Topic: Translational Safety Biomarker Pipeline (TransBioLine): Enabling development and implementation of novel safety biomarkers in clinical trials and diagnosis of disease

All information regarding future IMI Call topics is indicative and subject to change. Final information about future IMI Calls will be communicated after approval by the IMI Governing Board.

Topic details

Action type	Research and Innovation Action (RIA)
Submission & evaluation process	2 Stages

Specific challenges to be addressed

Early and reliable detection and monitoring of adverse events is essential for improving of patient safety, reducing late attrition of drug candidates, and enhancing understanding of toxic mechanisms. In particular, biomarkers that provide insights into mechanisms of tissue injury have the potential to revolutionise drug development as well as diagnosis of diseases. Therefore, the development of innovative, non-invasive biomarkers of tissue injury is of great interest to drug developers, regulators and the broader scientific community. Recent progress in biomarker development including the previous **SAFE-T** (http://www.imi-safe-t.eu/) that identified several promising biomarker approaches as well as the latest scientific advances in analysis of circulating microRNA provide excellent opportunities for biomarker research. Furthermore, the recent progress in regulatory science of biomarker qualification achieved by the Critical Path Institute and the Foundation for the National Institutes of Health (FNIH) provides a blueprint for conduct of formal qualification of emerging biomarkers via an innovative translational paradigm that relies on tissue injury caused by diseases and only limited clinical and non-clinical studies for assessment of biomarker performance. This approach optimises resource use and accelerates biomarker development.

Need and opportunity for public-private collaborative research

New biomarker approaches are needed to enable development of new therapeutic modalities and improve diagnosis of diseases. The development and qualification of biomarkers is a costly and time-consuming

process. It requires developing new innovative scientific approaches and analytical technologies as well access to appropriate human populations for biomarker qualification and assay validation, and other large-scale cross-institutional efforts. Therefore only large international scientific collaborative projects that include industry, academic researchers and regulators can be successful. For instance, the previous IMI project SAFE-T in the EU and the Critical Path Institute's Predictive Safety Testin Consortium (PSTC) yielded several promising biomarker candidates that are under review by the regulatory agencies in Europe and USA. The PSTC approach that relies on human disease as approximation of chemical injury for evaluation of biomarker performance significantly reduced need for conduct of costly clinical trials. Furthermore, recent progress in circulating microRNA analysis and next generation sequencing has opened new avenues for development of mechanistic biomarkers and precision medicine. However, more research and robust datasets are necessary to qualify new biomarker approaches by regulatory agencies and to enable their implementation in clinical trials and diagnosis of disease. The proposed TransBioLine topic with funding from the Innovative Medicine Initiative 2 Joint Undertaking (IMI2) provides a unique platform for leading experts from industry, academia and regulators to design and execute necessary research needed for development and implementation of novel safety biomarkers in clinical trials and clinical practice. Furthermore, the topic provides an opportunity for partnering with Small- and Medium-sized Enterprises (SMEs), including diagnostic companies to enable the development of robust assays that are compliant with regulatory requirements for use in clinical laboratories decreasing the time needed for transferring biomarker discoveries from bench to bedside.

Scope

The TransBioLine project will focus on development of biomarkers of injury for liver, kidney, pancreas, vasculature, central nervous system (CNS) and the development of non-invasive liquid biopsies. The project will have four strategic goals:

1. **Develop data sets enabling the implementation of emerging safety biomarkers in clinical trials and/or diagnosis of disease:**

   The applicant consortium will be expected to develop robust learning and confirmatory datasets that will support appropriate "contexts of use" for the emerging biomarkers. The resulting datasets will form the foundation for formal biomarker qualification by the European Medicine Agency (EMA), Food and Drug Administration (FDA) and Pharmaceutical and Medical Devices Agency (PMDA).

2. **Develop non-invasive mechanistic biomarkers of tissue damage called "liquid biopsy" that will have a potential to revolutionise drug development and diagnosis of disease:**

   The applicant consortium will be expected to exploit the circulating cell free serum microRNAs for development of non-invasive tissue- and mechanism- specific diagnostic signatures/biomarkers. This effort will exploit state of the art technologies such as next generation sequencing in conjunction with systems biology approaches to gain insights into mechanisms of toxicity and disease, and risk assessment.

3. **Develop standardised assays and technologies for detection of biomarkers and data interpretation:**

   For biomarkers pursued by the TransBioLine project, the applicant consortium is expected to develop robust assays compliant with regulatory requirements for implementation in clinical laboratories for clinical trials and clinical practice including appropriate level of validation as well bioinformatics, sample and data management tools. This will provide opportunities for partnership with diagnostic companies and SMEs.

4. **Achieve regulatory acceptance for biomarkers:**

   The applicant consortium is expected to submit regulatory documentation that supports formal biomarker qualification with EMA, FDA and PMDA, and manage biomarker qualification process. Furthermore, the consortium will establish and maintain collaborative relationship with regulatory agencies (EMA, FDA, PMDA) organise workshops and meetings.

Research approach: The biomarker development for each target organ will concentrate on a specific context of use with limited number of already identified emerging biomarker candidates. The main focus of each individual target organ work packages will be on the development of learning and confirmatory datasets that are essential for supporting regulatory qualification and implementation of emerging biomarkers in clinical trials and diagnosis of disease. To enable application of biomarkers developed under TransBioLine project in routine clinical laboratories, assay development, statistics and an expertise in regulatory science, will be essential for achieving regulatory acceptance by EMA, FDA and PMDA. It is expected that these functions will be fully integrated with target organ work packages (WPs) to achieve maximum flexibility and impact. The appropriate resources for these activities in addition to project management will be allocated from individual target organ WPs budgets.

