TransBioLine
Translational Safety
Biomarker Pipeline

Innovative Medicines Initiative - TransBioLine
Drug-induced Vascular Injury Work Package
DDTBMQ000037

Letter of Intent

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Abbreviations

ANCA+	Anti-neutrophil cytoplasmic antibody-associated-positive
ANGP2	Angiopoietin 2
CALD1	Caldesmon 1
CNN1	Calponin
C-PATH	Critical Path Institute
Cq	Quantification cycle
CRP	C-reactive protein
CXL 10	C-X-C motif chemokine 10
DIVI	Drug-induced vascular injury
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ECLIA	Sandwich electrochemiluminescent assay
ELISA	Sandwich enzyme-linked immunosorbent assay
EMA	European Medicines Agency
FA	Fluorescein angiography
FDA	Food and Drug Administration
GCA	Giant cell arteritis
GroA	Growth regulated alpha protein
HCG	Human chorionic gonadotropin
IL6	Interleukin 6
IL8	Interleukin 8
IMI	Innovative Medicines Initiative
IP-LC-MS/MS	Immunoprecipitation coupled to nano-liquid chromatography tandem mass spectrometry
ITGB1	Integrin beta-1
LLOQ	Lower limit of quantitation
LOS	Letter of Support
miRBase	microRNA database
miRNAs	microRNAs
MMP3	Matrix metalloproteinase 3

MPO	Myeloperoxidase
MPO-ANCA+	myeloperoxidase anti-neutrophil cytoplasmic antibody–positive
MRI	magnetic resonance imaging
^{23}Na-MRI	Sodium MRI
NGAL	Neutrophil gelatinase associated lipocalin
NGS	Next-generation sequencing
NIBP	noninvasive blood pressure
NK	Natural killer
OCT	Optical coherence tomography
PET	positron emission tomography
PCR	polymerase chain reaction
PSTC	Predictive Safety Testing Consortium
PTX3	Pentraxin-related protein
QTcF	QT interval corrected by Fridericia's formula
RT-qPCR	Quantitative reverse transcription PCR
SAFE-T	Safer and Faster Evidence-based Translation (consortium)
SAP	Statistical Analysis Plan
SELP	P-selectin
SLE	Systemic Lupus Erythematosus
SMTNb	Smoothelin isoform b variant
SOP	Standard Operating Procedure
SPREC	Standard preanalytical coding for biospecimen
TAK	Takayasu's disease
THBD	Thrombomodulin
TIMP1	Metalloproteinase inhibitor 1
ULoQ	Upper limit of quantitation
UniProt	Universal Protein Resource
VEGF	Vascular endothelial growth factor
VEGFA	Vascular endothelial growth factor-A
VI	Vascular injury
VCAM1	Vascular adhesion protein 1
WP4	Work Package 4

1 ADMINISTRATIVE INFORMATION

1.1 Submission Title: Letter of Intent for biomarkers of drug-induced vascular injury

1.2 Requesting Information:

Requesting Organization

Name: Innovative Medicines Initiative (IMI) TransBioLine
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1.3 Submission Dates:

LOI submission date: September 27, 2019

2 INTRODUCTION

There is a high unmet need for safety biomarkers that could be used to sensitively and specifically detect vascular injury (VI) in drug development. Drug-induced vascular injury (DIVI), characterized by damage to the vascular endothelium, smooth muscle damage, and associated inflammation, is a poorly detectable and a difficult to predict side effect of new and existing medications from diverse chemical and pharmacological classes. FDA (2016) and EMA (2017) issued a Letter of Support (LOS) to the Innovative Medicine Initiative (IMI), Safety and Faster Evidence-based Consortium (SAFE-T) and the Critical Path Institute (C-Path), Predictive Safety Testing Consortium (PSTC) for further study and development of safety biomarkers for detecting DIVI in early clinical development. In 2018, IMI approved a subsequent VI Work Package (WP4) under the Translational Safety Biomarker Pipeline (TransBioLine 2019-2024), which is an IMI-

2 project with a remit to qualify biomarkers for drug-induced injury of five prioritized organ systems. As there is no current standard clinical lab test for accurate safety monitoring of VI, qualification will require a novel and challenging approach.

Work Package 4 aims to develop and qualify, using patient populations with acute vascular disease as a surrogate for drug-induced vascular injury, a panel of biofluid-based vascular injury safety biomarkers (VI biomarker panel), which will be used to sensitively detect DIVI (vascular endothelial and smooth muscle damage, and inflammation) in healthy subjects in early clinical trials; in conjunction with the totality of preclinical and/or clinical information available, these biomarkers will support dosing decisions. In addition to a subset of biomarkers for which SAFE-T received an LOS, the candidate biomarker panel includes new biomarkers. Selection of the candidate biomarker panel is based on the nature of the biomarker panel biology to capture response over injury duration, across vascular compartments, and shared histomorphologic outcomes to injury regardless of the pathomechanism of insult. The candidate panel includes high sensitivity C-reactive protein, which serves as a clinical lab reference point for current practice but is not specific for vascular injury, as well as smooth muscle biomarkers for which assays previously were not available. Patients with systemic or localized vasculitides will serve as a surrogate population to demonstrate the performance of the vascular injury biomarkers to detect drug-induced vascular injury. It is anticipated that the signature may vary according to pathogenesis, but collectively they will be able to detect early end-organ vascular injury.

