IMI1 Final Project Report
Public Summary

Project Acronym: SAFE-T

Project Title: SAFER AND FASTER EVIDENCE-BASED TRANSLATION

Grant Agreement: 115003
Project Duration: 15/06/2009 - 14/06/2015

1. Executive summary

1.1. Project rationale and overall objectives

The SAFE-T project addressed the current lack of sensitive and specific clinical tests to diagnose and monitor drug-induced injury of the kidney, liver and vascular system, which present major hurdles in drug development. For that, the consortium has

- Evaluated the utility of safety biomarkers for monitoring DIKI, DILI and DIVI in humans,
- Developed assays for clinical application of safety biomarkers,
- Compiled significant evidence supporting qualification of safety biomarkers for regulatory decision making in clinical drug development and in a translational medicine context,
- Gained evidence for usage of safety biomarkers in the diagnosis of diseases

Overall objectives of SAFE-T:

- Define a generic scientific qualification strategy for translational safety biomarkers.
- Develop and validate assays for the quantification of biomarkers in clinical samples.
- Select mechanistic biomarkers for drug-induced kidney, liver and vascular injuries (DIKI, DILI, and DIVI).
- Qualify translational biomarkers using clinical cohorts for regulatory decision making.
- Create a database for clinical data, drug related annotations and biomarker profiles.
- Provide a unique Biobank of the material collected for clinical qualification of biomarker candidates.
- Obtain acceptance by regulatory authorities for the use of the safety biomarkers in specific translational and clinical contexts.

1.2. Overall deliverables of the project

The main deliverables of SAFE-T were:

1. Publication of a scientific strategy for qualification of safety biomarkers
2. Strategy reports for DIKI, DILI and DIVI biomarker qualification
3. Report on the integrated assay validation strategy for DIKI, DILI, and DIVI
4. Implemented database for human safety biomarker profiles
5. Clinical data, samples and quantitative biomarker data from exploratory studies conducted for DIKI, DILI and DIVI
6. GCP validated assays for confirmatory studies
7. Clinical data, samples and quantitative biomarker data from confirmatory studies conducted for DIKI, DILI and DIVI
8. Interpretation and report on biomarker data from exploratory and confirmatory studies
9. Submission of biomarker data to health authorities for regulatory approval
10. Biobank with human samples suitable for qualification of additional safety biomarker candidates
11. Public presentations of SAFE-T (website, presentations) and SAFE-T results (publications and presentations)
12. Safety biomarkers for specific clinical context of use in DIKI, DILI and DIVI, approved by health authorities EMA and FDA.
13. Dissemination of the SAFE-T results to a well identified target audience through defined dissemination, communications, and training plan.

Deliverables 1-6 and 10 have been fully achieved. In particular, the database established by SAFE-T contains comprehensive data on more than 60 biomarker candidates significantly supporting further research on drug-induced kidney, liver, and vascular injuries. A unique, centralized and well-controlled repository of human samples dedicated to the qualification of translational DIKI, DILI, DIVI biomarkers has been created. Sample information is directly linked to anthropometric and clinical data. A significant fraction of those samples is still available for further research, facilitating assessment of new biomarker candidates or expansion of data on selected SAFE-T biomarkers.

Deliverables 7-9 and 11-13 have as yet been achieved only partially, since analysis of data is ongoing while this final consortium report is being written. Moreover, given initial delays in study start and difficulties in patient recruitment, completion of confirmatory studies was not possible as planned originally for DIKI and DILI programs. Submission and publication of results has been delayed due to significant challenges with data cleaning and data management, and is now planned for end of October 2015. Finally, it is expected that at least DIKI and DILI biomarkers will not yet achieve full qualification, but instead aim at obtaining a Letter of Support by the agencies, confirming the usefulness of the markers and encouraging sponsors to apply the new biomarkers in an exploratory setting in order to generate more data for final qualification at a later time. DIVI markers are planned to be submitted for a limited qualification; however, at the time of report finalization, this is still subject to discussions within the DIVI group and with the agencies, given some recent challenges in assay performance for some of the markers.

