Reviews • POST SCREEN

A generic operational strategy to qualify translational safety biomarkers

Katja Matheis[1], David Laurie[2], Christiane Andriamandroso[3], Nadir Arber[4], Lina Badimon[5], Xavier Benain[6], Kaïdre Bendjama[7], Isabelle Clavier[6], Peter Colman[8], Hüseyin Firat[7], Jens Goepfert[9], Steve Hall[8], Thomas Joos[10], Sarah Kraus[4], Axel Kretschmer[11], Michael Merz[2], Teresa Padro[5], Hannes Planatscher[9], Annamaria Rossi[8], Nicole Schneiderhan-Marra[9], Ina Schuppe-Koistinen[12], Peter Thomann[7], Jean-Marc Vidal[13] and Béatrice Molac[7]

[1] Boehringer-Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
[2] Novartis Pharma AG, Basel, Switzerland
[3] Interface Europe, Brussels, Belgium
[4] Tel-Aviv (Souraski) Medical Center, Tel-Aviv, Israel
[5] Barcelona Cardiovascular Research Center (ICCC-CISC), Barcelona, Spain
[6] Sanofi-Aventis, Paris, France
[7] Firalis SAS, 35 rue du Fort, 68330 Huningue, France
[8] Pfizer Ltd, Sandwich, UK
[9] Natural and Medical Sciences Institute, Reutlingen, Germany
[10] Experimental & Diagnostic Immunology GmbH, Reutlingen, Germany
[11] Bayer Schering Pharma AG, Leverkusen, Germany
[12] AstraZeneca R&D, Södertälje, Sweden
[13] EMA, London, UK

The importance of using translational safety biomarkers that can predict, detect and monitor drug-induced toxicity during human trials is becoming increasingly recognized. However, suitable processes to qualify biomarkers in clinical studies have not yet been established. There is a need to define clear scientific guidelines to link biomarkers to clinical processes and clinical endpoints. To help define the operational approach for the qualification of safety biomarkers the IMI SAFE-T consortium has established a generic qualification strategy for new translational safety biomarkers that will allow early identification, assessment and management of drug-induced injuries throughout R&D.

Introduction

The Safer and Faster Evidence-based Translation (SAFE-T) consortium is a public–private partnership comprising 20 partners from the pharmaceutical industry, small–medium enterprises, academic institutions and clinical units of excellence with representatives from the European Medicines Agency (EMA) as external observers and advisors. It operates under the framework of the EU Innovative Medicines Initiative Joint Undertaking (IMI-JU)[a] (http://www.imi.europa.eu).

The SAFE-T consortium proposes a generic qualification strategy for translational safety biomarkers (TSBM), outlining proposals on how to generate sufficient preclinical and clinical evidence to qualify new TSBM for regulatory decision making in defined

Corresponding author:. Molac, B. (beatrice.molac@firalis.com)

[a] IMI–JU is partly funding the research activities under the SAFE-T project.
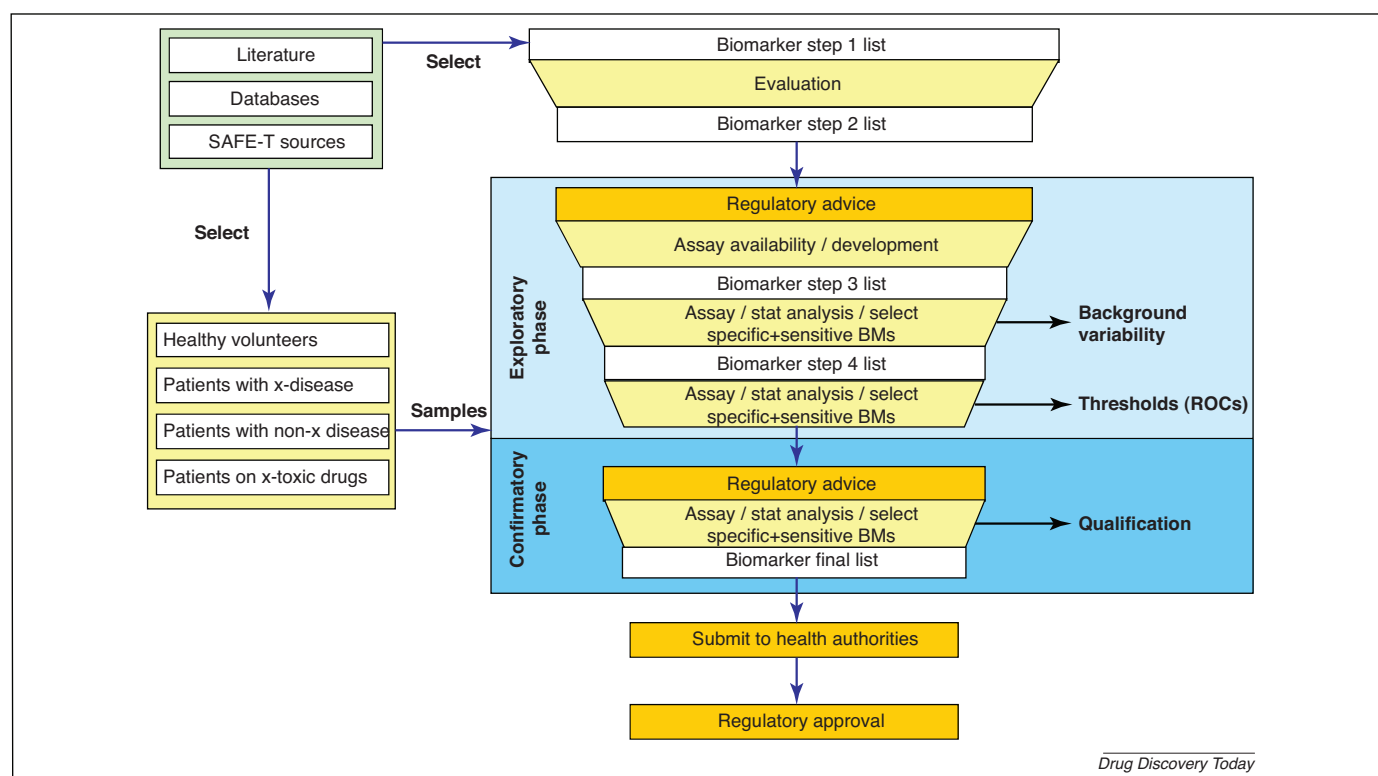
FIGURE 1