The biomarker qualification plan includes a learning and confirmatory phase; the aim of the learning phase is to narrow down the biomarker panel. Additive statistical approaches, such as a composite measure, will be assessed to evaluate the potential added value to that of the individual biomarker performance, in detecting VI. To achieve specificity to the vascular system while maintaining sensitivity, we propose use of a biomarker panel with minimally ≥ 1 biomarker from each vascular compartment (endothelial, smooth muscle, inflammation).

WP4 will also concurrently investigate the potential for circulating microRNAs (miRNAs) to be used as VI biomarkers. The aim is to select a few miRNAs from NGS data in the discovery phase, and to “bridge” results to a targeted RT-qPCR, which is designed to measure only the selected miRNA biomarkers. Thus, by selecting a small subset of candidate miRNAs from the discovery phase, the potential complexity of adding miRNAs to the protein-based biomarker panel will be manageable.

In order to align TransBioLine’s qualification work with FDA’s expectations, the VI Work Package is seeking early and continued discourse with CDER to facilitate a successful qualification effort.

3 DRUG DEVELOPMENT NEED STATEMENT

There is a high unmet need for safety biomarkers that could be used to sensitively and specifically detect VI in drug development. DIVI, characterized by vascular endothelial and smooth muscle damage, and inflammation, is a poorly detectable and a difficult to predict side effect of new and existing medications from diverse chemical and pharmacological classes. The relevance of DIVI in nonclinical animal toxicology studies to humans is often uncertain. While heart rate and blood pressure can be used as biomarkers of VI for those compounds causing systemic hemodynamic changes, this is not true for compounds with only localized vasoactivity or with a different mode

of action. Other circulating biomarkers, like C-reactive protein, are non-specific indicators of some VI. Further, while sporadic occurrences of clinical DIVI have been reported for a large number of drugs, these occurrences manifest as a vasculitis syndrome that cannot always be differentiated from idiopathic vasculitides. Thus, pharmaceutical companies seldom advance compounds with a nonclinical DIVI liability into clinical trials, especially at or near clinically relevant exposure multiples, because the occurrence of this injury usually cannot be detected or conclusively ruled out in patients. It is estimated that on average, approximately 2.5% of the typical pharmaceutical company's nonclinical portfolio is affected by DIVI-related safety liability, leading to significant delays or project termination (Woodcock 2016, Rasi 2017).

DIVI is most commonly associated with vasoactive compounds, nonclinically and clinically. More recently, a smaller group of other compounds is thought to cause injury through other mechanisms at different anatomic sites (e.g., cytostatic agents and large molecules) but with shared histomorphologic outcomes to vasoactive compounds (Engelhardt et al. 2015; Frazier et al. 2015; Mikaelian et al. 2014). Such histomorphologic changes also are shared with a spectrum of systemic vasculitides affecting different vascular sizes and beds, including cutaneous leukocytoclastic vasculitis, anti-neutrophil cytoplasmic antibody-associated (ANCA+) vasculitis, giant cell arteritis (GCA) and Takayasu's disease (TAK), as well as with balloon angioplasty patients. In addition, vascular injury can manifest in the vascular beds in the eye and represent a component of glomerular response due to various insults. As such, better biomarkers based on shared histomorphologic outcomes, rather than on mechanism of injury, are needed to identify, characterize, and/or detect DIVI.

4 BIOMARKER INFORMATION AND INTERPRETATION

The learning phase of this project will identify a subset of the most promising protein-based biomarkers across the three categories and miRNA biomarkers that will be brought into the confirmatory phase.

4.1 Biomarker name

The biomarkers in the following list are molecular biomarkers.

Protein-based Biomarkers Scheme: UniProt (http://uniprot.org/)

Endothelial biomarkers

1. Angiopoietin 2 (ANGP2 O15123) – secreted by endothelial cells. Plasma and urine matrices.

2. P-selectin (SELP P16109) – component of the Weibel-Palade bodies of resting endothelial cells and alpha granules of resting platelets; component of cell membrane of activated endothelial cells and platelets. Plasma and urine matrices.

3. Thrombomodulin (THBD P07204) – component of endothelial cell membrane (receptor). Plasma and urine matrices.

4. Vascular adhesion protein 1 (VCAM1 P19320) – component of cell membrane of activated endothelial cell membrane (receptor). Plasma and urine matrices.

Smooth muscle biomarkers

1. Caldesmon 1(CALD1 Q05682) – component of thin filaments in mature smooth muscle cells and of stress fibers in fibroblasts. Plasma and urine matrices.

2. Calponin 1 (CNN1 P51911) – component of thin filaments in mature smooth muscle cells. Plasma and urine matrices.

3. Smoothelin isoform b variant (SMTNb P53814) – component of vascular smooth muscle cells. Plasma and urine matrices.

Inflammation biomarkers

1. C-reactive protein (CRP P02741) – secreted by hepatocytes as an acute phase protein. Serum matrix.

2. C-X-C motif chemokine 10 (CXL10 P02778) – secreted chemokine by several cell types including monocytes, endothelial cells and fibroblasts. Plasma matrix.

3. Growth regulated alpha protein (GroA P09341) – secreted chemokine by several activated cell types including (leukocytes (macrophages and neutrophils), endothelial cells and fibroblasts. Serum matrix.

4. Interleukin 6 (IL6 P05231) – secreted cytokine by activated leukocytes (T lymphocytes and macrophages), vascular smooth muscle cells, endothelial cells and osteoblasts; secreted myokine by activated skeletal muscle cells. Serum matrix.