1.3. Summary of progress versus plan since last period

In April 2015, the consortium secured three stand-alone tripartite scientific advice meetings for DIKI, DILI, and DIVI with both EMA and FDA, which provided helpful guidance on further qualification work and data submission. Feedback from both EMA and FDA was very positive. However, discussion with the agencies suggested that for DIKI and DILI, the data available as yet may not be adequate for a full qualification, but rather may entail a Letter of Support. For DIVI, there was confidence at both EMA and FDA that a limited qualification for a subset of markers may be achievable.

The DIKI group completed the statistical analysis of exploratory Stage Gate Analysis data and finished the biomarker prioritization process. As a result, eight biomarkers were selected to be tested on the extended exploratory and the confirmatory sample set.

A common strategy was established with the Kidney Injury Workgroup of the Predictive Safety Testing Consortium (PSTC) on developing a joint translational strategy with SAFE-T, delivering clinical data on kidney injury biomarkers which would lead to specific preclinical studies conducted by PSTC to assess the translational role of these clinical markers. The preclinical studies will be designed and conducted after the full readout of SAFE-T Work Package 2 data (i.e. in 2016).

A total of 98 patients completed the confirmatory contrast media study. Samples from exploratory and the confirmatory studies were analysed for the selected biomarkers and study monitoring and data management processes were completed on the key clinical data that will be used in regulatory submission packages.

Statistical analysis for the final submission package has started but is not yet completed due to delays because of data management issues. The final briefing book will be submitted to EMA and FDA in October 2015.

The DILI team completed adjudication of all acute DILI cases as well as biomarker analyses, ensured proper monitoring of all clinical sites for data cleaning, and finished data management by uploading all data in SDTM format. Statistical analysis of the data has been largely completed as well, preliminary assessment of the results suggest excellent performance for a subset of new liver safety biomarkers in terms of diagnostic and prognostic value. Preparation of submission documents is ongoing while this final consortium report is being written.

The DIVI group made significant progress towards completion of exploratory study analyses as well as generation of additional confirmatory data to support regulatory qualification. Additional funding was obtained from Pfizer and from Takeda to secure samples and associated patient data from HV samples from Bayer, balloon angioplasty (BA) patients (three time points each) from Tel Aviv (TASMC), and vasculitis patients from Paris (APHP).

A concerted effort was made to monitor all WP4 studies and to review and clean data in OpenClinica.

WP5 screened a large amount of samples for all three organ WPs during the last project year, despite significant challenges related to delayed availability of samples. A joint effort within WP5 enabled generation of an additional 6185 data points for DIVI, 12610 data points for DIKI and 12405 data points for DILI samples including assay quality control procedures and data submission before mid-June 2015.

WP6 finalized statistical analysis plans for DIVI, DIKI and DILI and completed analysis of stage gate data to select biomarkers for DIKI, DILI and DIVI. Analysis of exploratory data for DIKI and DILI as well as confirmatory data for DIVI is still ongoing while this final consortium report is being prepared.

In terms of data management, data import programs from Open Clinica to SAS were validated, data management and validation plans set up, and queries according to DVP and agreement with WPs were programmed and validated. The data query process was managed both automatically and manually. Data were mapped to SDTM format and transferred to the statisticians.

Electronic case report forms (eCRF) for remaining studies had to be developed, and data entry verified. On behalf of Data Management and Monitoring, extensive data curation was performed. As solution to data sustainability, SAFE-T's high quality data will be stored in the tranSMART database, initiated during the last project year in collaboration with ITTM (Information Technology for Translational Medicine), a spin-off of the IMI eTRIKS consortium. The database will be updated and extended with further study data as part of the SAFE-T sustainability plan.

The SAFE-T biobank took care of 27 sample shipments involving 17 different studies with a total of 49,054 tubes. A total of 46,218 aliquots were prepared, properly labelled and stored frozen at -80°C.