SAFE-T scientific qualification strategy for translational safety biomarkers (TSBMs).

contexts. The experience gained during the course of the SAFE-T project for three organ toxicities will be integrated into improvements for this initial generic approach.

In 2001, the US National Institute of Health (NIH) defined a biomarker as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic agent'. To avoid confusion, it has been agreed that the term 'validation' should only refer to the technical characterization and documentation of method performances, and the term 'qualification' is the evidentiary process of linking a biomarker with biological processes and clinical endpoints [1,2].

Most safety biomarkers in use today have not been formally qualified. There is no systematic scientific qualification strategy in place allowing for the accumulation of sufficient clinical and biological evidence for the acceptance of biomarkers independent of a specific drug. Regulatory agencies have established submission procedures for the regulatory review and endorsement of biomarker qualification, but have not yet defined the scientific standards and approaches needed.

The first submission of kidney safety biomarkers by the Predictive Safety Testing Consortium (PSTC) [3] also opened the door to a new framework of fit-for-purpose qualification of biomarkers instead of having an absolute 'all or nothing' qualification. With more data and evidence the limited context can be extended. This principle has been referred to as 'incremental', 'progressive' or 'rolling' qualification.

Thus, the SAFE-T consortium proposes broad principles of a research plan including assay method validation and biomarker qualification to develop biomarkers for regulatory decision-making in specific contexts (Fig. 1).

Each work programme (DIKI, DILI, DIVI, see Table 1) will follow the general qualification plan of a two-stage programme of studies: initial exploratory studies followed by confirmatory studies. The exploratory studies will explore reference population variation, biomarker variation in kidney, liver or vascular disease, biomarker variation in other organ diseases and biomarker variation in drug-induced organ injury, alongside technical validation of assays and biomarker characteristics (sampling and storage, among others). Biomarkers will be compared and then selected for the next stage of qualification based upon cumulative evidence showing acceptable (i.e. minimal) biological variation, evidence of a clear response in DIKI, DILI or DIVI, respectively, limited response in diseases affecting other organs, and evidence of some response in kidney, liver or vascular disease that is preferably pathophysiologically similar to drug-induced organ injury.

Following this selection, candidate biomarkers will be subject to a second round of focused, extensive and thorough confirmatory studies that will build upon the data and experience of the exploratory studies. Each study will be designed to support specific claims for biomarker use. Following these studies, where appropriate data exist, a submission to the regulatory authorities will be made that contains all relevant data on specific biomarkers of interest from the exploratory and confirmatory studies.

Overall, the qualification strategy follows a 'fit-for-purpose' principle. This means that evidence of the strengths and limitations of the biomarkers is accumulated in a focused clinical context to support specific claims for regulatory endorsement. In

TABLE 1

Desirable biomarker profile for drug-induced kidney, liver and vascular injuries

	Drug-induced kidney injury (DIKI)	Drug-induced liver injury (DILI)	Drug-induced vascular injury (DIVI)
Type of biomarkers to be clinically qualified	Preclinically qualified and exploratory biomarkers	Exploratory preclinical and clinical biomarkers	Exploratory biomarkers for DIVI and biomarkers for human vascular disorders
Definition of purpose	Risk prediction Early diagnostic Prognostic	Risk prediction Early diagnostic Prognostic	Risk prediction Early diagnostic Prognostic
Contexts of use	Preclinical, early clinical and clinical Candidate TSBMs will be evaluated in clinical studies	Preclinical, early clinical and clinical Candidate TSBMs will be evaluated in clinical studies	Preclinical, early clinical and clinical Parallel 'forward and reverse qualification' required
Current standards	Specific but not sensitive enough, lack of predictivity Current standards include serum creatinine and blood urea nitrogen	Sensitive but not specific enough, lack of predictivity and prognostic value Current standards include liver enzymes and bilirubin	Not sensitive nor specific enough Absence of standards for preclinical DIVI DIVI-like pathologies in human are diagnosed using clinical, pathological and biological criteria

subsequent studies, during the regular use of the biomarkers and with the help of additional studies conducted by other consortia and stakeholders, the context of use can be further extended or limited. Table 1 outlines the biomarker profiles desirable within the SAFE-T project.