5. Interleukin 8 (IL8 P10145) – secreted chemokine by several activated cell types including macrophages and endothelial cells (stored in Weibel-Palade bodies). Serum matrix.

6. Matrix metalloproteinase 3 (MMP3 P08254) – component of extracellular matrix. Plasma and urine matrices.

7. Metalloproteinase inhibitor 1 (TIMP1 P01033) – secreted preferentially by Th17 and Th1 cells. Plasma matrix

8. Neutrophil gelatinase associated lipocalin (NGAL P80188) – secreted by activated leukocytes (neutrophils and macrophages), kidney tubule cells, smooth muscle cells and endothelial cells. Plasma and urine matrices.

9. Pentraxin-related protein (PTX3 P26022) – component of several activated cell types, including monocytes and endothelial cells. Plasma matrix.

Circulating microRNAs Scheme: miRBase (www.mirbase.org)

1. MicroRNAs (miRNAs) are small non-coding RNA of approximately 22 nucleotides length that control gene expression in all human cells. Approximately2000 human miRNA genes are known currently. The specific types of miRNAs are defined based on the miRNA identifier (e.g. hsa-miR-126-5p), miRbase accession number (MIMAT0000444) and the RNA sequence (CAUUAUUACUUUUGGUACGCG). This information is conventionally stored in the "fasta" file format:

```
>hsa-miR-126-5p MIMAT0000444
CAUUAUUACUUUUGGUACGCG
```

4.2 Analytical methods

The following methods will be used to measure the proteins in urine and EDTA plasma or serum (as detailed in the Table 4-1 Biomarkers and analytical methods below):

1. Sandwich enzyme-linked immunosorbent assay (ELISA) with chromogenic reporters
2. Sandwich electrochemiluminescent assay (ECLIA) with electrochemiluminscent reporter
3. Immunoprecipitation coupled to nano-liquid chromatography and tandem mass spectrometry read-out (IP-LC-MS/MS)

Table 4-1 Biomarkers and analytical methods

	Biomarker		Matrix	Read Out
	ENDOTHELIAL BIOMARKERS			
1	Angiopoietin 2	ANGP2	EDTA plasma and urine	IP-LC-MS/MS
2	Vascular adhesion protein 1	VCAM1	EDTA plasma and urine	IP-LC-MS/MS
3	P-selectin	SELP	EDTA plasma and urine	IP-LC-MS/MS
4	Thrombomodulin	THBD	EDTA plasma and urine	IP-LC-MS/MS
	SMOOTH MUSCLE BIOMARKERS			
5	Caldesmon 1	CALD1	EDTA plasma and urine	IP-LC-MS/MS
6	Calponin	CNN1	EDTA plasma and urine	IP-LC-MS/MS
7	Smoothelin isoform b variant	SMTNb	EDTA plasma and urine	IP-LC-MS/MS
	INFLAMMATION BIOMARKERS			
8	C-reactive protein	CRP	Serum	Turbidimetric assay
9	C-X-C motif chemokine 10	CXL10	EDTA Plasma	ELISA
10	Growth regulated alpha protein	GroA	Serum	ELISA
11	Interleukin 6	IL6	Serum	ECLIA
12	Interleukin 8	IL8	Serum	ECLIA
13	Metalloproteinase inhibitor 1	TIMP1	EDTA Plasma	ELISA
14	Pentraxin-related protein	PTX3	EDTA Plasma	ELISA
15	Matrix metalloproteinase 3	MMP3	EDTA plasma and urine	IP-LC-MS/MS
16	Neutrophil gelatinase associated lipocalin	NGAL	EDTA plasma and urine	IP-LC-MS/MS
	CIRCULATING MicroRNAS			
17	microRNA	various	EDTA Plasma	NGS/RT-qPCR

4.3 Measurement units and limit(s) of detection

For the endothelial, smooth muscle and inflammation protein-based biomarkers listed in the previous table, the unit of measurement is ng/ml and currently the LLOQ is to be determined.

For the circulating microRNAs the measurement units and limit(s) of detection are provided in Table 4-2:

Table 4-2 Circulating microRNA – measurement units and limits of detection

Detection platform	Measurement units	Limits of detection
Next-generation sequencing	Read count	1 read count
Reverse-transcription quantitative PCR (RT-qPCR)	Quantification cycle (Cq)	Cq 40

4.4 Biomarker interpretation and utility

Currently the information is not available to provide the details for biomarker interpretation and utility. The plan is to use the learning phase as well as nonclinical data to investigate univariate and multivariate combination approaches to clinical interpretation of the panel of biomarkers and substantiate this approach in the confirmatory phase. The biomarker panel will minimally include ≥ 1 biomarker from each compartment (endothelial, smooth muscle, inflammation) to convey specificity to the vascular system as well as maintain sensitivity.

5 CONTEXT OF USE STATEMENT

A safety biomarker panel to aid in the detection of acute drug-induced vascular injury (DIVI) in early clinical trials in healthy volunteers when there is an a priori concern that a drug may cause DIVI in humans.

