant to 21 CFR 314.50 and 601.2. See section IV.B. of the guidance	Must be submitted pursuant to 21 CFR 314.81 in annual report and should be submitted pursuant to § 601.12 as synopses or abbreviated reports
Probable valid biomarker	Does not need to be submitted if not used by the sponsor in decision making. The FDA welcomes voluntary submission of such data in a VGDS	The FDA recommends submission, using algorithm in section IV.B. of the guidance	Must be submitted pursuant to 21 CFR 314.81 in annual report and should be submitted pursuant to § 601.12 as synopses or abbreviated reports
Exploratory or research pharmacogenomic data	The FDA welcomes voluntary submission of such data in a VGDS	The FDA recommends submission, using algorithm in section IV.B. of the guidance. The FDA welcomes voluntary submission of such data in a VGDS	The FDA welcomes voluntary submission of such data in a VGDS

*Data obtained from [101].

BLA: Biologics license application; CFR: Code of Federal Regulations; IND: Investigational new drug; NDA: New drug application; VGDS: Voluntary genomic data submission.

regulatory context. However, the guidance does not specify the validation process converting exploratory biomarkers into valid biomarkers, and probable valid into known valid biomarkers.

How should genomic biomarkers be validated?

The application and use of biomarkers closely tracks with the regulatory function of an agency. The FDA, as a regulatory agency charged with ensuring the safety and efficacy of drugs, should define a process (or processes) expected to be undertaken by sponsors to validate genomic biomarkers relevant to their submissions. Since no such process exists today for at least preclinical biomarkers, it is reasonable to expect that the regulatory agency will need to design a validation process that allows consensus-building with sponsors regarding the validity of biomarkers. It will also need to support the approval of genomic biomarker validation with the application of this process.

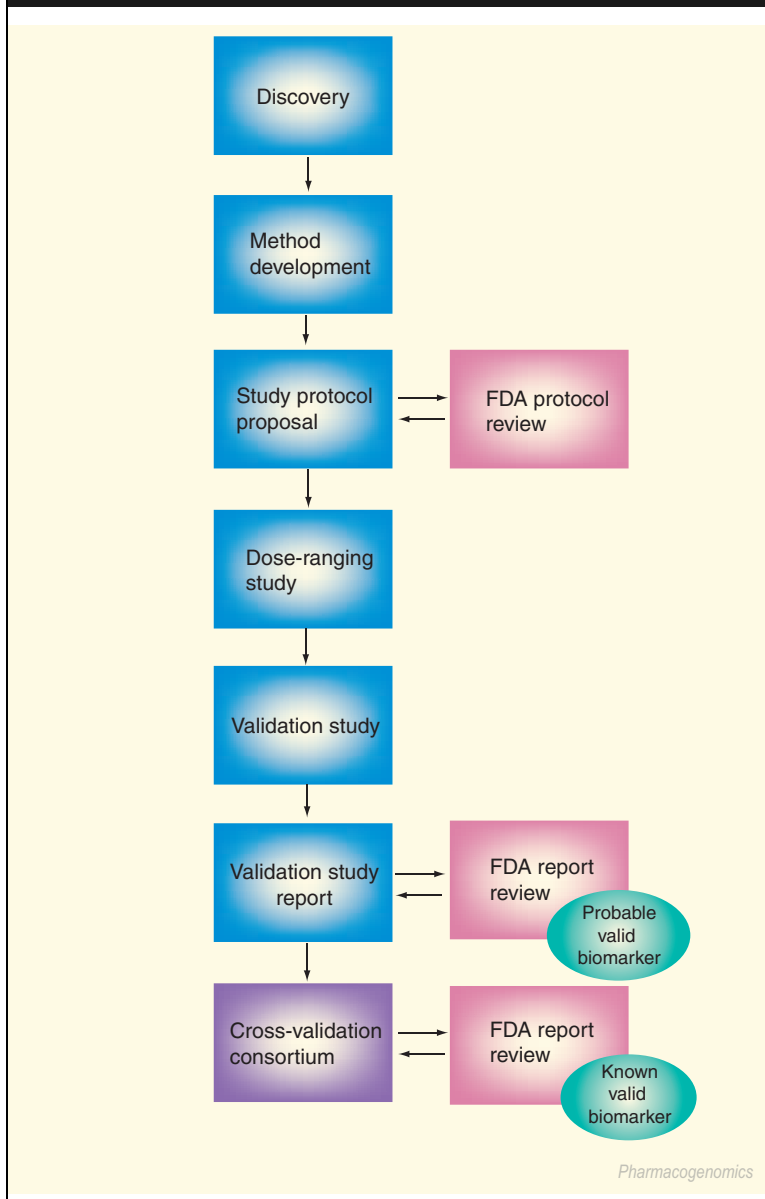
There are multiple areas for the application of genomic biomarkers. Process maps for the validation of preclinical and clinical genomic biomarkers are likely to share some steps and to have some steps that are likely to be specific for each area. Figure 1 shows a proposal for process maps for validation of preclinical genomic biomarkers. It is worthwhile to examine how this