Criteria relevant for qualification of translational safety biomarkers

Any biomarker candidate selected for the clinical qualification process (i.e. exploratory phase) has to be supported by solid scientific evidence and a clear rationale based on available preclinical and clinical data. Relevant clinical data to be generated includes sensitivity, specificity, positive and negative predictive values, as well as baseline variability in healthy volunteers versus patient populations, and the impact of clinically relevant covariates such as age, gender and body mass index. Similarly, preclinical data and knowledge of comparative molecular biology will aid the understanding of translatability and the molecular mechanisms underlying the biomarker (e.g. biomarker type, mechanism of toxicity, tissue specificity and reversibility of the biomarker), together with characteristics such as the time course and half life, concordance with histopathology, as well as special procedures and techniques that cannot be conducted in humans.

Preclinical data providing insight into the molecular mechanism of toxicity, tissue specificity and reversibility, concordance with histological findings, and characteristics such as time course and half life are significant information – wise to evaluate whether the selected biomarker is suitable for clinical application. Special consideration has to be given to whether the change of a biomarker is caused by, for example, cellular leakage, active shedding from pre-apoptotic cells, *de novo* synthesis, organ dysfunction from some other etiology, an immune reaction or change in biomarker clearance. Translation of any biomarker candidate from animal models to humans is facilitated by understanding associated mechanisms, pathways and kinetics across species. Accordingly, this information is a key element of the qualification process.

The specificity for the target organ and an enhanced sensitivity compared with current standards (e.g. known biomarkers such as ALT, AST, GLDH, BUN and creatinine) both need to be evaluated

in detail during the qualification process. Furthermore, information such as *in vivo* stability (metabolism of the biomarker and circadian rhythm effects), stability after sampling and the characteristics of the intended assay must be considered and included in the validation criteria.

In the confirmatory phase of biomarker qualification the performance of the biomarker should be continuously monitored and reassessed based on comparison with all the information from the exploratory phase.

Assay validation

Increasingly crucial decisions are probably to be taken on the basis of biomarker measurements. Therefore, the analytical methods used must be well characterized, validated and documented to yield reliable results that can be interpreted satisfactorily during the biomarker qualification process and, later on, during safety assessment in drug R&D and disease diagnosis.

Existing guidelines and recommendations from regulatory agencies on validation of bioanalytical methods are focused on the validation of assays intended to measure drug or drug metabolite concentrations in biological matrices and are biased toward chromatographic methods (e.g. LC–MS) [4–7]. There is regulatory guidance from the FDA and EMA specifically dedicated to genomic biomarker assays, but not for other types of platform (e.g. protein biomarkers) [8,9].

Because of the diverse nature of biomarker analysis, the validation method should be improved and adapted according to the characteristics of the candidate biomarkers for the needs of drug development and diagnostic applications. Thus, SAFE-T established a 'standard validation procedure for biomarker immunoassays' applicable to all three organ toxicity areas of interest. Although providing enough flexibility to allow the validation of assays for diverse types of biomarkers, this procedure ensures sufficiently consistent documentation of assay performance throughout the SAFE-T consortium to allow decision making and, ultimately, regulatory submission.

Two of the main features of the early phase of the SAFE-T project that are not addressed by the existing guidelines are the large number of candidate biomarkers to be studied and the use of ligand-binding assays (LBA) as the 'gold standard' for the measure-

ment of protein biomarkers. LBA are characterized by a non-linear relationship between the response and the concentration of analyte, which can be affected by a large number of variables – resulting in increased workload to standardize and validate those methods. Also, the complexity of the validation is further exacerbated by the introduction of multiplex immunoassays.

Both issues have been addressed during the American Association of Pharmaceutical Scientists workshop on biomarker assay validation where the use of a fit-for-purpose approach has been suggested [10]. This principle states that the extent of the validation process should demonstrate that a given method is 'reliable for the intended application'. A similar approach is adopted within the SAFE-T assay validation procedure. Adapted validation plans inspired by existing guidelines for bioanalytical method validation [4–6] are tailored to meet the different needs of biomarker qualification with a basic assay validation in exploratory studies and more-rigorous testing for confirmatory studies [11] in a later stage of the project. The procedure applies to assays developed within SAFE-T as well as to commercial assays, whether they are sold as 'research use only' assays or as in vitro diagnostic (IVD) products. Indeed, although IVDs are technically well characterized, SAFE-T needs to generate internal validation data to support the interpretation of data obtained from a population that might not have been the IVD target population.

Assay parameters that should be addressed during immunoassay validation include specificity, selectivity [12], accuracy, precision (intra- and inter-assay variation), sensitivity, robustness, linearity, parallelism and dynamic range (with a definition of an upper and lower limit of detection and quantification) [10]. Besides these parameters, stability of the analyte under standard handling conditions should be considered. Assay acceptance criteria for these parameters are defined in the standard validation procedure and are, if applicable, in accordance with EMA and FDA guidelines [5–7]. For each assay, performance data will be documented in a validation report. Adapted from existing published recommendations [13,14], acceptance criteria in the SAFE-T project are designed to be adjustable to the biomarker of interest and the potential particular constraints associated with this biomarker. Moreover, to ensure the stability of parameters the procedure will include a quality control policy and run acceptance criteria during testing. Once these parameters are established a quality control policy will be applied to ensure that the same standard is maintained throughout sample testing.

Validation data and characterization of assays will be the basis for the set-up of diagnostic tools based on the biomarkers that show an interesting profile in the SAFE-T studies. The SAFE-T and PSTC consortia are closely collaborating to harmonize many aspects of biomarker qualification process including study protocols, statistical analysis procedures, assay development and the exchange of biological materials. When needed, the consortia will perform joint activities to qualify new translational biomarker candidates in preclinical and clinical settings for the three drug-induced organ injuries.