6 ANALYTICAL CONSIDERATIONS

6.1 Immunoprecipitation coupled to nano-liquid chromatography and tandem mass spectrometry read-out (IP-LC-MS/MS)

A single bioanalytical platform will be used to generate an IP-LC-MS/MS multiplex of ANGP2, SELP, THBD, CALD1, CNN1, SMTNb, NGAL, TIMP1, and VCAM1. Based on a feasibility analysis already conducted, the remaining inflammation biomarkers in the VI biomarker panel

will be measured using a different assay solution (see Section 6.2). This IP-LC-MS/MS platform allows for low blood volumes and a single assay across nonclinical and clinical samples. This multiplex currently is under fit-for-purpose validation at a single laboratory, using serum, plasma, and urine matrices from nonclinical species and humans with the intention to make the multiplex publically available at a central laboratory / contract research organization (feasibility permitting).

6.2 Sandwich enzyme-linked immunosorbent assay (ELISA) with chromogenic reporters

The other analytical platforms used to generate the remaining inflammation biomarkers, incorporates sandwich ELISA procedures from Meso Scale Diagnostics (Il6, Il8 and Cxl10), RnDSystems (GroA, TIMP1, PXP3) and Roche Cobas (hsCRP). These are validated or under fit-for-purpose validation, using blood matrices in humans and will be performed in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory.

Fit-for-purpose validation includes examining intra- and inter-assay precision, dilutional linearity, limit of blank (analytical sensitivity), limits of quantitation (upper and lower), matrix/recovery, sample-freeze/thaw stability and stored sample stability. There will be a standard operating procedure (SOP) for sample collection and storage established prior to sample collection, and the assays are run under laboratory SOPs. The assays are not under review by the Center for Devices and Radiologic Health or the Center for Biologics Evaluation and Research.

6.3 Next generation sequencing/ Reverse-transcription quantitative PCR (RT-qPCR)

For circulating miRNAs, the assays that are used are designed to determine the concentrations (i.e. copies) of miRNA sequence molecules in plasma as continuous parameters. The matrix used for miRNA analysis is double-centrifuged EDTA plasma. SOPs have been generated, which describe the procedure for collection and storage of EDTA plasma for miRNA analysis by defining collection tubes and volume, incubation times, centrifugation parameters (temperature, speed, time), and storage (temperature). Sample quality control involves the assessment of hemolysis (spectrophotometric analysis) and enzyme inhibition (RT-qPCR of spike-in controls).

The analytical platform used in the learning phase for unbiased quantification of circulating miRNAs in EDTA plasma is small RNA next-generation sequencing (NGS). VI miRNA biomarker candidates that are selected in the discovery phase, will be analyzed during the confirmatory phase using reverse-transcription quantitative PCR assays (RT-qPCR). Both NGS and RT-qPCR were shown to have cross-species compatibility for a variety of mammalian species. Both NGS and RT-qPCR are undergoing fit-for-purpose validation using human plasma. The analytical fit-for-purpose validation plan for NGS includes accuracy (spike-in recovery), precision, the analytical measurement range (LLoQ, ULoQ), and multiplexed sequencing validation. The analytical validation plan for RT-qPCR includes accuracy (spike-in recovery and amplification efficiency), precision (repeatability and reproducibility), the analytical measurement range (LLoQ, ULoQ), parallelism (dilution linearity), sensitivity, and stability.

6.4 Sample handling, shipment and storage

Finally, the Central Biobank of the Charité (Berlin, Germany; ZeBanC) is responsible for sample and ID management. A harmonized SOP and instructions for sample handling and shipment will be established which will then be used by all recruitment sites. Furthermore, pre-designed kits for structured acquisition of biomaterials including colour-coded 2D barcoded tubes (FluidX/ Brooks) are provided.

These sample kits come with sample-related data to document standard preanalytical information (date and time of sampling, volume, duration until processing, centrifugation conditions). This is of utmost importance to assess the quality of the collected biomaterials for biomarker analysis in our consortium. The standard preanalytical coding for biospecimen (SPREC) facilitates the documentation of preanalytical quality parameters is used in our biobank and will provide consistent data. The recruitment centers will organize shipment. After notification the aliquots will be sent on dry ice with temperature monitoring.

In the ZeBanC the samples will be stored in monitored deep-freezing systems. Aliquot data and location are documented in the ZeBanc LIMS system and are traceable at any time.