proposal was developed, as well as how their expected outcomes compare with work on genomic biomarkers reported thus far.

Proposal for a process map for validation of genomic biomarkers of preclinical drug safety assessment

Genomic biomarkers have been embedded in preclinical drug development over the past decade [2]. An interesting dichotomy has developed over this period between the perception and use of genomic biomarkers of preclinical efficacy and genomic biomarkers of preclinical safety. Genomic biomarkers of preclinical efficacy have been actively developed and used in drug development [3]. Results from studies with these markers in the prioritization of compounds as ligands for specific targets, or in troubleshooting problems with these compounds in development, have often been reported both through regulatory submissions [4] and through publications in peer-reviewed journals [5]. Genomic biomarkers of preclinical efficacy are often proprietary and linked to the development of specific compounds within a single company. It is reasonable to assume that most pharmaceutical companies have, at one time or another, actively identified, developed and applied genomic biomarkers of efficacy during their drug development processes.

Figure 1. Proposal for a preclinical drug safety evaluation genomic biomarker validation process map.



The contrast to the corresponding application of genomic biomarkers of preclinical drug safety assessment is remarkable. Proprietary applications of most genomic biomarkers of efficacy in this context often lead sponsors to validation studies circumscribed to their own companies. Data on the validity of genomic biomarkers of efficacy will often be identified within one development program, one therapeutic area or one particular company. In contrast, it is reasonable to expect that data on the validity of genomic biomarkers of safety will apply to, and be easily exchanged by, multiple companies, across therapeutic areas and development programs. Consortia of multiple

companies for the cross-validation of genomic biomarkers of safety are likely to find common benefits for all members, as well as sufficient data to lead to ‘know valid biomarker’ status. These consortia will benefit from available public databases for genomic biomarkers.

At the FDA, exploratory genomic biomarkers of preclinical safety assessment have been reviewed to assess the potential of genomic technologies in ‘mock’ submissions [6], as well as to identify the statistical, biological and toxicological parameters that are likely to determine the success of these biomarkers in voluntary genomic data submissions (VGDS) [7,101]. The VGDS format has opened a scientific dialog between the FDA and sponsors on these biomarkers. Information leading to validation process map proposals has been freely exchanged in these meetings.

The proposal in Figure 1 consists of several intuitive steps leading from an exploratory to a known valid genomic biomarker for preclinical drug safety, as listed here:

- Discovery
- Method development
- Study protocol proposal
- Regulatory protocol review
- Dose-ranging study
- Validation study
- Validation study report
- Regulatory study report review
- Cross-validation consortium

Discovery

The identification and selection of genomic biomarker candidates for preclinical drug safety is often initiated with a high-density hybridization array. A toxic end point can be defined by dose, time, gender and genetic relationship. Biological pathways associated with mechanistic biomarkers are identified in this step, and a list of references supporting this association can be provided. Data submitted through a VGDS of the results from this step could be used to provide early feedback from the regulatory agency regarding additional work needed. Go/no go decisions over whether to proceed with the method development step will depend on the quality of the data and information collected in this step.