Clinical programme principles

The clinical programme is aimed at providing human biological and clinical data to support the qualification and validation of translational safety DIKI, DILI and DIVI biomarkers that could

impact drug development and regulatory decision making [15]. The programme will assess the clinical applicability of the selected biomarkers in predicting, diagnosing and monitoring drug-induced renal, liver and vascular toxicity in humans, and will allow the comparison of data obtained in human and animal studies for qualification of translational biomarkers.

A two-step approach is proposed: exploratory biomarker proof of translation (PoT) followed by confirmatory biomarker proof of performance (PoP) studies. PoT studies will be conducted with small groups of healthy subjects or patients with drug- and non-drug-induced pathologies for testing the translational value of selected biomarker candidates in comparison to current gold standards. PoP studies will be conducted in large patient populations with drug- and non-drug-induced pathologies and with common disorders to establish biomarker performance.

Criteria for the selection of biomarkers to be evaluated in the exploratory studies (mainly based on literature review) and in the confirmatory studies (based on results of the exploratory studies) will be defined before the start of the clinical activities. Clinical studies to be conducted will depend on the known characteristics of the selected biomarker to be assessed.

Biological samples and related clinical data will be obtained through either clinical studies specifically dedicated to the project or those conducted for drug development by the SAFE-T Pharma companies (extra samples). In addition, existing sample collection from all partners could be included in the qualification process if deemed appropriate.

Clinical investigations will be conducted in the following populations:

- Healthy subjects with stratification to allow for the assessment of the effect of various intrinsic and extrinsic factors – samples and data in healthy subjects collected in drug development studies will come from subjects in baseline periods, subjects receiving placebo or subjects receiving reference drug with known potential toxicity for the target organ of interest (e.g. ketoconazole in pharmacokinetic interaction studies) [16]. In addition, some dedicated studies might be conducted in healthy subjects, with or without drug administration.
- Patients – samples will come from patients on chronic treatment with drugs associated with an increased risk to cause injury to the target organ of interest, patients with well-defined pathologies of the target organs or patients with other well-defined pathologies.

Clinical study protocol outlines will be discussed with regulatory agencies before initiating specific exploratory studies, and their results will be presented to the regulatory agencies before progressing to the confirmatory phase.

All clinical studies will be conducted in accordance with the principles reported by the 18th World Medical Assembly (revised version of the Declaration of Helsinki, 1964) [17] and all applicable amendments laid down by the World Medical Assembly and the International Conference of Harmonization (ICH) guidelines for good clinical practice (GCP).

Integrative data analysis and project database

Statistical techniques that will be used for the SAFE-T studies will be chosen in accordance with the fit-for-purpose objectives of the SAFE-T consortium. All statistical analyses will be prospectively
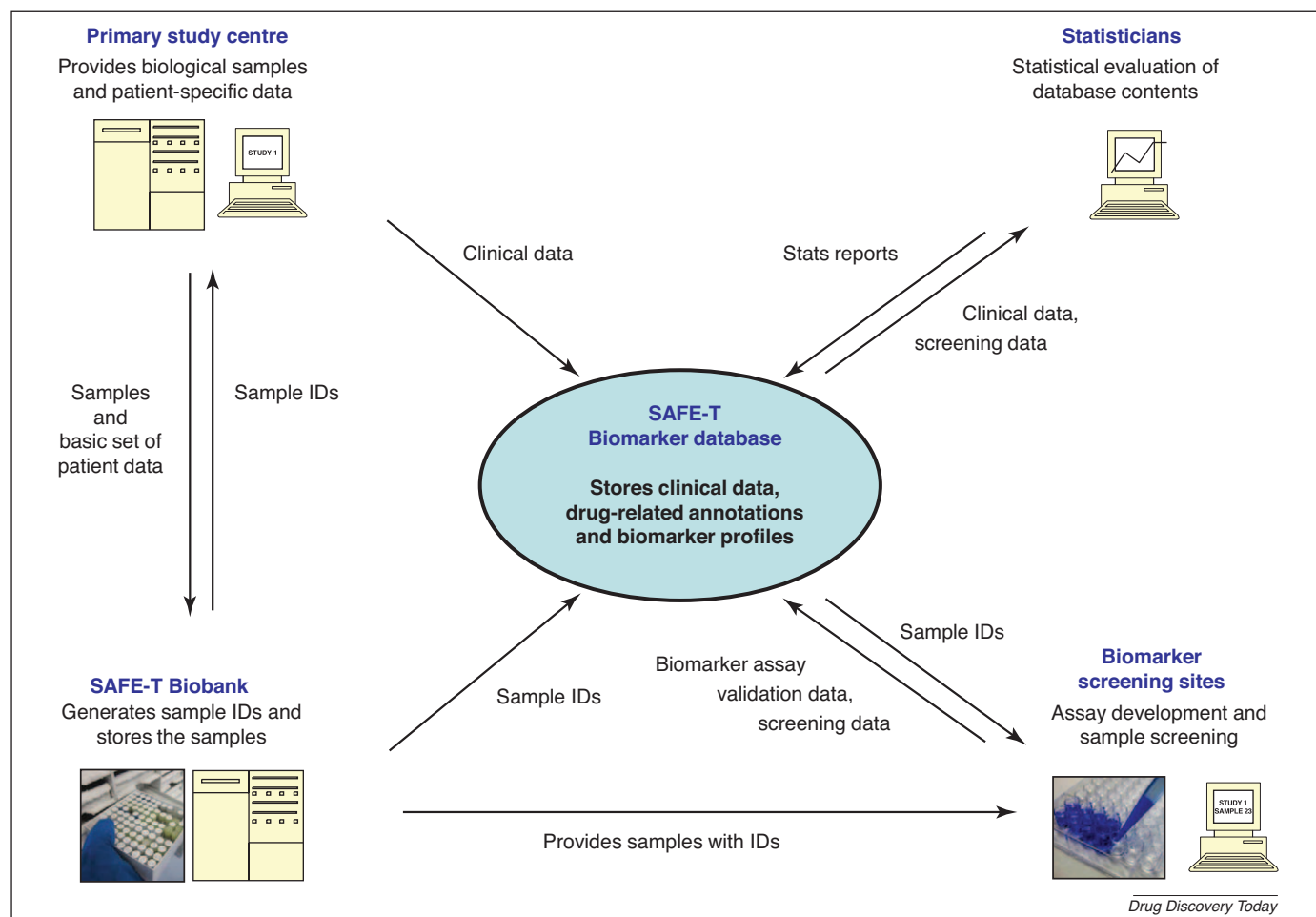
FIGURE 2