1.4. Significant achievements since last report

Significant achievements during project year 6 include, as outlined above, the successful completion of tripartite Scientific Advice meetings with EMA and FDA, which led to adapting the overall qualification strategy of the consortium, shifting the goals for DILI and DIKI from full qualification towards Letters of Support, and towards limited qualification for DIVI, overall goals that are, at this point in time, more realistically achievable than a full qualification.

Completion of clinical studies and selection of biomarker subsets across all three organ work packages with promising performance was another major milestone for SAFE-T. Although final statistical analysis has not yet been completed, from preliminary results it can already now be concluded that some of the markers will likely have significant impact on assessment of drug-induced organ injuries. For example, the DILI group identified a marker, acetylated HMGB1, as being highly prognostic of clinical outcome in patients with drug-induced liver injury. This achievement was possible through collaboration with colleagues of the US based Drug-Induced Liver Injury Network (DILIN), who shared samples of patients with severe clinical outcome, a patient group that was not well represented in the SAFE-T DILI studies, with the consortium.

The collaboration with PSTC has to be counted as a significant achievement as well. In particular during the last year of the project, the close collaboration between both consortia proved to be extremely valuable with respect to scientific output, but also regarding interactions with regulators, where both consortia spoke with one voice, contributing to harmonization of qualification processes between EMA and FDA. PSTC also helped to establish a closer, informal relationship to FDA by connecting SAFE-T to PSTC's Scientific Liaison at FDA CDER, who since then has been regularly involved in SAFE-T discussions and enabled informal communication with FDA's scientific experts.

Data capture and data management, as well as proper monitoring of the clinical studies were among the biggest challenges for the consortium, as neither the resource nor respective budget could be specified in detail in the original work plan. SAFE-T solved both issues via identifying external contractors providing the required support and ensured funding via internal budget reallocation and additional cash contributions from EFPIA partners. Despite some delays and difficulties, both activities could subsequently be completed by the contractors.

The most important SAFE-T outputs will be results of biomarker performance, as reflected in the regulatory submission documents, along with the associated raw data and clinical samples. Maintaining access to these consortium assets is of critical importance not only to address potential questions by EMA or FDA during regulatory review of the data, but also to facilitate future research on either the selected SAFE-T biomarkers or new marker candidates identified after the completion of SAFE-T. Ensuring sustainability thus was one of the key tasks to be tackled by the consortium during its final year.

SAFE-T has established a sustainability plan (see Section 5.3) to ensure access to data and samples, as well as maintenance of the consortium website for up to one year. The plan as approved by the SAFE-T Steering Committee is to establish a follow-up project, ideally under IMI2, taking care of sustainability of SAFE-T outputs after one year.

As yet, there is no dedicated guideline available for assay validation in the context of biomarker qualification. The assay validation process established by SAFE-T and PSTC is currently being discussed between both consortia, with the aim of integrating respective validation procedures into one common document. This should then be discussed with the FDA at a joint workshop, in order to have the consortium processes accepted and adopted as generic procedure for assay validation in the context of biomarker qualification. This would be another major achievement for both SAFE-T and PSTC. The SOPs for assay validation and quality control are already publicly available via the SAFE-T website and can be used by SAFE-T partners, collaborators and external laboratories for further assay development.

1.5. Scientific and technical results/foregrounds of the project

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.

Eight **DIKI** biomarkers with good performance in detecting acute tubular injury were chosen to test in a wider sample set that would include the confirmatory study of acute tubular injury following administration of contrast media as well as samples from healthy subjects and from patients with chronic renal diseases such as diabetic nephropathy and autosomal dominant polycystic kidney disease.

Final results are not yet available but statistical analysis is ongoing. A final data package will be submitted to EMA and FDA in 2015. It is expected that this data package will support the use of a number of the tested biomarkers to aid in monitoring for acute tubular injury in early Phase 1 clinical studies. These biomarkers would allow the safe progression of new molecular entities shown to have preclinical nephrotoxicity into early clinical development.