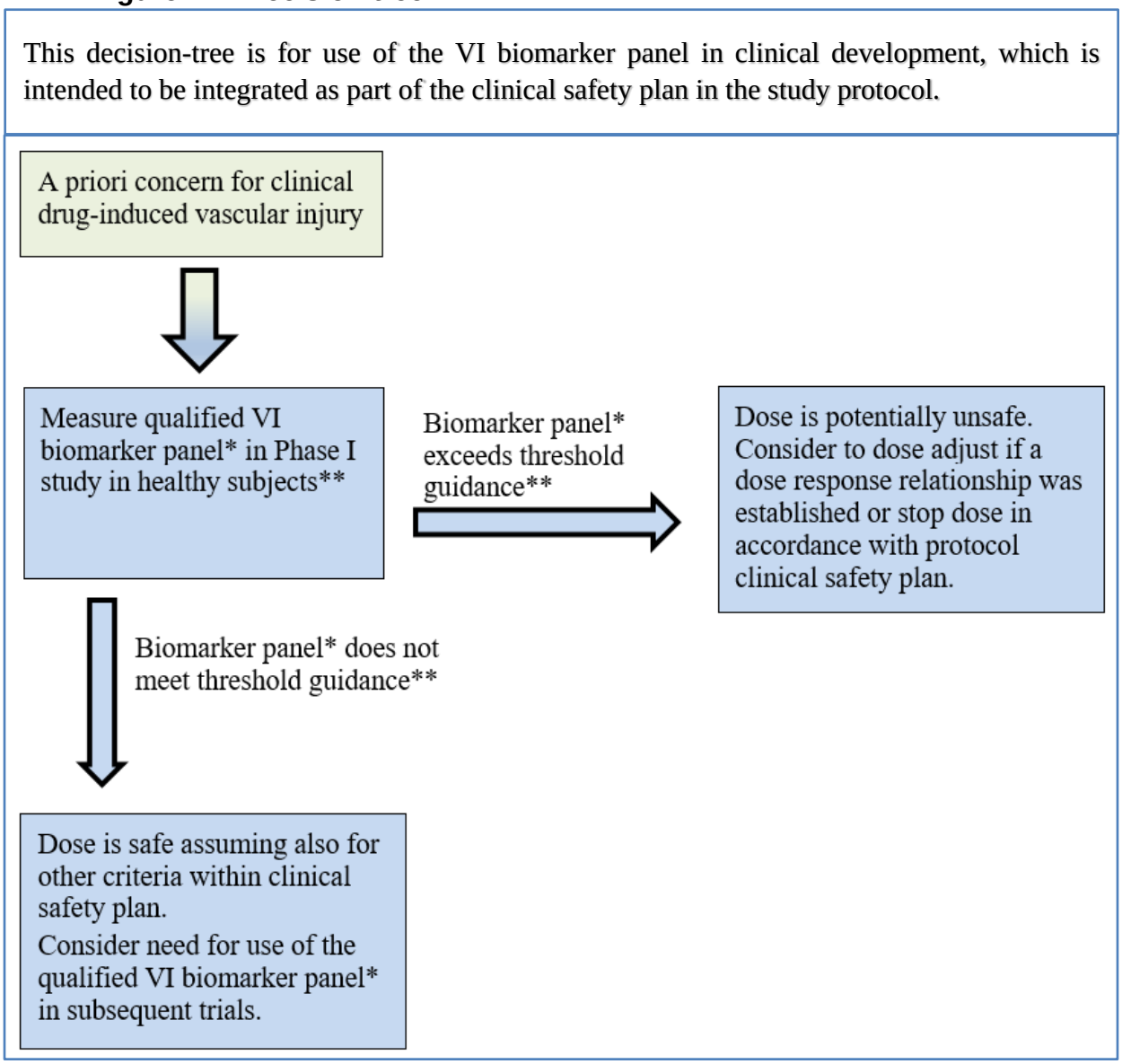
7 CLINICAL CONSIDERATIONS

7.1 Use in Drug Development

The qualified VI biomarker panel, in conjunction with the totality of preclinical and/or clinical information will support dosing decisions. This biomarker panel will sensitively detect acute onset of DIVI (vascular endothelial and smooth muscle damage, and inflammation) in clinical evaluation of healthy subjects in early clinical trials.

The following decision tree (Figure 7-1) for the qualified VI biomarker panel is intended to be integrated into the clinical study protocol as part of the clinical safety plan and support dosing decisions when there is an *a priori* concern for DIVI. This decision tree is not meant to be used for biomarker selection or biomarker development within the TransBioLine project.

Figure 7-1 Decision tree



* The vascular injury (VI) biomarker panel will minimally include ≥ 1 biomarker from each compartment (endothelial, smooth muscle, inflammation) to convey specificity to the vascular system as well as maintain sensitivity.

** The decision tree is intended to be integrated as part of the clinical safety plan in the study protocol.

7.2 Patient population or drug development setting

The qualified VI biomarker panel is intended for use in early clinical trials in non-smoking healthy subjects.

7.3 Clinical validation

7.3.1 Biological and Clinical Relevance

The proteins in the VI biomarker panel originate from vascular endothelium, smooth muscle, and associated inflammatory cells (leukocytes or other cells) involved in the cascade of response to vascular injury and can be measured in blood (plasma or serum as appropriate). As submitted in the Summary Data Package supporting Clinical Biomarkers of DIVI (2017), it was demonstrated that compared to control rats, circulating levels of several of these endothelial and inflammation biomarkers change in response to various vascular toxicants with different mechanisms of injury and correlate with shared histomorphologic outcomes of vascular injury (defined by endothelial apoptosis/degeneration/necrosis; endothelial hypertrophy/hyperplasia; smooth muscle degeneration/necrosis; smooth muscle hypertrophy/hyperplasia; inflammation). The biomarkers measured also return to baseline compared to control rats on recovery from vascular injury as determined by histopathology (defined as reconstitution of the vasculature). Although not measured in blood, the smooth muscle biomarkers demonstrate decreased expression by immunohistochemistry in a pilot rat toxicity study (internal data), suggesting that the smooth muscle proteins are released into circulation and thus will be measurable in blood in response to VI. The exploratory data set included in SAFE-T submission for the LOS currently may be augmented by the PSTC through a confirmatory data set in rat toxicology studies and opportunistic large animal toxicology studies.

We infer that the biomarkers correlate to the same histomorphologic endpoints in humans irrespective of mechanism of toxicity or disease pathogenesis as defined by standard endpoints of imaging, functional tests, and/or established circulating biomarkers. The exploratory clinical data set using patients with systemic vasculitides generated by SAFE-T to support the Letter of Support that was issued, indicates this inference is likely true. Further, several of the same biomarkers demonstrated a response in clinical trials of patients with systemic vasculitides, either to diagnose and/or indicate response to treatment (Monach 2014; Monach et al. 2013).