General overview of the SAFE-T biomarker database structure and actions.

planned and the trial objective will ultimately drive the estimation of sample size, when relevant, and other aspects of study design.

All biomarkers will be characterized, in the first instance, using a set of various analyses. Effects of intrinsic and extrinsic factors will be determined using generalized linear models. Variability components (i.e. within-subject, between-subject and total variability) will be estimated and the ratio of within-subject versus between-subject variability will then be calculated for each biomarker [18]. Changes over time in a healthy population and after organ-toxic drug administration will be investigated using graphical tools. Density plots and special cut-off (limit of normality) determination will be provided for each biomarker.

Next, the performance of each biomarker, or any combination of biomarkers (combination of biomarkers being considered in the same way as a single biomarker in the following analyses), will be assessed by estimating their empirical sensitivity and specificity. Subjects having organ toxicity will be identified by 'gold standard' biomarkers (i.e. histopathology in case of biopsy) or at least using highly specific biomarker(s) in the case of flawed biomarkers (i.e. using non-sensitive but specific biomarkers). In the absence of a specific biomarker the performance of each biomarker will be assessed by estimating the positive and negative percentages of agreement [19] between biomarkers, for instance using some parametric approaches of mixture models with latent class variable

[20,21] or non-parametric approaches of cross-classified biomarkers [22,23].

For each biomarker, based on the above estimation, each pairing of sensitivity and specificity will be used to construct the ROC curves. As a single index to summarize the accuracy of the test [24], the ROC AUC, or partial ROC AUC, will be computed for each biomarker and then they will all be compared, also against gold- or flawed-standard biomarkers if there are any available. Other performance criteria [25] such as the Youden index, efficiency, likelihood ratio and/or subjective quantities, such as the cost of misclassification (MCT), might be provided.

The performance of each candidate biomarker assessed during the exploratory phase will be used to select the most promising biomarkers for their confirmation in the later phase of the SAFE-T consortium.

Finally, in confirmatory studies, the best cut-off value [25] will be determined for each biomarker based on the best ratio between the sensitivity and specificity and their associated cost (penalties caused by high false positive and false negative rates).

Figure 2 depicts the data flow expected in the project. To achieve regulation and consistent data collection at all sites, SAFE-T will set up a common electronic data capture (EDC) system (OpenClinica). The re-use of forms (electronic case report forms; eCRFs) in multiple studies will facilitate data integration and cross-study analysis.

This solution, an internet-accessible instance of OpenClinica, is offered to all studies participating in SAFE-T. However, every sponsor is allowed to use another EDC system, if the data are then mapped to CDISC SDTM and CDISC LAB, which are recommended standards for data submission to the regulatory authorities. To achieve term consistency throughout the study, sites need to use predefined established coding dictionaries. All standard compliant data will then be uploaded to a data warehouse accessible to all SAFE-T partners.

All uploaded data will be source-traceable and all activities on the database will be logged for regulatory compliance. Upon completion of the SAFE-T project, all data will be locked against further updates. The data will then be made available to other parties as stipulated by the SAFE-T Steering Committee.

Principles of biobanking

A key element for translational biomarker qualification is the availability of well-classified, large enough patient cohorts and the establishment of a quality-controlled biobank together with an IT-based infrastructure for the management of samples and required data.

To this respect, the SAFE-T consortium will establish a unique consortium sample repository to ensure adherence to common biobanking standards on quality, access and availability in the field of qualification of translational DIKI, DILI and DIVI biomarkers (Fig. 2). The SAFE-T repository will contain a large, well-controlled and centralized collection of samples from healthy volunteers and patients with various types of drug-induced and non-drug-induced systemic and/or common diseases together with well-documented but anonymous (coded) clinical and biological data from the sample donors.

As described in Box 1, the SAFE-T Biobank will centralize and harmonize all steps of sampling, storage and shipping, data management and legal issues, providing well-tracked biomaterials and associated clinical and biological patient data. For every step, specific standard operating procedures have been established by the SAFE-T consortium. A biobank informatics core will encode identifiers in a robust, centralized database. A controlled web portal will ease data entry for biobank contributors.

The availability of a repository, as described for the SAFE-T Biobank, will foster reliable sample preservation and appropriate quality to preserve the original nature of the recovered biological materials.