Method development

The platform applied in discovery is developed into an analytical platform. For example, lower density hybridization or quantitative polymerase chain reaction (PCR) arrays could be used to

measure expression level changes for a subset of genes discovered using the original platform. A go/no go decision over whether to proceed with the study protocol proposal step will depend on whether specifications for biological matrices, sample volumes, assay sensitivity, specificity and reproducibility can be satisfactorily determined.

Study protocol proposal

Key sections of the validation protocol to be provided to the regulatory agency should include:

- Goal
- Analytical assay definition
- Identification of samples in which the biomarkers will be measured
- Statistical analysis plan outlining method and acceptance criteria
- Definition of data set for validation
- Rationale for sampling
- Exposure data
- Definition of use of biomarker as a mechanistic, diagnostic or predictive candidate
- Power calculations for accuracy, specificity and sensitivity
- Plan for future cross-species comparison
- Preliminary dose-ranging step
- Knowledge about compound pharmacology
- Knowledge about compound toxicity
- Number of compounds
- Replicate animals in final study
- Necropsy plan
- Clinical pathology
- Histopathology

A go/no go decision to submit the study protocol proposal to the regulatory agency for protocol review will be made depending on the availability of supporting data and a complete draft protocol.

FDA protocol review

The design of validation protocols is context-specific. An open dialog between the sponsor and the agency is important in the review of validation protocols by the FDA. The details of a process for submission and feedback are likely to include guidance about the document to be submitted with written feedback and a face-to-face meeting. Multiple review functions within the agency will participate in this review process. The Genomics Group will be joined in this review function by pharmacology/toxicology reviewers, as well as by reviewers from clinical divisions. It is anticipated that this could be an iterative process: the go/no go point for this review will include an approval or rejection of the protocol or the request for additional information before a second review cycle.

Dose-ranging study

The dose-ranging study is designed to set standards and quality control metrics for the subsequent validation study. These would include minimal evidence that the dose selection will have an effect on the marker and current standards, such as histopathology. Previously published studies would suggest the starting doses for this study. Available data on drug quality and formulation as well as exposure would be assessed at this step. Dose multiples would be selected through classical criteria: a low dose close to the pharmacological dose and a high dose with target organ toxicity confirmed by histopathology. A go/no go point to proceed with the validation study would be reached if the data confirms dose choices and target toxicity.

Validation study

A validation study should include a compound set with diverse structural classes sharing similar toxicological end points. Doses for these compounds should span ranges over which a range of lesion severity will occur. The concept of validation-in-context is particularly critical in a validation study: a diagnostic biomarker where the temporal dependence of a signal coincides with that for the toxicological end point will require study protocols shorter than those for a predictive marker that may eventually be developed into a predictive test. The validation protocol should also include control compounds from the same structural classes not capable of inducing lesions in the target organ. Replicate samples are required to allow the detection of statistically significant differences in expression levels for genomic biomarkers candidates. A go/no go point will be reached if the study results confirm the proposed mechanistic, diagnostic or predictive context for genomic biomarker candidates.

How would a sponsor know at this stage that a validation study has succeeded in this confirmation? This is the same question that reviewers at the agency will need to ask when results of these studies are evaluated. A protocol designed following guidelines outlined in the last paragraph will probe the context in which a biomarker may be considered valid. In the context of toxicogenomics, a validation protocol design for a mechanistic biomarker may focus on how changes observed in expression levels for a genomic biomarker or biomarker set reflect specific biological pathways associated with the development of a lesion, and whether these changes are also associated with the biological mechanism-of-action of the compound tested. Diagnostic biomarker validation in toxicogenomics would require protocols with which to

establish a concurrent correlation between an established metric for damage, such as histopathology, and changes in expression levels for genomic biomarkers or biomarker sets. Predictive biomarkers would require protocols that could rigorously show a correlation between the early detection of changes in expression levels and eventual development of histopathology. It is reasonable to expect that the complexity and cost of validation protocols for predictive biomarkers will be greater than those for diagnostic or mechanistic markers.