7.3.2 Aims/Goals

The TransBioLine Drug-Induced Vascular Injury (Work Package 4) research plan is intended to support the following goals:

- Establish a reference range in healthy subjects, characterizing inter- and intra-subject variability.
- Develop and qualify a VI biomarker panel using surrogate patient populations with acute vascular disease for the detection of DIVI in healthy subjects in early clinical trials to support dosing decisions.
- The following pilot studies will be conducted to provide supportive information for qualification of the VI panel:
  - Pilot study to determine if circulating biomarkers can be detected in response to acute VI in the eye for which imaging methods provide a strong endpoint against which to evaluate VI biomarker panel performance.

- Pilot study to determine if urinary biomarkers can be detected in response to VI in the kidney glomerulus, for which imaging methods and biopsy (wherein feasible) provide a strong endpoint against which to evaluate VI biomarker panel performance in urine.

7.3.3 Overview of the Clinical Research Plan

7.3.3.1 Learning phase evaluation of potential VI biomarker panel

In the learning phase, we will test a larger VI biomarker panel using cross-sectional and longitudinal studies with a limited number of patients with systemic vasculitides (small-medium vessel vasculitis including ANCA-associated vasculitis and leukocytoclastic vasculitis; large vessel vasculitis) and mechanical vascular injury (balloon angioplasty). This will establish the VI biomarker panel performance in the presence of VI (associated with shared histomorphologic outcomes of endothelial and smooth muscle injury and inflammation) and determine its correlation relative to diagnostic determinants of the disease state (acute onset / acute flare and quiescent state). This will inform selection of a more narrow VI biomarker panel for further evaluation in confirmatory phase. Circulating miRNAs will be explored using discovery approach by NGS and a small set of miRNAs will be selected for confirmatory phase to narrow down biomarkers.

7.3.3.2 Confirmatory phase evaluation of DIVI biomarkers

In the confirmatory phase, selected biomarkers from the learning phase will be further qualified using cross-sectional and longitudinal studies with larger patient cohorts with the same diseases and conditions. In the vasculitides patients, the biomarkers will be compared between acute flare and remission/steady state to capture acute vascular injury above chronic quiescent state, as well as compared between acute flare to healthy volunteer reference range from Healthy Volunteer Study to capture acute vascular injury above normal state. Similar comparisons will be made for balloon angioplasty patients between pre- and post-mechanical injury, and post-mechanical injury and healthy volunteer reference ranges.

The learning and confirmatory phases will be complemented by the clinical pilot imaging studies of the kidney and eye (please see below) and by rat and non-rodent toxicity studies using glomerular toxicant with histopathology as a gold standard and a bio-imaging endpoint to analyze biomarker performance in the urine as well as in blood. These studies are not considered pivotal for the qualification, but can provide insight of biomarker response in relation to the tangible endpoints of imaging and microscopic evaluation of the target vasculature.

7.3.3.3 Reference range data for healthy volunteers

Respective reference ranges will be established in healthy volunteers for the VI panel in both blood and urine matrices. The reference range will be used as described in the learning and confirmatory phases, as well as the pilot imaging studies.

7.3.3.4 Exploratory analysis of novel imaging methods for glomerular injury/vasculitis

7.3.3.4.1 Glomerular vascular injury/vasculitis

This pilot study can provide insight of biomarker response in relation to the tangible endpoints of imaging and microscopic evaluation of the target vasculature. This study uses the capacity of novel MRI imaging techniques to detect vascular injury of the kidney glomerulus in SLE (Systemic Lupus Erythematosus) glomerulonephritis, which represents clinical models of DIVI, through the quantification of renal blood flow, renal tissue oxygenation, fibrosis and edema. In addition, ^{23}Na-MRI will be used to follow the progression of renal function during glomerular vascular injury, because with an increasing number of injured tubules subsequent to glomerular injury, the typical sodium gradient between medulla and cortex can no longer be maintained (Maril N. 2006, Nangaku M. 2006, Dahlmann A. 2015). The pilot study uses a similar approach as described in the confirmatory phase with the addition of imaging and renal biopsy from this patient cohort to potentially provide more robust endpoints against which to evaluate biomarker performance in the urine and blood in response to glomerular injury, which includes vascular injury. This pilot study will include matched healthy volunteers with imaging analysis and analysis of the full biomarker list in the urine and blood.

7.3.3.4.2 Ocular vascular injury/vasculitis

This pilot study can provide insight into biomarker response in relation to the tangible endpoints of imaging evaluation of the target vasculature. This study uses the capacity of imaging modalities of the posterior eye, including fluorescein angiography (FA) and optical coherence tomography (OCT), to detect vascular injury in association with inflammatory eye disease with a vascular injury component. This study also investigates the feasibility of circulating biomarkers to detect injury to the ocular vascular beds as compared to healthy volunteer reference range. The pilot study uses a similar approach as described in the confirmatory phase with the addition of imaging to potentially provide a more robust endpoint against which to evaluate biomarker performance in the blood in response to vascular injury in the eye. This pilot study will include matched healthy volunteers with imaging analysis and analysis of the full biomarker list in the blood.