Since disease approximate chemical injury, TransBioLine project will rely mainly on samples from subjects with tissue injury caused by appropriately selected diseases. Only targeted clinical and non-clinical studies with drug/chemical induced organ injury will be used as supportive evidence for assessment of biomarker performance. The clinical sample set for analysis of biomarker performance will predominantly consist of remaining samples from clinical studies and remaining samples from subjects with appropriate disease phenotypes collected during medically indicated examinations. This approach limits effects of storage on biomarker stability, optimises resource use and accelerates biomarker development. It requires close collaboration with clinics and clinical researchers, especially to enable accurate diagnosis and anchoring endpoint evaluations, adherence to inclusion/exclusion criteria, ensuring correct patient consent, and correct sample collection. The state of the art next generation sequencing and system biology with proven track record in identifying miR signatures in human subjects and normalization approach to enable consistent quantification will be necessary for development of miR-based liquid biopsies. A partnership with SMEs and diagnostic companies will be required for development of robust assays that will enable application of the studied biomarkers in routine clinical laboratories. Since the qualification of biomarkers by regulatory agencies is essential for implementation of biomarkers in clinical trials and diagnosis of disease, strong expertise in regulatory science and established relations with Health Authorities by the applicant consortium will be required. To achieve the TransBioLine goals appropriate sample and data management systems, statistical and bioinformatics tools and strategy will need to be integrated throughout TransBioLine WPs.

Expected key deliverables

The TransBioLine primary objective is the development of datasets enabling formal biomarker qualification and biomarker implementation in clinical trials and/or diagnosis of disease. The key deliverables will consist of:

- Biomarker qualification submissions to EMA, FDA and PMDA for specific high priority context of uses defined by TransBioLine for liver, kidney, vascular, pancreas and Central Nervous system (CNS);
- Datasets that will enable the acceptance of emerging safety biomarkers by regulatory agencies for specific drug development programs under individual Investigational New Drug (INDs) even before the biomarkers are qualified as drug development tools;
- A new paradigm-changing non-invasive biomarker approach for interrogating mechanisms of toxicity and disease via miR-based “liquid biopsies”. This will include (a) detailed characterization of cell free serum miR-nome in healthy subjects and in subjects with diseases and (b) system biology platform applicable for addressing safety in clinical trials that will enable investigators to de-convolute observed miRs signatures to biological pathways in specific tissues;
- Robust biomarker assays compliant with regulatory requirements defined as “Research Use Only” (RUO), “Laboratory Developed Tests” (LDT) and/or “In Vitro Diagnostics” (IVD) as appropriate. The long term goal is to have assays broadly available in clinical laboratories world-wide;

- To facilitate the biomarker qualification by regulatory agencies, the TransBioLine will organise an annual biomarker qualification workshop with EMA, FDA and PMDA. It is expected that the workshop will have a significant impact on harmonization of biomarker qualification across regions, maintain organisational timelines, and facilitate global collaboration and global reach;
- To promote application of new biomarkers in clinical practice, publications in high quality peer-reviewed journals as well as presentations at various national and international meetings is expected.

Expected impact

The biomarkers developed during TransBioLine are expected to accelerate drug development by providing innovative drug development tools and also significantly improve diagnosis of disease by enabling non-invasive interrogation of disease mechanisms. The availability of qualified biomarkers as drug development tools will have a broad positive impact on patient safety in clinical trials as well. The TransBioLine will open new markets by introducing new commercially available diagnostic products and services by diagnostic companies and SMEs. This will strengthen the competiveness and industrial leadership of Europe. TransBioLine will enable the development of new innovative biomarker approaches derived from genomics applicable as non-invasive "liquid biopsies" providing tools for precision diagnosis of mechanisms of toxicity or disease at the molecular level. Although the formal qualification of biomarkers as drug development tools by regulatory agencies is the ultimate deliverable, the biomarker data produced by TransBioLine will enable the acceptance of biomarkers by regulatory agencies under individual INDs even before the biomarkers are fully qualified.

Potential synergies with existing Consortia

Applicants should take into consideration, while preparing their short proposal, relevant national, European (both research projects as well as research infrastructure initiatives), and non-European initiatives and consortia. Synergies should be considered in order to incorporate past achievements, available data and lessons learned where possible, thus avoiding unnecessary pitfalls, overlap and duplication of efforts.

Biomarker development and biomarker qualification by regulatory agencies is a recognised unmet medical need. In fact, IMI JU funded its first translational biomarker project **SAFE-T** (http://www.imi-safe-t.eu/) that yielded several biomarker candidates. Among current and future IMI consortia, **TransQST** (http://transqst.org) and TransBioLine WP liquid biopsies have an excellent opportunity to bridge in non-clinical and clinical systems toxicological approaches and realise synergies in the development of systems toxicology tools. Furthermore, several international organizations and consortia in the EU and US are actively working in this space. Most notable are the Predictive Safety Testing Consortium of Critical Path Institute in Tucson, AZ, and Biomarker Consortium of FNIH in Washington, DC, that have made significant progress in biomarker qualification for selected liver and kidney biomarkers and are collaborating with the FDA on developing a regulatory framework for biomarker qualification and more recently, biomarker assay validation. In addition, several technical committees at the Health and Environmental Science Institute in Washington, DC, are working on evaluating analytical technologies and developing best practices. Recently, Japan's NIHS initiated a large collaborative project in liver biomarkers. Therefore, developing collaborative partnerships with these organizations when applicable throughout duration of TransBioLine project will be important. In contrast to the previous and current biomarker development efforts, the proposed TransBioLine will focus on enabling implementation of emerging biomarkers in clinical trials via qualification by EMA, FDA and PMDA and integrating the progress in regulatory science with the development of unique state-of-the-art mechanism-based biomarkers and clinical assays.