Validation study report

This report is a comprehensive summary of study results. The report will summarize data supporting the specificity and sensitivity of the candidate biomarker relative to the accepted measurement standard, such as histopathology. It should exhaustively characterize the tissue in which the biomarker is being validated. It should also define in detail the diagnostic or prognostic use of the biomarker. A go/no go point will be reached when the sponsor considers the report is to be ready for review by the FDA.

FDA study report review

Criteria for the evaluation of study results will include the number of compounds as well as the sensitivity, specificity and reproducibility of the biomarker measurement. The review will be performed by the regulatory team that reviewed the study protocol proposal. This is likely to be an iterative process: the go/no go point for this review will include approval or rejection of the study report or request for additional information before a second review cycle. This step will be completed when a recommendation is made for classification of this biomarker as ‘probable valid’ and for cross-validation.

Cross-validation consortium

A specific example of a validation process map for genomic biomarkers of preclinical drug safety assessment is likely to focus on a full validation of the exploratory biomarker through the known valid biomarker level. The biomarker is a successful agent for change within drug development when it is broadly accepted and used. Broad acceptance will be encouraged by a cross-validation at this stage, for example, supported by data from pharmaceutical company cross-validation consortia such as the Toxicogenomics Cross-Validation Consortium organized by the Critical Path Institute [102]. In general, the cross-validation process includes the independent validation of the pre-

clinical biomarker by two or more companies being able to replicate the findings of the initial report filed by the submitting party. A full report for the results of this cross-validation will be evaluated by the FDA (as in the regulatory study report review) to assess whether the biomarkers may be considered ‘known valid’.

Proposal for a process map for validation of genomic biomarkers in clinical trials

A clinical counterpart to the preclinical process map proposal is likely to differ considerably from the preclinical proposal. In addition, it is also likely that there will be several clinical genomic biomarker validation process maps, depending on whether the biomarker is being validated as part of a codevelopment effort or in an independent validation effort. Hereby, it is reasonable to expect that biomarkers that are being developed during the drug development process, and, consequently, are necessary for drug approval, are also being validated via the drug development process. In this case, the regulatory agency will review the biomarker validation package in the context of the usefulness of the biomarker to make a therapeutic decision.

However, it is also possible that biomarkers are developed and validated outside of a related drug development effort. For example, the recent evaluation of the usefulness of uridine diphosphate glucuronosyltransferase (*UGT1A1*) genotype [8] to assess the possible risk of experiencing an adverse event during irinotecan therapy, or genotyping of cytochrome P450 (*CYP2C9*) and vitamin K epoxide reductase complex (*VKORC1*) [9] to determine an optimal maintenance dose for warfarin therapy, illustrate cases in which genomic biomarkers help better guide therapy of drugs already on the market. In this case, acceptance of a biomarker as a known valid biomarker and a recommendation of its use, for example, via a description of the biomarker and its therapy-specific use in the drug label, is likely to include an advisory committee meeting.

The process for identifying clinical known valid biomarkers may focus on the application of pharmacogenomic biomarkers described in drug labels. A list of known valid pharmacogenomic biomarkers in the context in which their applications are specified in the labels of specific drugs is shown in Table 2. Each row captures data from a different biomarker context. Columns describe context in label, drug associated with this label,

Table 2. Biomarker validity in the context of drug labels .