7.3.3.5 MiRNA Signature

In addition to the soluble protein biomarkers, we will broadly investigate circulating microRNA signatures for VI. MicroRNAs are small non-coding RNAs of approximately 22 nucleotides length that modulate post-transcriptional gene expression. Out of approximately 2000 known human microRNAs, several microRNAs are known to exhibit highly tissue-specific transcription patterns. This includes microRNAs that are specifically transcribed in vascular and microvascular endothelial cells (e.g. hsa-miR-126-3p), as well as vascular smooth muscle cells (hsa-miR-143, hsa-miR-145). Active or passive release of microRNAs from cells, specifically upon tissue injury, enables minimal-invasive detection of such microRNAs in the circulation. Hence, we hypothesize that circulating microRNAs might serve as sensitive and specific biomarkers for VI. We will apply next-generation sequencing as non-targeted analytical platform to select VI microRNA biomarker candidates in the learning phase. A subset of promising miRNAs will be brought forward from the learning phase to the confirmatory phase.

Table 7-1 Overview of Planned Studies

Summary Study Objective	Study number	Study	Description	Objective(s)
Reference range	1	Healthy volunteers	Analysis of 3 serial individual blood and urine samples collected from healthy volunteers meeting recruitment criteria (n = 125).	Establish reference range for healthy subjects and evaluate influence of sex, ethnicity, age and intra- and inter-subject variability to support qualification.
Mechanical vascular injury	2	Patients with vascular lesions	Analysis of blood from subjects with arterial or venous occlusion that undergo balloon angioplasty, comparison of pre- and post-interventional samples (n = 75).	Evaluate biomarker performance in a controlled setting of mechanical vascular injury to support qualification.
Large-vessel vasculitis	3	Patients with proven large-vessel vasculitis (TAK, GCA)	Analysis of blood from subjects with large vessel vasculitis, comparison of samples at diagnosis or flare (active disease) and remission (quiescent disease) (n = 75).	Evaluate biomarker performance for large vessel injury to support qualification.
Medium- and small-vessel vasculitis	4	Patients with proven medium- and small-vessel vasculitis (ANCA-associated vasculitis, leukocytoklastic vasculitis)	Analysis of blood from subjects with medium- and small-vessel vasculitis, comparison of samples at diagnosis or flare (active disease) and remission (quiescent disease) (n = 75).	Evaluate biomarker performance for medium/small vessel injury to support qualification.

Summary Study Objective	Study number	Study	Description	Objective(s)
Vascular injury of the kidney glomerulus	5	Patients with proven renal involvement (glomerulonepehritis) of systemic vascular disease (SLE–associated vasculitis); healthy volunteers	Analysis of blood and urine from subjects with MPO ANCA-associated vasculitis, comparison of samples at diagnosis or flare (active disease) and remission (quiescent disease) (n = 20), comparison with matched healthy volunteers (n = 20) and evaluation against biopsy (wherein feasible) and renal imaging that quantifies renal damage and function (MRI).	Evaluate biomarker performance for medium/small vessel injury with special focus on renal involvement for which imaging methods and biopsy (wherein feasible) provide strong endpoints against with to evaluate biomarker performance. Pilot study.
Vascular injury of the eye	6	Patients with proven ocular involvement of the posterior and anterior/intermediate eye by vasculitis, healthy volunteers	Analysis of blood from subjects with anterior/intermediate or posterior vascular inflammation of the eye (n = 20), comparison with matched healthy volunteers (n = 20), and evaluation against ocular imaging (OCT, fluoroscopy).	Evaluate biomarker performance for medium/small vessel injury with special focus on eye involvement for which imaging methods provide strong endpoints against with to evaluate biomarker performance. Pilot study.
Develop rodent ANCA model	7	Anti-MPO rat model study	Analysis of blood from rodent models of MPO ANCA-associated vasculitis, comparison with control group, and evaluation against histopathology.	Develop a nonclinical model that complements the clinical study in these patients to evaluate biomarker performance against histopathology. Pilot study.
Develop/evaluate imaging agent in rodent	8-10	Rat vascular injury model with imaging studies (n=3)	Analysis of blood and urine from rat vascular injury model, comparison to control group, and evaluation against histopathology and imaging that quantifies vascular injury.	Develop a nonclinical model that complements the clinical renal imaging study for which imaging methods (such as MMP3/elastase PET tracer) and histopathology provide strong endpoints against with to evaluate biomarker performance. Pilot study. Conduct potential rodent clinical enabling study if needed.

7.3.3.6 Characteristics of Patient populations

7.3.3.6.1 Healthy volunteer cohort (Study number 1):

- Inclusion criteria:
  - Healthy according medical history (also: no bariatric surgery for oral compound)
  - Normal vital signs: e.g (May vary per protocol) NIBP systolic; diastolic; breaths/min normal etc.
  - Normal ECG: sinus rhythm, QTcF male and female. may differ per protocol, QRS, and PR (may vary per protocol)
  - Negative beta HCG
  - Negative alcohol, urine tox screen, negative cotinine test
  - Negative for HIV1-2/hepA+B+C, sometimes hepE
  - No meds with DILI potential; associated w renal injury or w/alteration of creatinine excretion
- Exclusion Criteria:
  - Out of age range 18-80 years of age
  - No informed consent
  - Known diseases specifically: chronic disease (including that induced by any aftermath of an acute disease), metabolic syndrome, GI disease (e.g. salivary, pancreatic or liver disease), acute disease in previous 12 months, skin diseases
  - No allergies for medication or other allergies

7.3.3.6.2 Balloon angioplasty (mechanical injury) patient cohort (Study number 2):

- Inclusion criteria: Patients with confirmed occlusive venous or arterial disease, not due to an inflammatory/autoimmune, malignant or infectious process with an indication for balloon angioplasty. Age 18-80yrs. Able to give informed consent.
- Exclusion Criteria: Not fulfilling inclusion criteria.