For **DILI**, the major achievements were the recruitment of patients with acute DILI from various clinical partners in France, Germany, Switzerland, Spain, Israel and UK, as well as control groups consisting of patients with chronic disease of non-hepatic origin, and healthy volunteers with no evidence of liver disease. The biomarkers under study were analyzed in an initial stage-gate cohort and were narrowed down to the most promising candidates for subsequent evaluation in DILI patients recruited within SAFE-T. In addition, 166 samples from DILI patients recruited by the US DILIN network were contributed. The DILIN samples were of particular interest since they comprised idiosyncratic DILI cases only, 10% of which progressed to liver failure.

Of the biomarkers studied in the project, various new candidates were identified as offering comparable sensitivity to ALT in terms of diagnosing liver injury, and – more importantly – allowing discrimination of patients who recovered spontaneously from those that developed liver failure. The biomarkers performed differently depending on the DILI causing drug, thus shedding light on the mechanisms behind the liver injury.

The **DIVI** group has tested candidate biomarkers in surrogate populations of humans with vascular disease or injury, including the human vasculitides and other relevant vascular disorders, working under the hypothesis that the human vasculitides are associated with histopathological alterations similar to those observed in DIVI despite different pathophysiologic mechanisms. WP4 identified relevant clinical biomarkers for evaluation in clinical conditions that have morphologic similarities to preclinical DIVI, and consortium partner Firalis SAS validated assays for these markers which were then measured in clinical samples. Univariate statistical analysis resulted in poor performance for the ability of individual biomarkers to discriminate disease samples from healthy volunteers. Several multivariate statistical methods were then used to identify combinations of biomarkers with improved performance. In one example, AUROC (Area under the Receiver Operating Characteristic) of the 5 best combinations was greater than 0.8. Regulatory qualification of the DIVI biomarkers was not achieved during the course of the SAFE-T consortium because of some technical issues that arose late in the project. However, WP4 is still discussing its options for regulatory submissions based on the data already generated.

WP5 selected appropriate assay platforms for the individual analytes across the three organ work packages, identified and tested commercial kits and assay reagents, and developed assays for those biomarkers for which no established assays or kits were available. In certain cases assay reagents needed to be generated, purified and applied to the assay development procedure at the different screening sites. The heterogeneous nature of the analytes for DILI, DIKI and DIVI analytes required the use of a number of different assay platforms, e.g. immunoassays, ELISAs, LC-MS, qPCR or colorimetric assays, which were successful implemented for the screening procedures.

All assays were thoroughly tested and validated before approved by a dedicated team of WP5 members from different partners for application in sample screening. The validation procedures were developed by WP5 considering commonly used guidelines. A fit-for-purpose approach was realized for the needs of assay validation within SAFE-T. The sample analysis was accompanied by a quality control procedure to help ensure data consistency of the different sample screening phases throughout the project lifetime and beyond.

The final assays were then used to screen large amount of samples for the different sample sets (stage gate, exploratory, confirmatory) selected by the individual organ work packages. Altogether, within SAFE-T WP5 provided more than more than 64,575 data points generated with the validated assays to the data management team for statistical analysis.

In terms of analysis of the biomarker data, different statistical approaches, the building of predictive models and their validation were evaluated during the initial period of the project. Exploratory data were analysed and a briefing book shared and discussed with the health authorities in February 2015.

For DIVI, recent technical problems with the analytical platform (MSD) for the determination of the biomarker concentrations are currently preventing finalization of the statistical analyses and the submission of the qualification packages with limited content of use to the health authorities.

For DILI and DIKI, the original intention of confirming the performance of selected biomarkers has been modified to conducting exploratory analyses to identify biomarkers worthy of further study. The aim

of the submission now is to get a Letter of Support from the Regulators supporting further work on selected biomarkers.