Industry consortium

The industry consortium will provide expertise and assets in developing large data sets derived from subjects in clinical trials, access to healthy volunteer populations, and data generation for full characterization of biomarker performance. The use of samples from prospective clinical trials run by the member companies will bring significant savings to the project notwithstanding limiting the need for unnecessary clinical investigations. Because of the global nature of clinical studies run by the industry consortium, the TransBioLine project will be able to evaluate performance of biomarkers in variety of populations. Furthermore, the member companies will contribute targeted non-clinical studies, targeted clinical studies, and clinical and non-clinical datasets. Additionally, the industry consortium will contribute expertise in assay validation and a regulatory perspective, expertise in conduct of clinical investigations, experience with biomarker use in preclinical and clinical studies, study data that will support development of novel imaging agents, and managing processes and samples among various laboratories participating in the project. The expected industry consortium contributions will also include biomarker assays when applicable, expertise and scientific leadership.

Indicative duration of the action

The indicative duration of the action is 60 months.

Applicant consortium

The applicant consortium will be selected on the basis of the submitted short proposals.

The applicant consortium is expected to address all the objectives and make key contributions to the defined deliverables in synergy with the industry consortium, which will join the selected applicant consortium in preparation of the full proposal for stage 2. This may require mobilising, as appropriate the following expertise:

- Expertise with a demonstrated track record via publications in peer-reviewed journals in pertinent biomarker assay technologies needed to conduct TransBioLine research;
- Demonstrated analytical capabilities such as immunoassays, Liquid Chromatography-Mass Spectrometry (LC-MS), next generation sequencing etc;
- Expertise and capabilities in sample management systems, patient compliance statements, data management including database systems that comply with managing clinical data, state-of-the-art statistical and bioinformatics tools including tolls for next generation sequencing data;
- For the liquid biopsy approach, extensive expertise and proven track record in peer-reviewed literature in analysis and normalisation of circulating miRs in human subjects using next generation sequencing and state-of-the-art bioinformatics with demonstrated expertise in generating signatures of circulating miRs for specific disease phenotypes and/or toxicities in human subjects;
- To achieve regulatory acceptance of biomarkers by regulatory agencies, extensive expertise in regulatory science with proven track record in biomarker qualifications including preparation of regulatory submissions to regulatory agencies (EMA and/or FDA), and interactions with regulatory agencies world-wide;
- Ability to prospectively enrol the remaining samples from subjects with disease phenotypes defined by individual WPs to assess the biomarker performance pertinent to the TransBioLine research;
- Capability to identify, retain and manage remaining serum, Cerebrospinal fluid (CSF) and urine samples from healthy subjects and subjects with relevant disease phenotypes, including a broad range of etiologies and/or treated with a variety of therapeutic modalities as specified by individual WPs;
- Capability to obtain appropriate patient consent forms, access detailed medical records data for all subjects/samples, and adjudicate the data;
- Ability to potentially recruit subjects treated with appropriate drugs for conduct of limited prospective studies;

- Proven expertise in efficiently managing and maintaining time lines for large, multi-institutional scientific projects and proven expertise in project management.

In addition to academic groups, relevant Small- and Medium-sized Enterprises (SMEs) with relevant proved expertise are encouraged to participate in the applicant consortium. SMEs can be of great benefit to IMI projects and, inter-alia strengthen the competitiveness and industrial leadership of Europe. Their involvement might offer a complementary perspective to industry and the academia, and help deliver the long-term impact of the project. For these reasons, applicants should consider engaging SMEs throughout the proposal. Under this topic, the contribution of SMEs would be considered especially beneficial in areas that include bioanalytical expertise for diagnostic assay development, bioinformatic analysis, data mining, and data and sample management.

The size of the consortium should be proportionate to the objectives of the project.

Suggested architecture of the full proposal

In the spirit of the partnership, and to reflect how IMI2 JU call topics are built on identified scientific priorities agreed together with EFPIA beneficiaries/large industrial beneficiaries, these beneficiaries intend to significantly contribute to the programme and project leadership as well as project financial management.

The final architecture of the full proposal will be defined by the participants in compliance with the IMI2 JU rules and with a view to the achievement of the project objectives. The allocation of a leading role within the consortium will be discussed in the course of the drafting of the full proposal to be submitted at stage 2. To facilitate the formation of the final consortium, until the roles are formally appointed through the consortium agreement, the proposed project leader from among EFPIA beneficiaries/large industrial beneficiaries shall facilitate an efficient negotiation of project content and required agreements. All beneficiaries are encouraged to discuss the project architecture and governance and the weighting of responsibilities and priorities therein. The architecture outlined below for the full proposal is a suggestion. Different innovative project designs are welcome, if properly justified.

The consortium is expected to have a strategy on the translation of the relevant project outputs into regulatory practices, regulatory, clinical and healthcare practice. A plan for interactions with Regulatory Agencies/health technology assessment bodies with relevant milestones, resources allocated should be proposed to ensure this e.g. qualification advice on the proposed methods for novel methodologies for drug development, qualification opinion.

A plan for aspects related to sustainability, facilitating continuation beyond the duration of the project should also be proposed.

Work package 1 – Biomarkers of Kidney injury

Context of Use:

A panel of qualified urinary kidney safety biomarkers may be used together with sCr and BUN in subjects with normal kidney function or in patients with some pre-existing kidney disease (and not just normal healthy volunteers**)** as a more sensitive and/or earlier biomarker to monitor for both glomerular as well as renal tubular safety in clinical trials. These biomarkers will be used when such injury has been demonstrated to be monitorable by the biomarkers in animal studies of similar duration with the same test agent. Applying the biomarkers in initial single and multiple ascending dose clinical studies, or in continuous dosing clinical studies could enable or restrict initial dose level selection and planned dose escalations, or drive decisions to interrupt or continue dosing.