Ethical issues

The qualification and validation of translational safety biomarkers in clinical settings is generally supported by a clinical programme where biological samples and related clinical data are collected through either clinical studies specifically dedicated to the project or other clinical studies conducted for drug development. The collection, use and retention of blood samples through sampling, integrative data analysis, database and biobank establishment raise the following ethical issues: consent, personal data confidentiality and security, handling and storage of data.

Clinical studies in SAFE-T will be conducted by applying all relevant national and EU legislations and regulations. The collection, use and retention of blood samples will be compliant with the EU Charter of Fundamental Rights [26], the Clinical Trials

---

BOX 1

Overview of the SAFE-T Centralized Biobank activities

(i) Guidelines for sample collection and handling

- Type of human sample: body fluids (blood and urine) and biopsy material.
- Generic form for patient informed consent: the clinical centres where the trials are conducted should adapt the consents based on local ethics requirements (and requirements from the project and according to EU standards).
- Unique patient number (UPN) and unique sample number (USN) will be used for keeping coded patient information.
- Standard operating procedures (SOPs) for sample collection and handling at the collection site, and shipping to the Centralized Biobank.

(ii) Storage of the samples at the SAFE-T Centralized Biobank

- Information on volume, quality and availability of samples for biomarker (BM) qualification.
- Security system for continuous control of good sample storage conditions.
- Strategy for emergency response in case of freezer failure.
- Legal requirements for human sample storage.

(iii) IT biobank management system for control and retrieval of samples

- Coded information of patients (anamnesis and clinical history) and related samples.
- Control of sample location (e.g. in the freezers).
- Continuous track of the samples.

Directive 2001/20/EC [27] of the European Parliament and of the Council and Directive 95/46/EC [28] on the protection of individuals.

The procedure for obtaining informed consent will be applied by the consortium when the collection of new samples is required. Regarding the collection of information from a subject's medical records, the consent will mention who will access the records (further research projects), what information will be obtained, how the patient's confidentiality will be protected and whether data are to be shared with other stakeholders.

Research using archived collections

The consortium will make sure that established collections used in SAFE-T are obtained in an ethical manner (consent originally obtained with mention of use for other research projects).

The SAFE-T consortium will ensure that confidentiality of a subject's personal data is preserved and measures will be taken to encode or anonymize banked biomaterial as far as possible and as early as possible in the data processing. The consortium will have the responsibility to ensure that procedures and security arrangements are sufficient to prevent breaches of confidentiality.

Adequate security for storage and handling of such data will be addressed (SAFE-T Centralized Biobank). Once archived, material or data will be afforded the same level of protection as and when in active use.

TABLE 2

Overview of biomarker interactions with regulatory agencies

Interaction process		Region		
		Europe	USA	Japan
Early consultation (informal) meeting	Process	Briefing meeting with PGWP	VXDS briefing meeting with IPRG	Informal meetings with PMDA
	Cost	Free	Free	Free
	Timeline	Within eight weeks of request; written summary within four weeks after meeting. If joint meeting: briefing meeting within ten weeks of request; joint meeting summary within four weeks	Usually within eight weeks of request; no formal timelines for meeting or meeting report	Unknown
Formal consultation meeting	Process	Scientific advice from CHMP on future protocols and methods for biomarker qualification; managed by EMA's Scientific Advice Working Party	New draft biomarker qualification data submission (BQDS) process includes a 'consultation and/or advice' stage	Pharmacogenomics and/or biomarker consultation on future protocols and methods for biomarker qualification
	Cost[a]	€72 800 (40k for subsequent advice)	Free	3.03 million Yen (~US$30 000)
	Timeline	Four months	No stated timelines	Six months
Biomarker qualification	Process	Biomarker qualification review by qualification team managed by EMA's Scientific Advice Working Party. Public opinion by CHMP	Draft process includes a 'review' stage; managed by FDA biomarker qualification review team (BQRT) Public statement of qualification by CDER	Pharmacogenomics and/or biomarker consultation on data for biomarker qualification
	Cost[a]	€72 800 (40k if there was previous scientific advice)	Free	3.03 million Yen (~US$30 000)
	Timeline (approx.)	Four months for EMA review, plus three months for public consultation	No stated timelines. Could be about six months. Public comment period	Six months

[a] 90% fee reduction for small and medium-sized enterprises (SMEs).

Regulatory strategy

Before and during the development of a biomarker it is strongly recommended to consult with regulatory agencies. It is important that regulatory agencies recognize and endorse biomarkers for their proposed context of use in drug development and discussions with regulators at key milestones of the development process will ensure that studies are designed in accordance with regulatory agency expectations. The formal qualification of biomarkers will require assessment of data by regulatory authorities that have released guidance on the process, and application structure has been published by the EMA [29], FDA [30,31], PMDA [32] and by ICH [33]. These processes might be suitable not only for the qualification of biomarkers that have broad utility (e.g. organ function biomarkers) but also for biomarkers for a single drug or class of drugs.

Interactions with regulatory authorities

During the biomarker development programme several possibilities are available for meeting and/or consultation with regulatory agencies (Table 2).

Early consultation

EMA and FDA can provide early informal advice on biomarker development, for example aspects of the scientific approaches, techniques and standards.