To capture clinical data, WP6 established eCRFs (OpenClinica) for more than 30 prospective studies and up to 6 sites. For the exchange of data, a CrushFTP server was set up. This server hosts all data in SAFE T collected and generated, like study protocols, regular OpenClinica exports, biomarker screening results, results of statistical analysis, etc. In the tranSMART database, all high quality data (data after monitoring, cleaning and management) are stored and will be further improved by uploading the latest data sets during the next months. This database easily allows cross study searches and searches for patients with specific clinical data.

With its biobank and database, SAFE-T has created a unique, centralized and well-controlled repository of human samples and data dedicated to the qualification of translational DIKI, DILI, DIVI biomarkers.

1.6. Potential impact, main dissemination activities and exploitation of results

"It is estimated that 197,000 deaths per year in the EU are caused by ADRs *(Adverse Drug Reactions)* and that the total cost to society of ADRs in the EU is €79 billion." (EU commission, MEMO/08/782, 10 Nov 2008). Among the side effects most challenging to drug developers and prescribers alike are drug-induced injuries to kidney, liver, and vascular system.

SAFE-T has made a significant contribution to detect such adverse reactions as early and as specifically as possible, through the extensive characterization of new serum, plasma and urinary biomarkers. These now standardized biomarkers have great potential to not only improve drug safety, but in the future can likely be applied to non-drug related diseases of liver, kidney and vascular system as well. Their usage will enable close monitoring of affected patients and predict their clinical outcome. This is of crucial importance to make therapeutic use of drugs safer, to increase the likelihood that respective risks to patients are detected while a new compound is still in development and can be managed properly, and to ultimately reduce economic burden to public health significantly.

Among the clinical indications that will benefit from these new biomarkers are some of the most dreaded scourges to public health such as diabetic nephropathy, acute kidney injury, non-alcoholic steatohepatitis (NASH), chronic hepatitis C infection, liver fibrosis and cirrhosis, peripheral and coronary artery disease. Samples available in SAFE-T's biobank cover nineteen different patient populations, and can be used for further biomarker research. The data analysed across the consortium's biomarker qualification program provides comprehensive insight into performance and variability of 105 new soluble biomarkers (initial screening for DIKI: 20 proteins; DILI: 15 proteins, 2 enzyme activities, 3 miRNAs, 41 bile acids; DIVI: 25 proteins).

Application of SAFE-T biomarkers both in drug development and in clinical practice will likely make drugs safer, reduce late stage attrition in drug development, and improve diagnosis and management of acute and chronic diseases highly relevant to public health not only in Europe, but globally.

The strategy for clinical biomarker qualification established and tested by SAFE-T, biomarker assays developed by SAFE-T SMEs, and state-of-the-art processing and storage of human biospecimens for qualification by the SAFE-T biobank will have significant impact on biomarker research and substantially ease qualification efforts in the future.

SAFE-T results are being presented at major European and US scientific conferences and published in high profile peer-reviewed journals. Assay validation standards developed by the consortium are already available on the SAFE-T web page and assay specifications will be made publically available. Lessons learned by SAFE-T will help to streamline future qualification activities and steer clear of rocks SAFE-T was faced with.

Assay developers will be able to capitalize on the results of the consortium to develop commercial assays that can be used in clinical studies and, as more data are gathered in future consortia, extend the use of the novel organ injury biomarkers to a hospital setting.

1.7. Lessons learned and further opportunities for research

Qualification of new safety biomarkers needs prospective clinical studies with repeated sampling across a variety of different patient populations. Considerable expertise in drug development, capacities to recruit large numbers of specific patients in a short time span, experience and capabilities to test, develop and validate a multitude of new assays, and close collaboration with regulators are crucial prerequisites to successfully qualify new biomarkers. This cannot be done by individual companies, SMEs or academia in isolation, but needs large scale collaboration across a variety of larger and smaller enterprises and academic institutions, in close alignment with regulatory agencies. The IMI Public Private Partnership program provides a highly efficient framework to facilitate such collaborative endeavours.