Specific Goals:

1. **Progressive Qualification of Translational Tubular Injury Biomarkers in Patients with Mild Pre-existing Kidney Dysfunction:**

Given that diabetes and hypertension are the two known top causes of chronic kidney disease (CKD) most worthy of being considered for advancing confidence in the use of tubular injury biomarkers in patients (and not just normal healthy volunteers (NHVs)), cohorts of such hypertensive and diabetic patients could be targeted for testing, such that comparable thresholds and significant fold-change from baseline performance results are seen as comparative data to reference against data on translational kidney safety biomarkers that have already been collected for NHVs and patients with normal renal function [1]. There is also anticipated value in comparing these tubular injury urine protein biomarkers that are currently being clinically qualified by IMI and FNIH/PSTC with the FDA and EMA. A goal is to complete and expand the context of use for protein urine biomarkers undergoing qualification presently and also to open the door for potentially exploring additional promising new biomarkers that may appear in blood and to derive an optimal panel for detection of drug-induced tubular injury in humans and that may not therefore also require urine collections.

Expected Applicant Consortium Contribution:

This is an example of contributions that will be required to support proposed project. Since cisplatin has been used to benchmark thresholds and biomarker performance results in subjects with normal renal function (IMI-SAFE-T, FNIH Kidney Team) the proposal is made to assess cisplatin next in patients with hypertension and diabetes. Lung cancer patients often have a long history of smoking preceded by chronic obstructive pulmonary disease requiring corticosteroid treatment. Such patients frequently have concomitant hypertension and/or steroid induced diabetes. Lung cancer patients presenting with CKD1 or CKD2, who are eligible for cisplatin therapy could provide urine and plasma samples following treatment with cisplatin. Assessment of biomarker baseline values, variability, and responses associated with standard of care cisplatin treatment would be compared to the data generated from similarly treated subjects with normal renal function that have already been assessed [1]. Primary hypotheses should focus on statistical power for subgroups of patients based upon hypertension and diabetes and that secondary hypotheses could include investigation as to whether higher BMI (> 30 kg/1.73 m^2) and eGFR < 60 ml/min may pose challenges to interpretation of biomarker responsiveness and utility.

Industry contribution:

(a) Pre-diabetic/diabetic, (b) hypertensive, (c) obese, (d) metabolic syndrome patients familiar to clinical research units already well benchmarked for their CKD1/ 2 status would be a valuable source for benchmarking baseline variability for this kidney safety biomarker research, (e) Additionally, monitoring of these injury biomarkers following initiation of therapy with new oral hypoglycemic Sodium Glucose Co-Transporter (SGLT2) inhibitors [2], which appear to be potential intervention agents against CKD progression, is also proposed for consideration to generate data to investigate a hypothesised baseline-elevated set of biomarkers, and post-intervention return of these biomarkers toward normal to support expanding the evidence for such biomarkers for qualification in a weight-of-evidence strategy.

2. **Advancing the Qualification of Translational Glomerular Injury Biomarkers:**

There is also value in advancing the qualification of novel early biomarkers of drug-induced glomerular injury to support drug development. Translational urinary protein biomarkers of drug induced glomerular injury have shown promising results (e.g., albumin, cystatin C, clusterin) in rodent studies [3][4], and it is hypothesised that small RNAs that may be measured in blood for the liquid biopsy project to strengthen the urinary protein biomarker data.

Expected Applicant Consortium Contribution:

Several types of studies are suggested for consideration within a proposal from academia to generate the appropriate clinical samples to support drug-induced glomerular injury biomarker qualification [5]. At least two of the following studies or other more appropriate suggestions are welcomed: (a) Renal

adverse effects following mechanistic target of rapamycin (mTOR) inhibitor therapy of breast cancer are often preceded by hyperlipidemia, and may present with asymptomatic proteinuria increase to full nephrotic syndrome (15%), elevated serum creatinine (44%), or acute renal failure. (b) Renal adverse effects following anti-VEGF therapies may present as hypertension, asymptomatic proteinuria (23%), and, rarely, nephrotic syndrome or acute renal failure, suggesting a rich source of patient urine samples for detecting biomarkers of the earliest signals of glomerular change. (c) Elevation of serum creatinine is not uncommon in patients with hypercalcemia of malignancy or osteolytic bone metastases (breast cancer, multiple myeloma) receiving I.V. bisphosphonate therapy [6]. (d) Pre-eclampsia manifesting as proteinuria and hypertension can be observed in 5-10% of pregnancies. Such patients considered as high risk for such, would be expected to be readily detected by standard of care pre-natal blood pressure monitoring and urinalyses.

Industry contribution:

Industry member conduct of Non-human primate studies using the same agents as for those selected clinical glomerular injury studies, and using the same biomarker assays as for the human biomarkers to generate translational biomarker performance data supported by histopathologic analyses, would be highly supportive of a favorable regulatory qualification decision and to inform the optimised scheduling of clinical sampling.

Work package 2 – Biomarkers of liver injury

Context of Use:

1. **Risk of progression:**

Biomarker X or a panel of liver safety biomarkers anticipate a risk of progression from hepatocellular injury to severe Drug-Induced Liver Injury (DILI) in patients in whom an initial DILI diagnosis has been established based on elevations of the standard marker Alanine transaminase (ALT) alone or in combination with Total Bilirubin (TBIL). Applying the biomarkers to compounds with an identified hepatotoxic risk may allow prospective monitoring and identification of a DILI signal. Biomarker levels will be correlated with subsequent clinical outcome to allow prognostic assessment of patients with idiosyncratic DILI. This may drive decisions to interrupt or continue dosing or to implement intensified monitoring according to risk stratification (progression – recovery – adaptation).