Genomic biomarker	Context in label for which this biomarker is valid	Drug for which this label was issued	Other drug labels with similar context for this biomarker	Ref.
C-KIT expression	Gastrointestinal stromal tumor c-Kit expression predicts response to the tyrosine kinase inhibitor Gleevec® (imatinib mesylate). <i>In vitro</i> , imatinib inhibits proliferation and induces apoptosis in GIST cells, which express an activating c-kit mutation.	Imatinib mesylate		[10,11]
CYP2C19 variants	CYP2C19 variants (PM and EM) with genetic defect lead to change in drug exposure. Patients should be monitored to determine if it is necessary to adjust the dosage of drugs when taken concomitantly with omeprazole – PRLOSEC.	Omeprazole	Pantoprazole; voriconazole; esomeprazole; proguanil and atovaquone; nelfinavir; delavirdine; lansoprazole; amoxicilli; clarithromycin; lansoprazole; lansoprazole and naproxen	[12–14]
CYP2C19 variants, alternative context	CYP2C19 variants (PM and EM) with genetic defect lead to change in drug exposure (do not significantly influence rabeprazole clearance, clinical efficacy or potential for drug interactions – see ref PMID 12495367). In a clinical study in Japan evaluating rabeprazole in patients categorized by CYP2C19 genotype (n = 6 per genotype category), gastric acid suppression was higher in PMs as compared with EMs. Clarithromycin susceptibility testing should be done when used in combination therapy with this drug.	Rabeprazole		[15]
CYP2C9 variants	CYP2C9 variants – PM and EM genotypes; patients who are known or suspected to be P450 2C9 poor metabolizers based on a previous history should be administered celecoxib with caution as they may have abnormally high plasma levels due to reduced metabolic clearance.	Celecoxib	Warfarin	[16–18]
CYP2C9 variants, alternative context	CYP2C9 metabolism and drug–drug interactions. Fenofibrate is a weak inhibitor of CYP2C19 and CYP2A6, and mild-to-moderate inhibitor of CYP2C9 at therapeutic concentrations.	Fenofibrate	Bosentan; fluvastatin	[19–20]
CYP2D6 variants	CYP2D6 PM individuals have genetic defects leading to reduced levels of activity of P450 2D6. Fluoxetine, like other agents that are metabolized by P450IID6, inhibits the activity of this isoenzyme, and thus may make normal metabolizers resemble ‘poor metabolizers’. Therapy with medications that are predominantly metabolized by the P450IID6 system and that have a relatively narrow therapeutic index should be initiated at the low end of the dose range if a patient is receiving fluoxetine concurrently or has taken it in the previous 5 weeks.	Fluoxetine HCl	Fluoxetine HCl and olanzapine; atomoxetine; cevimeline hydrochloride; tolterodine; terbinafine; tramadol and acetamophen; clozapine; venlafaxine; aripiprazole; risperidone; metoprolol; propranolol; carvedilol; tiotropium bromide inhalation; propafenone; tamoxifen; thioridazine; timolol maleate; protriptyline HCl; vicoprofen-hydrocodone bitartrate and ibuprofen	[21–26]
DPD deficiency	Dihydropyrimidine dehydrogenase deficiency. Screening prior to therapy to avoid risk	Capecitabine	Fluorouracil cream; fluorouracil topical solution and cream	[27–30]

CYP: Cytochrome P450; EGFR: Epidermal growth factor receptor; EM: Extensive metabolizer; G6PD: Glucose-6-phosphate dehydrogenase; GIST: Gastrointestinal stromal tumor; HCl: Hydrochloride; Her: Human epidermal growth factor receptor; NADH: Nicotinamide adenine dinucleotide; NAT: N-acetyltransferase; PM: Poor metabolizer; TPMT: Thiopurine methyltransferase; UCD: Urea cycle disorder; UGT: UDP glucuronosyltransferase; VKORC: Vitamin K epoxide reductase complex.

Table 2. Biomarker validity in the context of drug labels (cont.).