During the course of the SAFE-T project, benefits but also challenges associated with the IMI PPP model became apparent. Lessons learned in SAFE-T during six years of collaborative research should significantly help to streamline future biomarker qualification efforts. The following sections provide an overview on some key learning. A more detailed list of issues and lessons learned has been compiled in slide sets from SAFE-T partners and collaborators which had been discussed in depth at SAFE-T's final face-to-face meeting in Basel in June 2015. These will be made available to the IMI-JU upon request. A more comprehensive analysis of challenges and lessons learned is also planned to be published as a joint effort of consortium and collaboration partners after final feedback from regulatory agencies and IMI-JU has been received.

Lessons learned

Project planning and initiation

Biomarker qualification is a complex undertaking and needs adequate planning time. Time span available to carefully plan the qualification strategy, set up a suitable budget, and develop a comprehensive Description of Work was just a couple of months. With hindsight, more extensive

planning time may have helped to avoid some difficulties that became apparent during the course of the project.

Collaboration with external partners, such as other consortia, or specialist contractors, is crucial for a project of SAFE-T's size and complexity in order to capitalize on synergies and avoid undue duplication of efforts. For SAFE-T, the close interaction with C-Path Institute's Predictive Safety Testing Consortium (PSTC) was recognized to be of particular importance, as PSTC was focusing on preclinical biomarker qualification across the same organ areas as SAFE-T, whereas SAFE-T's primary objective was clinical qualification.

Hence, a Confidentiality Agreement between both consortia was signed already in the first project year. However, signature of the final legal framework agreement could be completed only three years later, given differences in in consortia structure and relevance of IP considerations. Once the legal framework agreement was in place, collaboration between both consortia turned out to be highly efficient and beneficial for both sides. DIKI, DILI, and DIVI groups in SAFE-T and PSTC worked on joint project plans addressing key regulatory feedback and requirements; both consortia developed shared objectives and a common vision of a translational safety biomarker strategy, and openly shared knowledge and information across consortium borders. The collaboration was hallmarked by mutual respect and understanding of strengths of diverse participants and stakeholders, and, through collaboration with FDA and EMA, contributed substantially to clearer and more harmonized regulatory processes.

A crucial lesson learned is that, as soon as a project has been approved, **collaborations across consortia, both within and outside IMI, need to be planned well in advance of the project start**, and any legal or organizational barriers resolved ideally by the time the project is kicked off.

A particular challenge for SAFE-T was the absence of regulatory guidance at project start. Guidance from both EMA and FDA was published only once the project was well underway. The qualification program had to be planned without being sure that it is fully aligned with regulatory expectations. It is therefore of great importance to have a close dialogue with the regulators throughout the project lifetime. This was ascertained by having dedicated liaisons available to the consortium as advisors from both agencies. For future projects, it will be highly relevant to **ensure close regulatory involvement already during the planning phase**.

Expertise and competencies

Core competencies relevant to biomarker qualification include study planning, protocol and report writing, patient recruitment, data capture, data management, data analysis, programming, and study monitoring. Respective expertise needs to be on board the consortium from the beginning. Ideally, this would include **a clinical CRO** on either the applicant or the industry side, taking care of some or all of the above. SAFE-T resolved the challenge of not having a CRO as a consortium partner via subcontracting of data management and monitoring work during the project. Respective funds were made available via internal reallocation and additional cash contributions from EFPIA.

In terms of patient recruitment, in particular the DIKI and DILI work packages faced significant challenges to include a sufficient number of patients within the planned timeframe. For future consortia focusing on biomarker qualification it will be crucial to **ensure at project start the availability of an adequate number of clinical sites with proven track record for recruiting suitable numbers of patients across the target populations of interest**.