Specific goal: As evidenced by the EMA and FDA Letters of Support [7], biomarkers suitable for this context of use include macrophage colony stimulating factor receptor 1 (MCSFR1), total and hyperacetylated high mobility group box 1 (HMGB1), osteopontin, and total and caspase cleaved keratin 18 (K18 and ccK18).

2. **Mechanism of DILI:**

Biomarker X may be incorporated into clinical trials to assess the mechanism of hepatotoxicity induced by (i) compounds, which cause DILI in patients, and (ii) compounds that have shown hepatotoxicity in preclinical species or in in vitro investigations. The focus will be placed on any of the following mechanisms of intrinsic DILI: (a) mitochondrial toxicity, (b) reactive metabolite generation/ oxidative and endoplasmic reticulum (ER) stress, (c) inhibition of transporters such as the bile salt export pump (BSEP). For inhibition of BSEP, serum bile acid profiles should be measured and correlated with parameters such as drug dosage, clinical outcome, pattern of DILI and preclinical findings.

3. **Causality assessment:**

Biomarker X or an *in vitro* assay will assess causality of a suspected DILI causing drug in patients in whom a diagnosis of DILI has been established. There is no test available which allows causality assignment of a suspect drug to the onset of DILI in patients.

Specific goal:

To carry out a proof-of-concept study with a test system that assesses the causality of a suspected drug in the context of acute DILI. In patients in whom a diagnosis of DILI has been established based on elevations of the standard markers ALT, AST, ALP and bilirubin, potentially hepatotoxic medications administered to the patient should be assessed individually in a personalised medicine approach in material derived from the patient. With this *in vitro* assay, causality of DILI with a suspected drug can be confirmed; conversely, a drug that is falsely suspected to cause or contribute to DILI, can be de-risked if the result is negative.

Expected Applicant Consortium Contribution:

- Applicant consortium will provide samples from patients with acute severe DILI identified in clinical routine, and – if available – disease controls (e.g. non-alcoholic steatohepatitis (NASH), fibrosis, autoimmune hepatitis). In addition, the academic consortium will prospectively enroll samples from subjects with disease phenotypes that resemble chemical injury for evaluation of biomarker performance. Academic involvement includes protocol design and writing and close collaboration with the work package lead. This will allow the generation of a sufficiently large clinical sample set;
- Academic labs and/or SMEs should provide biomarker assays according to the context of use statements above. Assay providers should aim to achieve GLP/GCP standard validation during the course of the consortium. Academic partners are expected to have a track record in DILI research, and extensive experience with DILI samples is mandatory for any biomarker or assay provider.

Industry contribution:

Clinical samples from patients who developed hepatotoxicity in phase I-III, clinical samples from placebo-treated patients and – if available – disease controls (ongoing trials for liver disease, e.g. NASH, fibrosis, autoimmune hepatitis); additional clinical or preclinical data (including biomarker data) for compounds for which biomarker measurements are performed in human samples, FTE support (e.g. for work package leads, statistical support, medical writing and data management support).

Work package 3 – Biomarkers of pancreas injury

Context of use:

A panel of serological pancreas safety biomarkers may be used together with enzymatic Amylase and Lipase in normal healthy volunteers in early phase clinical trials as a more sensitive and specific biomarker to monitor pancreas acinar cell degeneration/necrosis. These biomarkers will be used when such injury has been demonstrated to be monitorable in animal studies of similar duration with the same test agent.

Specific goals:

1. **Delivery of robust validated assays suitable for use in human plasma or serum:**

   Prioritised pancreatic safety biomarkers shall consist of candidate proteins showing at least preliminary evidence of an association with acinar damage and pancreas-specific microRNAs (e.g. 216a-5p/216b-5p) [8][9]. It will be critical that reliable and sustainable assays are available to support clinical testing. Non-clinical versions of these assays are also desired.

   Expected Applicant Consortium Contribution:

   Academic collaborators with assay development expertise may contribute to the development and/or validation of relevant assays. Specific expertise with miRNA quantification is desired to help address important platform-related issues (e.g. qPCR) and supply suitable clinical assays.

   Industry contribution:

   Industry members will be expected to contribute platform expertise in assay development and analytical fit-for-purpose validation for clinical and/or non-clinical use. Industry participants will also provide

important experience that will guide the transfer of assays to reliable commercial vendors or contract research organisations including SMEs to provide sample analysis to the consortium.

2. **Biomarker baseline determination:**

Characterization of biomarker variability, and effects of potential confounding variables (e.g. gender, age, body mass, etc.) present in populations representative of volunteers in early phase clinical trials. In order to confidently interpret significant changes in the biomarkers, relevant reference ranges in cohorts representative of these volunteers must be generated.

Expected Applicant Consortium Contribution:

Academic collaborators with access to unique cohorts of volunteers (e.g. various ethnicity, gender, age) may contribute to the assessment of sample sets, as well as and analytical support for the generation of these baseline assessments.

Industry contribution:

It is envisioned that industry members will provide a majority of the samples required from normal healthy volunteers with relevant metadata for sample analysis.

3. **Provide proof of concept that the biomarkers can detect pancreatic acinar cell degeneration/necrosis:**

Provide determination of biomarker variability in diseases known to be associated with such injury (e.g. pancreatitis, pancreas transplant, pancreatic cancer, alcoholism). Establishing a greater understanding of candidate biomarkers with respect to disease onset, progression, or resolution is an important component of the work package that would support extending the context of use to later phase clinical trials.