For the EMA a briefing meeting can be held with the Pharmacogenomics Working Party (PGWP) [34]. The FDA arranges briefing meetings with the FDA's Interdisciplinary Pharmacogenomic

Review Group (IPRG) under their Voluntary eXploratory Data Submission (VXDS) process [30]. The format of these FDA and EMA meetings is informal and non-binding but provides valuable information during the qualification process. Joint EMA and FDA briefing meetings/VXDS videoconferences are also possible so that feedback or debate can be received simultaneously [35]; however, the feedback from the two agencies is independent and not necessarily aligned.

Formal scientific advice procedures

Each agency for the ICH regions (FDA, EMA and PMDA) has established formal or pilot procedures [29,31,32] to allow formal scientific advice on biomarker development programmes that should lead to data adequate for qualification. At present these are independent although the agencies can share intermediate assessment reports. A pre-submission meeting could be held to obtain support on formulating the questions, to obtain the clearest advice.

The estimated timelines for EMA and PMDA scientific advice are 4–6 months from submission to feedback: The FDA has a consultation process but not set timelines. If there is a scientific rationale several biomarkers can be included in one submission. In such cases this should be made clear in the 'intention to submit' letter to the regulatory agencies.

Biomarker qualification process

Each ICH agency (FDA, EMA and PMDA) has recently set up formal or pilot procedures [29,31,32] to enable them to review the final

data and reach opinions on qualification of biomarkers for specific contexts of use. If successful, each agency will release a public statement on the agreed context of use. The biomarker qualification review team in each agency includes drug review experts and a final decision is made by the agency's senior expert panel (e.g. EMA's Committee for Medicinal Products for Human Use, FDA's Center for Drug Evaluation and Research Board) to provide a thorough assessment of the practical utility or biomarkers in drug development programmes.

Pre-submission meetings could be held to obtain advice on formulating the text for claims and how to present the data to support them. This could be useful if the agencies perceive any major issues that could be addressed by modification of the claims or even, in extreme cases, if the agency considers that the data are not yet mature and that another round of scientific advice and further qualification work is more appropriate.

Recommended documentation

Regulatory agency advice

In general, briefing meetings and scientific advice meetings will require: an agreed list of questions to be addressed at the meeting, a list of attendees, background information on the topics to be discussed and justification of any proposals, supported by available preliminary and/or background data [29,31,33].

Biomarker qualification applications

Applications for biomarker qualification guidance are available under the ICH [33]. It indicates that biomarker qualification dossiers should include data summaries and an overview that critically assesses the strengths and limitations of the data. The study reports should also be included in the dossier, preferably as summary reports, with the actual study reports and raw data files available on request.

It is important to state if the submitted biomarker is supplementing, or replacing, the current standards. It is unlikely that a biomarker would be qualified in one step for all possibilities within a context and so a progressive, step-wise approach to qualification might be more appropriate. The ICH E16 [33] guidance recommends that the description of the 'context of use' follows a taxonomy approach. A biomarker could have more than one context (e.g. non-clinical and clinical predictive biomarkers).

Regulatory standards and/or principles and/or expectations

At present there are no formal regulatory guidelines available on principles of safety biomarker qualification (e.g. standards, analyses, sampling and replication). Therefore, it is important to discuss the expectations of the regulatory agencies on a case-by-case basis.

Concluding remarks

The operational qualification strategy described in this manuscript should enable a generation of sufficient clinical evidence for selected kidney, liver and vascular injury biomarker candidates in clinical studies. The biomarker diagnostic performance estimated in the exploratory phase will be confirmed using several samples from different types of patient populations. Once approved by regulatory agencies for prediction, diagnosis and monitoring of organ injuries in a drug development context, these biomarkers could also be used for diagnosing renal, hepatic and vascular diseases in a clinical setting.

The early involvement of all stakeholders is needed to agree on a prospective and specific guidance on the weight and specificity of data that would have to be submitted to qualify biomarkers for each purpose under proposed conditions. The SAFE-T consortium proposes a continuously improved guidance by implementing the experience acquired during the qualification activities that are aligned with the recommendations from regulatory agencies.

Disclaimer

The views expressed in this paper are the personal views of the author(s) and should not be understood or quoted as being made on behalf of, or reflecting, the position of the EMA or one of its committees or working parties.

References

1 Wagner, J.A. et al. (2007) Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. Clin. Pharmacol. Ther. 81, 104–107

2 Lesko, L.J. (2007) Paving the critical path: how can clinical pharmacology help achieve the vision? Clin. Pharmacol. Ther. 81, 170–177

3 Dieterle, F. et al. (2010) Renal biomarker qualification submission: a dialog between the FDA-EMA and Predictive Safety Testing Consortium. Nat. Biotechnol. 28, 455–462

4 FDA 62 FR 27464. FDA, US Food and Drug Administration (1996) Guidance for Industry: Q2B Validation of Analytical Procedures: Methodology. Available at http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM128049.pdf (accessed April 2011)

5 EMEA/CHMP/EWP/192217/2009. EMA, European Medicines Agency (2009) Draft Guideline on Validation of Bioanalytical Methods (EMEA/CHMP/EWP/192217/2009). Available at http://www.ema.europa.eu/pdfs/human/ewp/19221709en.pdf (accessed April 2011)

6 FDA, US Food and Drug Administration (2001) Guidance for Industry – Bioanalytical Method Validation. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf (accessed April 2011)

7 EMEA/CHMP/ICH381/95. EMA, European Medicines Agency (1995) Note for Guidance on Validation of Analytical Procedures: Text and Methodology. Available at http://www.ema.europa.eu/pdfs/human/ich/038195en.pdf (accessed April 2011)