Regarding the need for both, a CRO and for more clinical sites of excellence facilitating optimal patient recruitment, the IMI two stage process of selecting a defined applicant consortium from a range of competitors which then joins the associated industry consortium can turn into a significant challenge, if none of the applying groups match key requirements. SAFE-T addressed that challenge by subcontracting work and adding clinical partners during the course of the project. However, since the consortium budget was fixed for the entire project lifetime, the required funds had to be made available via internal reallocation and additional cash contributions from EFPIA, as pointed out above.

A lesson learned particularly for biomarker qualification is that it may be **preferable to have a joint applicant/industry consortium applying for IMI funding in a one-stage process**, ensuring that all key expertise is available either on the applicant or industry side from the beginning.

Project management

SAFE-T was governed by the Steering Committee (SC) with representatives from all partners. Supporting the SC were a leadership team consisting of a project coordinator and a scientific coordinator, both from EFPIA, a Scientific Advisory Committee, an Ethics Committee, and an IP Committee. It turned out that there was a need for a more inclusive governance body supporting the SC and the leadership team. To address this need, a SAFE-T Project Management Team (PMT) was established in 2012 with representation from EFPIA, academic, and SME partners, helping to prepare SC discussions and providing consensus-based recommendations to the SC. The PMT's support turned out to be very efficient, and was acknowledged and appreciated by the consortium.

For future projects, the recommendation would be to **establish a governance or advisory team such as the PMT right from project start**.

In terms of budget it would have been highly desirable to **keep a defined fraction of the budget flexibly available and maintained by the managing entity** to cover any upcoming needs not anticipated at project start.

Legal provisions of both the IMI Grant Agreement and the SAFE-T Project Agreement were not necessarily well known by all participants. This applies not only to details, but also to some basic principles of these documents. For future consortia, it is **recommended to prepare a Consortium "Legal Handbook"** summarising the main provisions relevant to the consortium and providing precise references (ownership of results, access rights, rules for publications, governance bodies and decision making rules, …). Dedicated webinars organized by the IMI-JU may be helpful as well.

What was sadly missed on SAFE-T was a **shared project management tool** similar to e.g. MS Project to monitor the advance of all WPs (deliverables, milestones). Future consortia should insist on having that available right from the start.

Sustainability of the project, and consequences of a limited sustainability, was not anticipated by all consortium partners, and although the need for sustainability was emphasised at an early stage, financial planning was only initiated at a rather late project stage, given uncertainties around associated costs.

In future biomarker consortia, a **sustainability working group should be created at an early stage of the project**, working on all key issues: scientific, technical, legal, financial, operational.

EFPIA contribution

A major obstacle for SAFE-T was the impracticality to properly estimate a realistic in-kind value of archived or prospectively collected EFPIA samples, particularly in the absence of clear respective guidance at project start. In many cases, both the true need for and the true value of such samples was significantly overestimated by EFPIA partners, leading to substantial inflation of the expected total EFPIA in-kind contribution to the consortium, and subsequently to significant gaps in committed versus actual in-kind contributions by individual partners. A key conclusion was that **archived samples may be less useful and more challenging to provide to the consortium than anticipated**.

In the initial SAFE-T budget, EFPIA in-kind support was defined at rather high level, lacking both granularity and specificity. To properly plan and conduct a clinical biomarker qualification program, however, it needs to be known as early as possible, for instance how many samples across which populations will be needed, and who is committing to provide those samples.

For future qualification efforts, it will be **crucial to properly assess in-kind value of samples upfront and to provide realistic estimates of who will provide which type and number of samples**.

There is also a clear **need for defined expert support**, such as data management, including provision of data capture systems, which for SAFE-T had to be built from scratch using open source software. On top of all that, an open **commitment to e.g. run special studies for the consortium**, such as drug challenge studies, or to **provide cash funding for specific items** in addition to public money would be highly desirable.

Finally, working on an IMI project such as SAFE-T can be time-consuming and challenging, which **needs to be fully reflected in people's individual objectives** and properly taken into account for resource planning.