Expected Applicant Consortium Contribution:

Disease samples from medical centers/institutions. In cases of patients presenting diseases such as pancreatitis, longitudinal sampling and the recording of disease outcomes are essential. In addition, it is expected that standard of care tests (e.g. amylase, lipase, c-reactive protein (CRP)) will be collected. Academic partners are encouraged to investigate the candidate markers using clinical samples to probe the influence of co-morbidities and factors that affect biomarker clearance from circulation.

Industry contribution:

Analytical and experimental support using non-clinical species to support reverse translation of qualified clinical markers of pancreatic acinar injury.

Work package 4 – Biomarkers of vascular injury

Context of Use:

The panel of vascular injury safety biomarkers used in conjunction with the totality of preclinical and clinical information in healthy volunteers to monitor for vascular safety in early clinical trials to help inform dose level selection, dose escalation, or decision on continuation of dosing.

Approaches and biomarkers of interest:

1. A panel of biomarkers will be selected from candidate biomarkers identified by SAFE-T and PSTC [10]. The panel includes endothelial, smooth muscle and inflammation markers. The performance of the panel will be assessed in subjects with existing vascular diseases to evaluate the ability of a biomarker panel to detect vascular injury specifically and the role of the presence of co-morbidities. Although immunoassay technology may be supplementary, the applicant consortium should explore LC-MS analytical technology for biomarker assay development. LC-MS technology is important for smooth muscle biomarker assay development, cross-species translation and low sample volume requirements. The applicant consortium should build on progress of LC-MS assay development and validation made by the PSTC consortium.

Sufficient level of assay validation should be completed prior to generating biomarker results beyond the learning phase.

2. The second objective is to explore a more accurate means to measure active vascular injury against which to qualify the emerging biomarker panel in humans, as currently we lack a standard, non-invasive, specific biomarker for vascular injury [11]. This may include optical coherence tomography (OCT), fluorescein angiography (FA), ultra-widefield FA, and fundus photographic evaluations in the ophthalmic vascular disease manifestations or imaging with contrast or bio labelled agents, such as PET tracers, in systemic vascular disease manifestations.

Specific goals:

1. **Select subset of vascular injury biomarkers in learning phase and use to advance the clinical qualification in patients with systemic vascular conditions to detect acute vascular injury:**

   The patient populations should include systemic vasculitides especially focused on active and untreated patients with non-infectious cutaneous leukocytoclastic vasculitis, anti-neutrophil cytoplasmic antibody-associated (ANCA+) vasculitis (PR3-ANCA+ and MPO-ANCA+ subtypes of granulomatosis with polyangitis [GPA] and microscopic polyangitis [MPA], giant cell arteritis and Takayasu's Disease, as well as balloon angioplasty (acute mechanical injury) as previously evaluated [9][10][12]. For those patients with ocular vascular injury manifestations of these vasculitides, this presents the opportunity to also measure the biomarkers against ocular vascular injury endpoints (see goal 4). The learning phase should be focused on a subset of these patients to provide a robust data set by which to select a subset of the panel of biomarkers to be used in the confirmatory phase, as well as the subsequent goals.

   Industry contribution:

   Expertise in data analysis and analytical support.

   Expected Applicant Consortium Contribution:

   Expertise in clinical vasculitides and/or dermatology research and access to unique cohorts of patients with the clinical vasculitides of interest, conducting observational cohort studies, clinical outcomes research, or pilot clinical projects that may contribute to the confirmatory data set. The clinical vasculitides academic collaborators should also have expertise or ready access to expertise in ophthalmology, as outlined in goal 4. Additionally, collaborators can provide analytical support.

2. **Augment healthy volunteer reference range data:**

   The panel of biomarkers selected in the learning phase of goal 1 will be assessed in subjects without detectable disease across age, gender and ethnic cohorts and with lower body mass index.

   Industry contribution:

   Industry members will provide samples from normal healthy volunteers with relevant metadata for sample analysis.

   Expected Applicant Consortium Contribution:

   Academic collaborators with access to unique cohorts of normal healthy volunteers (e.g. ethnic background, pediatric, or elderly samples) may contribute to the assessment of sample sets, as well as provide analytical support for the generation of these baseline assessments.

3. **Complement the clinical qualification of vascular injury biomarkers with imaging tools:**

   Generate an exploratory data set to provide foundation for future confirmatory studies to enable clinical qualification of non-invasive imaging tools to support diagnosis and monitoring of clinical vasculitis, preferably PR3-ANCA+ vasculitis in combination with a panel of circulating vascular injury biomarkers

(from goal 1). Imaging tools will include biomarkers already with compelling performance, such as MMP3, or that provide increased specificity to the vascular bed, such as an endothelial-specific tracer.

Expected Applicant Consortium Contribution:

Expertise in clinical vasculitides research and access to unique cohorts of patients with clinical vasculitis, preferably PR3-ANCA+ vasculitis, conducting observational cohort studies, clinical outcomes research, or clinical projects that may contribute to the exploratory data set. Academic collaborators would provide analytical support and clinical imaging capabilities to support a small scale exploratory clinical study to assess the diagnostic performance/value of a novel imaging agent of PR3-ANCA-associated vasculitis in combination with circulating vascular injury biomarkers. Additionally, academic collaborators with radiochemistry or medicinal/synthetic organic chemistry resources could also support the development and evaluation of potential radionuclides for vascular imaging.

Industry contribution:

Industry members will provide the exploratory MMP3-based imaging tools or support academic imaging candidates with a foundational preclinical qualification package demonstrating proof of concept in animal models and preclinical studies required to enable the clinical use of the imaging tool.