8 EMEA/CHMP/PGxWP/201914/2006. EMA, European Medicines Agency (2007) Reflection Paper on Pharmacogenomic Samples, Testing and Data Handling (EMEA/CHMP/PGxWP/201914/2006). Available at http://www.ema.europa.eu/pdfs/human/pharmacogenetics/20191406en.pdf (accessed April 2011)

9 FDA, US Food and Drug Administration (2006) Docket No. 2006D-0012. Draft Guidance for Industry and Food and Drug Administration Staff; Pharmacogenetic Test and Genetic Tests for Heritable Markers. Federal Register Notices 71, 6779–6781 [FR Doc. E6-01787]. Available at http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071075.pdf (accessed April 2011)

10 Lee, J.W. et al. (2006) Fit-for-purpose method development and validation for successful BM measurement. Pharmaceut. Res. 23, 312–328

11 Lee, J.W. and Hall, M. (2009) Method validation of protein biomarkers in support of drug development or clinical diagnosis/prognosis. J. Chromatogr. B 877, 1259–1271

12 Lee, J. and Ma, H. (2007) Specificity and selectivity evaluations of ligand binding assay of protein therapeutics against concomitant drugs and related endogenous proteins. AAPS J. 9, 164–170

13 DeSilva, B. et al. (2003) Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharmaceut. Res. 20, 1885–1900

14 Rocci, M.L. *et al.* (2007) Confirmatory reanalysis of incurred bioanalytical samples. *AAPS J.* 9, 336–343

15 Goodsaid, F.M. and Mendrick, D.L. (2010) Translational medicine and the value of biomarker qualification. *Sci. Transl. Med.* 2, 44–47

16 Pasqualetti, G. *et al.* (2010) Healthy volunteers and early phases of clinical experimentation. *Eur. J. Clin. Pharmacol.* 66, 647–653

17 Enserik, M. (2000) Helsinki's new clinical rules: fewer placebos, more disclosure. *Science* 290, 418–419

18 Harris, E.K. (1974) Effect of intra- and interindividual variation on the appropriate use of normal ranges. *Clin. Chem.* 20, 1535–1542

19 *FDA Guidance – Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests.* Available at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm (Accessed March 2007)

20 Choi, Y.K. (2006) Bayesian inferences for receiver operating characteristic curves in the absence of a gold standard. *J. Agric. Biol. Environ. Stat.* 11, 210–219

21 De Meyer, G. (2010) Diagnosis-independent Alzheimer's disease biomarker signature in cognitively normal elderly people. *Arch. Neurol.* 67, 949–956

22 Wang, C. (2007) Nonparametric estimation of ROC curves based on Bayesian models when the true disease state is unknown. *J. Agric. Biol. Environ. Stat.* 12, 128–146

23 Zhou, X.H. (2005) Nonparametric estimation of ROC curves in the absence of a gold standard. *Biometrics* 61, 600–609

24 Lasko, T.A. (2005) The use of receiver operating characteristic curves in biomedical informatics. *J. Biomed. Informatics* 38, 404–415

25 Greiner, M. (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prevent. Vet. Med.* 45, 23–41

26 The EU Charter of Fundamental Rights (June 1999). Available at: http://europa.eu/legislation_summaries/human_rights/fundamental_rights_within_european_union/l33501_en.htm (accessed April 2011)

27 The Clinical trials Directive 2001/20/EC of the European Parliament and of the Council. Available at http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:en:PDF (accessed April 2011)

28 The Directive 95/46/EC on the Protection of Individuals. Available at http://ec.europa.eu/justice_home/fsj/privacy/docs/95-46-ce/dir1995-46_part1_en.pdf (accessed April 2011)

29 EMEA/CHMP/SAWP/72894/2008 Corr. European Medicines Agency Committee for Human Medicinal Products (2009). *Qualification of Novel Methodologies for Drug Development: Guidance to Applicants.* Available at http://www.emea.europa.eu/pdfs/human/biomarkers/7289408en.pdf (accessed April 2011)

30 FDA, US Food and Drug Administration (2005). *Guidance for Industry. Pharmacogenomic Data Submissions.* Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079849.pdf (accessed April 2011)

31 FDA Guidance for Industry (2010) *Qualification Process for Drug Development Tools (Draft Guidance).* Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597.pdf (accessed April 2011)

32 PMDA Notification No. 0331004 (31st March, 2009). *Implementation Procedures for the Face-to-face Advice Services, Inspections for Certification and Other Services Provided by the Pharmaceuticals and Medical Devices Agency. (Attachment 3: Implementation Procedure for the Pharmacogenomics/biomarker Consultations that are Provided as Face-to-face Advice Services)*

33 ICH (2010) *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Harmonised Tripartite Guideline. Genomic Biomarkers Related to Drug or Biotechnology Product Development Response: Context, Structure and Format of Qualification Submissions (E16).* Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E16/Step4/E16_Step_4.pdf (accessed April 2011)

34 EMEA/CHMP/PGxWP/20227/2004. *CHMP Guideline on Pharmacogenetics Briefing Meetings*, 27 April 2006. Available at http://www.emea.europa.eu/pdfs/human/pharmacogenetics/2022704en.pdf (accessed April 2011)

35 EMEA, FDA, EU Commission Guiding Principles (May 19, 2006) *Processing Joint FDA EMEA Voluntary Genomic Data Submissions (VGDSs) within the Framework of the Confidentiality Arrangement.* Available at http://www.emea.europa.eu/pdfs/general/direct/pr/FDAEMEA.pdf (accessed April 2011)