Opportunities for further research

SAFE-T generated a vast amount of data on 105 initial biomarker candidates, of which more than 20 showed promising performance either individually or as part of marker panels. Given challenges outlined above, most of these selected markers will be submitted to EMA and FDA not to achieve full qualification yet, but rather to obtain a Letter of Support, confirming the practical utility of the markers and encouraging sponsors to use them in an exploratory setting in order to generate more data that

can then help to support a **qualification at a later point in time**. Together with data coming from prospective studies, which can build on the protocols developed by SAFE-T, such a qualification could very likely be achieved within a rather short time frame, given the groundwork done by SAFE-T.

Utilization of the new biomarkers in drug development, however, is only one of a range of potential applications. Given the diverse physiological and pathological processed reflected by the new markers, it is highly likely that they will have practical utility also in a hospital or clinical practice setting, helping to improve diagnosis, prognosis, and disease management across a variety of acute and chronic illnesses. Examples include diabetic nephropathy, acute kidney injury, renal or liver transplant rejection, non-alcoholic steatohepatitis (NASH), chronic hepatitis C infection, liver fibrosis and cirrhosis, peripheral and coronary artery disease. **Systematic assessment of diagnostic and prognostic value of the new markers in medical care** will be a major task for academic research in the coming years.

Drug-induced injury to kidney, liver, and vascular system are representing major drug side effects, but there are others that are of increasing concern as well, such as **drug-induced pancreatitis, muscular or skin injury**. All of these areas of toxicity will likely benefit from qualified new biomarkers as well, supporting mechanistic understanding, diagnosis, prognosis, and risk management. Building on SAFE-T's qualification strategy and lessons learned, another joint effort of clinical research, pharmaceutical and biotech industry could develop and qualify markers for new toxicities of interest within a rather limited timeframe, and in a highly efficient manner. To some extent, samples still available in the SAFE-T biobank might even be utilized to support that work.

SAFE-T, in close collaboration with PSTC, laid the foundation for translational safety biomarker qualification, bridging preclinical to clinical assessment of drug safety. However, a missing piece still is **application of the new markers in an *in vitro* setting**, helping to understand mechanisms of toxicity and predicting side effects at the discovery and compound selection phase already. Research efforts are ongoing already at different sites to develop and validate new, advanced mechanistic in vitro models e.g. for drug-induced liver injury. These models may benefit significantly from new endpoints based on the SAFE-T biomarkers.

Similarly to the *in vitro* setting, there are now *in silico* models available, allowing to model and simulate drug-induced organ injuries, such as the Hamner Institute's DILIsym™. As yet, these models use standard endpoints for prediction of human side effects. **Incorporating and testing SAFE-T biomarkers in *in silico* models and simulation platforms** may help to significantly enhance mechanistic understanding and improve predictions of human drug safety profiles.

Finally, for some drug side effects, monitoring patients as closely as possible is key to timely detection of toxicity and prevention of serious outcomes. As an example, drug-induced, idiosyncratic, hepatocellular injury is occurring rarely, but is often associated with life-threating or fatal outcome. Regular monitoring of safety biomarkers such as aminotransferases in serum is required to detect injury as early as possible and stop or adapt treatment to prevent more serious damage to the liver. However, there are limits to monitoring intervals suitable in clinical practice. Acceptance of taking blood samples more frequently than on a monthly basis is usually very limited both on the patient and the prescriber side (1). On the other hand, serious idiosyncratic DILI can develop within time spans shorter than one month (2), thus, there is an urgent need to facilitate close monitoring in patients at

risk to experience serious DILI. Ideally, this would be achieved using point-of-care or bedside testing. Indeed, a paper-based, inexpensive, and easy-to-use test for bedside testing of aminotransferases has been developed recently (3, 4) and is expected to received regulatory approval soon. **Developing point-of-care technology for a subset of more sensitive and specific safety biomarkers** such as some of the SAFE-T liver markers may well entail a paradigm shift in liver safety monitoring, allowing weekly or even daily monitoring of biomarkers with a finger prick blood test, and transmission of the readout via e.g. smartphone to the treating physician.

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