4. **Augment the clinical qualification of vascular injury biomarkers in patients with acute non-infectious ocular diseases with vascular injury** [13]**:**

This exploratory data set may enable an easier, more sensitive monitoring scheme and a patient population without underlying chronic vascular injury to support the clinical regulatory qualification outlined in goal #1. The biomarker levels in circulation may be compared to those in the ocular fluid. Diseases of interest include those with pathophysiology of vasculitis and microangiopathy with vascular leak that are not of infectious origin, including wet acute macular degeneration (AMD) diabetic retinopathy (DR) and various forms of acute uveitis (iritis, iridocyclitis, choroiditis, retinal vasculitis, chorioretinitis, anterior/intermediate/posterior uveitis, and drug-induced/idiopathic/immune-mediated uveitis). Clinical evaluation can be augmented by preclinical evaluation especially for drug-induced uveitis.

Expected Applicant Consortium Contribution:

Expertise in ophthalmology diagnostics (to include ultra-widefield FA, FA, OCT and fundus photographic evaluations), research, and access to unique cohorts of patients with the clinical ocular diseases of interest, conducting observational cohort studies, clinical outcomes research, or clinical projects that may contribute to the exploratory data set. Additionally, collaborators can provide analytical support.

Industry contribution:

Expertise in data analysis and analytical support and samples from ophthalmology programs.

Work package 5 – Biomarkers of CNS injury

Context of use:

The recent tragedy associated with the BIAL clinical trials in France underscores the critical need for more sensitive preclinical biomarkers predictive of neurotoxicity that can be readily translated to clinical trials. Thus, the goal of the current proposal is to evaluate the potential of non-invasive, fluid-based biomarkers (in human blood, urine, and/or CSF) to predict clinical neurotoxicity risk.

Approach and biomarkers of interest:

Several fluid-based biomarkers have been studied in attempts to improve diagnosis and prognosis of CNS injury and disease. Some of these biomarkers have also been studied in preclinical models of neurotoxicity. However, none have been qualified for a specific clinical use. One of the reasons for this is that these candidate biomarkers have not been thoroughly evaluated in large enough numbers of human samples in order to fully characterise background variation or the influence of age, sex, body weight, ethnicity,

comorbidities, drug treatments, etc. The successful applicant consortium will have: 1) demonstrated clinical experience with CNS injury/disease biomarkers and associated assays, 2) past experience with bioanalytical method validation of assays per Industry guidance; 3) the capacity to obtain relevant clinical samples for analysis.

Specific goals:

1. Select and validate a panel of biomarker assays consisting of 4-5 proteins (e.g. GFAP, UCH-L1, tau) and 4-5 cytokines (e.g. IL1-β, TNF, IL10, TGF-β2) for use with human blood (serum or plasma), CSF or urine samples. Other classes of biomarkers (e.g. isoprostanes) may also be proposed. The focus should be on achieving GLP/GCP standard validation (full or partial as needed) of up to 10 assays.

   Industry contribution:

   Expertise in development of biomarkers of CNS injury, analytical expertise, samples from clinical studies (healthy volunteers), data from preclinical studies.

   Expected Applicant Consortium Contribution:

   Expertise in CNS biomarkers, development and validation of biomarker assays, access to relevant samples.

2. Fully characterise the baseline variation of biomarkers in samples from "healthy" volunteers using assays validated in Goal 1 to establish reference ranges including assessing the influence of age, sex, body weight, ethnicity, etc. Depending on sample availability this objective will require up to 500 samples for each assay from volunteers with no known CNS disease or injury. In collaboration with the Liquid Biopsies work package, specific miRNA biomarkers (4-5 miRNAs) will also be identified for further evaluation.

   Industry contribution:

   Expertise in the development of biomarkers of CNS injury, samples from clinical trials (healthy volunteers).

   Expected Applicant Consortium Contribution:

   Clinical expertise in development of biomarkers of the CNS and access to samples from healthy subjects across various populations.

3. Evaluate the influence of 2-3 injury/disease states and 1 neurotoxic chemical treatment on biomarkers identified in stages 1 and 2: this will include samples from patients with brain injury (e.g. stroke, TBI), neurodegenerative disease (e.g. AD, MS, etc.) and chemical-induced neurotoxicity. Depending on sample availability, this objective will utilise ~100 samples from each type of patient.

   Industry contribution:

   Expertise in the development of biomarkers of CNS injury.

   Expected Applicant Consortium Contribution:

   Clinical expertise in appropriate disease phenotypes, ability to identify and access relevant samples from subjects with appropriate disease phenotypes, conduct biomarker studies.

Work package 6 – Liquid biopsies

Availability of non-invasive methods capable of differentiating underlying mechanisms of toxicity and/or disease is an unmet medical need. Micro RNAs (miRNAs) are regarded as a promising source of tissue-specific biomarkers that are released to circulation as a result of tissue damage and/or active secretion. Although some miRs showed promise as tissue specific leakage biomarkers [14] the recent progress in multiplexing technologies and next generation sequencing uncovered a paradigm changing potential of miRs to provide insights into pathogenesis of disease and/or mechanism of toxicity. Thus measuring miR profiles or

signatures has been proposed as liquid biopsies capable of detecting injury in distal tissues including their mechanistic context [15]. It has been shown that , panels of miRs were able to differentiate APAP overdose from ischemic liver injury [16], diagnose types of diabetes [17], chronic heart failure [18], and Parkinson and Alzheimer disease [19][20]. Recently, next generation sequencing enabled unbiased interrogation of the whole miRNome including structural modifications of miRNAs, also called isomiRs. Interestingly, changes in the relative isomiR distribution have been associated with specific developmental stages and disease progression [21], APAP-induced liver injury [22] and hepatocellular carcinoma tissues [23] and a variety of liver impairments [15]. Therefore the proposed project will focus on evaluation of miR profiles as liquid biopsies that would be applicable for interrogation of mechanisms of toxicity and etiology and pathogenesis of diseases. Since liquid biopsies WP will require the development of new innovative approaches that include sequencing of a large number of serum samples and development of bioinformatic tools, it is expected that significant resources up (to 40%) will be committed to this WP.