Genomic biomarker	Context in label for which this biomarker is valid	Drug for which this label was issued	Other drug labels with similar context for this biomarker	Ref.
EGFR expression	EGFR presence or absence – predictive value to response. Patients enrolled in clinical studies for cetuximab were required to have immunohistochemical evidence of positive EGFR expression using the DakoCytomation EGFR pharmDx™ test kit.	Cetuximab		[31,32]
EGFR expression, alternative context	EGFR presence or absence.	Erlotinib	Gefitinib	[33,34]
G6PD deficiency	G6PD deficiency. Screening prior to therapy, since G6PD-deficient patients are at higher risk of hemolysis.	Rasburicase	Dapsone	[35]
G6PD deficiency, alternative context	Hemolytic reactions (moderate-to-severe) may occur in G6PD-deficient patients. Individuals with erythrocytic G6PD deficiency or NADH methemoglobin reductase deficiency should be observed closely for tolerance if primaquine phosphate is prescribed.	Primaquine	Chloroquine	[36]
Her2/neu overexpression	Overexpression of Her2/neu – necessary for selection of patients appropriate for drug therapy.	Trastuzumab		[37–39]
NAT variants	N-acetyltransferase slow and fast acetylators. Slow acetylation may lead to higher blood levels of the drug, and thus, an increase in toxic reactions.	Rifampin, isoniazid, and pyrazinamide	Isosorbide dinitrate and hydralazine hydrochloride	[40,41]
TPMT low and intermediate activity, common non-functional alleles are – TPMT*2, TPMT*3A and TPMT*3C	TPMT deficiency or lower activity due to mutation at increased risk of myelotoxicity. TPMT testing is recommended and consideration given to either genotype or phenotype data patients for azathioprine therapy.	Azathioprine	Thioguanine, mercaptopurine	[42–47]
UCD deficiency disorders	Urea cycle disorders. Prior to the initiation of valproate therapy, evaluation for UCD should be considered.	Valproic acid	Sodium phenylacetate and sodium benzoate	[48]
UGT1A1*28 allele	UGT1A1 mutation. Higher drug and active metabolite level affect susceptibility to toxicity.	Irinotecan		[8,49,50]
VKORC1 variants	Polymorphisms of vitamin K epoxide reductase complex subunit (VKORC1) identify warfarin-sensitive patients who require a lower dose of drug, allowing personalized warfarin treatment. (recommendation from FDA Advisory committee of Pharmaceutical Sciences, Nov 2005)	Warfarin		[51,56]

CYP: Cytochrome P450; EGFR: Epidermal growth factor receptor; EM: Extensive metabolizer; G6PD: Glucose-6-phosphate dehydrogenase; GIST: Gastrointestinal stromal tumor; HCl: Hydrochloride; Her: Human epidermal growth factor receptor; NADH: Nicotinamide adenine dinucleotide; NAT: N-acetyltransferase; PM: Poor metabolizer; TPMT: Thiopurine methyltransferase; UCD: Urea cycle disorder; UGT: UDP glucuronosyltransferase; VKORC: Vitamin K epoxide reductase complex.

Highlights

- The US FDA pharmacogenomic guidance defines different categories for biomarkers, but not how to validate them.
- A validation process requires a consensus on an efficient and transparent process map for genomic biomarker validation.
- Different validation process maps are likely for preclinical and clinical biomarkers.

other drugs with similar context in their labels, as well as references supporting each of these label contexts. A close link with context definition in drug labels integrates this list within the scientific and regulatory consensus leading to drug labels.

Conclusions

The acceptance of new genomic biomarkers in the past has been a slow and tortuous process and a new, more formal process is needed to accommodate the speed at which genomics is entering the drug development process. The validation of genomic biomarkers in drug development is particularly important for the pharmaceutical industry and regulatory agencies:

reaching consensus on the validity of a genomic biomarker will accelerate the acceptance of the biomarker itself, as well as the drug development process, helping us to bring new and better drugs to the market faster. To achieve this goal, the authors have proposed validation processes for preclinical genomic biomarkers. Scientific consensus over the validity of biomarkers requires an open and active dialog between sponsors in industry and reviewers in regulatory agencies. The Critical Path document [104], and the enabling policies and regulatory documents emerging from this effort, have set the stage for the dialog that can lead to this consensus.

Outlook

Efforts being undertaken today will result in the inclusion of novel biomarkers in drug development and regulatory review, leading to more efficient drug development processes.

Disclaimer

The opinions expressed in this publication are those of the authors and not necessarily those of the US FDA.

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