Specific goals:

1. **Characterization of NextGen platform:**

   There are at least three categories of concern with miRNA sequencing which need to be addressed by the consortium such as (a) ligase bias, (b) effect of potential inhibitors of cDNA synthesis and qPCR present in serum and/or plasma samples and (c) characterisation of NextGen sequencing method performance. It is expected that the applicants will justify the selected sequencing platform with available published data and when not available outline studies that will sufficiently characterise selected sequencing technology.

   Industry contribution:

   Expertise in method development and validation.

   Expected Applicant consortium contribution:

   Nextgen sequencing methodology and data analysis expertise.

2. **Characterise circulating miRnome in healthy subjects:**

   The interpretation of the miR-based liquid biopsy approach is dependent on detailed characterization of the circulating miRNome in healthy subjects including evaluating the potential influence of age, sex, ethnicity, longitudinal variability, inter and intra-individual variability, effect of food etc. Since the characterization of circulating miR-nome will provide a foundation for the development of tissue damage-specific signatures, a large cohorts of subjects (500-2000) will need to be interrogated.

   Industry contribution:

   Serum samples from healthy volunteers of various specifications, expertise in design of studies, data interpretation, bioinformatics.

   Expected Applicant Consortium Contribution:

   Serum samples from healthy subjects (ages, sex, ethnic groups), Next gen sequencing methodology and data analysis.

3. **Develop specific target organ injury miR signatures:**

   This will require obtaining a sufficient number of samples from subjects with characterised impairments of various etiologies. The focus of this specific goal is expected to be in line with target organ WP. If tissue biopsies are available, tissue and liquid biopsy miRNA profiles will be compared. To be successful large numbers of subjects will need to be included in this project.

Industry contribution:

Serum samples from healthy volunteers of various specifications, expertise in design of studies, data interpretation, bioinformatics.

Expected Applicant Consortium Contribution:

Serum samples from subjects with specific diseases, Next gen sequencing methodology and data analysis.

4. **Develop an informatics platform that allows the deconvolution of miR based signatures to pathways and mechanisms:**

The goal is to develop a user-friendly system that will enable researchers to interrogate miR profiles for meaningful mechanistic information. This objective will need to utilise available databases of miR tissue distribution across human and non-clinical species that are available publicly or from member companies.

Industry contribution:

Data from existing databases (miR atlas), expertise in design of studies, data interpretation, bioinformatics.

Expected Applicant Consortium Contribution:

Bioinformatic expertise, development of databases and search engine approaches for data mining.

Work package 7 – Assay development, sample and data management, and statistical support

This WP will be integrated with individual WPs and provide backbone support for other WP activities. This might be an opportunity for including SMEs with appropriate expertise. Availability of robust assays that are transferable to research and ultimately to clinical laboratories for commercial use is essential for implementation of safety biomarkers in clinical trials and diagnosis of diseases. It is important to note that the level of validation will need to reflect the stage of the biomarker candidate [24]. For example, a kidney marker intended for regulatory review differs from the qualification of an emerging vascular or CNS biomarker candidate. These issues will be addressed through assay validation plans co-authored by consortia partners and the SME or laboratory performing a particular validation. This WP will provide opportunity to engage SMEs to standardise assay methodologies for particular stages of development and potentially develop commercially available diagnostics. Furthermore, appropriate sample and data management tools that are compliant with data management and patient privacy standards and statistical support will be essential for TransBioLine success. This WP will provide necessary support across individual target organ- and liquid biopsies-based WPs.

Specific goals:

1. **Coordinate the development of standardised validation procedures and SOPs for all biomarker assays and sample management;**

2. **Prioritise most impactful biomarker assays for development of diagnostics as laboratory developed tests (LDTs) or potentially as in vitro diagnostics (IVDs):**

Industry contribution:

Assay validation, SOP and quality control.

Academic/SME Grant Support:

Assay development, validation, LDT and potentially IVD development capabilities.

3. **Provide sample and data management support for TransBioLIne project:**

   Industry contribution:

   Expertise in compliance.

   Expected Applicant consortium contribution:

   Sample management and distribution across laboratories. Data warehousing in compliant databases.

4. **Statistical support for individual WPs:**

   Industry contribution:

   Expertise and conduct of statistical analysis

   Expected Applicant consortium contribution:

   Expertise and conduct of statistical analysis

Work package 8 – Regulatory acceptance of biomarkers

Achieving regulatory acceptance of emerging safety biomarkers by EMA, FDA and PMDA is essential for their application in clinical trials and diagnosis of disease. Although the biomarker qualification process utilises evidentiary standards that were recently formalised [1][24], it is necessary to develop and maintain a dialog and collaborative relationship with regulatory agencies via consultations, meetings and workshops. In addition, establishing connections and relationships with stakeholders from wider scientific and health care communities will be essential for dissemination of and implementation of novel biomarkers in clinical practice.

Specific goals:

1. Develop biomarker qualification strategy for all safety biomarkers in the TransBioLine project;
2. Develop individual biomarker qualification packages and manage submissions to regulatory agencies;
3. Organise annual workshops with regulatory agencies to discuss biomarker qualification.

Industry contribution:

Support writing regulatory documents, expertise in regulatory interactions.

Expected Applicant Consortium Contribution:

Expertise in regulatory science, managing submissions to regulatory agencies, organizing workshops and meetings with regulatory agencies.

Work package 9 – Project management

The goal of this work package is the overall project coordination, integration and dissemination.

Specific goals:

1. Financial management, maintain timelines, and execute on deliverables and milestones;
2. Legal and contractual management;
3. Communication to the scientific community and the public.

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