7.3.3.6.3 Systemic vasculitides patient cohort, large-sized vessel (Study number 3):

- Inclusion criteria: Confirmed diagnosis of a large vessel vasculitis (giant cell arteritis, Takayasu`s disease), either confirmed by biopsy or suitable imaging method. Age 18-80yrs, no concomitant malignant, psychiatric or infectious disease. No other autoimmune disease (apart from polymyalgia rheumatica, which may co-incide with GCA). Able to give informed consent. Inclusion at i) flare/active disease (either newly diagnosed or already established diagnosis; with follow-up sample in remission) or ii) at remission with follow-up sample at flare-up/active disease.

• Exclusion Criteria: Not fulfilling inclusion criteria.

7.3.3.6.4 Systemic vasculitides patient cohort, small- and medium-sized vessel (Study number 4):

• Inclusion criteria: Confirmed diagnosis of a small- to medium-vessel vasculitis (leukocytoclastic vasculitis, confirmed by biopsy; ANCA-associated vasculitis), either confirmed by biopsy or suitable imaging method. Age 18-60 yrs, no concomitant malignant, psychiatric or infectious disease. No other autoimmune disease. Able to give informed consent. Inclusion at i) flare/active disease (either newly diagnosed or already established diagnosis; with follow-up sample in remission) or ii) at remission with follow-up sample at flare-up/active disease.

• Exclusion Criteria: Not fulfilling inclusion criteria.

7.3.4 Statistical Plan

The objective of the Statistical Analysis Plan (SAP) is to describe a statistical strategy for the validation of the biomarkers under consideration for vascular injury. The assessment will be done by considering reliability and validity of each biomarker individually or as a composite set. The goal is to identify the panel of biomarkers that consistently detect changes due to acute vascular injury. The strategy is mainly on evaluation of the data on distinguishing capacity of the biomarkers for differences between healthy and vascular injury patients. Relevant changes (effects) within subject's overtime period and between subjects across groups will be evaluated using controlled clinical studies. This SAP assumes that the assays or platforms that generate each of the biomarker data achieve validation as fit-for-purpose. Thus, it is not our plan to describe the global assessment of the precision, accuracy, limit of detection, limit of quantification, specificity, linearity and range, ruggedness and robustness of the assays that generate the data (this will be provided in a separate validation document).

7.3.4.1 Establishing reference range for healthy volunteers

Reference ranges for each of the biomarkers will be established in healthy volunteers in the respective blood and urine matrices.

Subjects: See previous section for the characteristics of the patient populations.

The goal is to assess variability in each of the biomarker measures within (using longitudinal measures over 3 time points) and across subjects to define properties of variability and reproducibility in healthy subjects. Differences among sex, ethnicity and age groups will be evaluated. If there is evidence of difference among the groups, then separate reference ranges will be constructed.

The inter- and intra- subject coefficients of variations will be calculated to quantify variability over time periods and between subjects.

The normality and homogeneity of variances will be checked using Shapiro-Wilk's and Leaven's tests, respectively. If the data passes both assumptions, repeated measures ANOVA model will be used to test differences among sex, ethnicity and age groups. If one of the assumptions fail, then the data will be log transformed and the assumptions will be rechecked for the log transformed data. If the log transformed data passes both assumptions, repeated measures ANOVA model will be used on the log transformed data. In case, one of the assumptions fail on the log transformed data, then the nonparametric Friedman's ANOVA on the original data will be used to test differences among sex, ethnicity and age groups. All statistical tests will be conducted at 5% level of significance. When the repeated measure ANOVA or the nonparametric Friedman's ANOVA indicate significance differences among the subgroups of sex, ethnicity and age, separate reference ranges will be constructed for each group, otherwise, a single reference range will be constructed according to the procedure described in the EP28 guideline of Clinical Laboratory Standards Institute (CLSI, 2008).

Reference ranges based on parametric approach will be calculated if the Shapiro-Wilk's test suggest that the normality assumption is satisfied either on the original or log transformed data. Otherwise, nonparametric approach will be used to calculate the reference intervals. Regardless of the approach, both lower (L_l) and an upper (U_l) reference limits which assumed to enclose a 95% of the values for healthy subjects will be estimated.

7.3.4.2 Learning phase evaluation of potential DIVI biomarkers

In the learning phase, we will test a larger panel of biomarkers in a limited number of patients with systemic vasculitides (small-to-medium vessel vasculitis including ANCA-associated vasculitis and leukocytoclastic vasculitis; large vessel vasculitis) and mechanical vascular injury (balloon angioplasty) to assess performance and select a smaller biomarker list for further evaluation in confirmatory phase. Patients' data from those experiments at the learning phase will be matched with the healthy volunteers' data. Healthy volunteers' data will be stratified by age, sex and ethnicity and will be randomly selected to match the patients' data if it is the case that those factors are important determinants.

The statistical objective in this learning phase is to describe the relationship between a biomarker and the subjects' disease status as an indicator of vascular injury and then select biomarkers which are medium to highly related/correlated to the subject's disease status for further analysis at the confirmatory stage of the project. A biomarker may be selected in its performance individually or in combination with others (composite biomarkers). The statistical approaches that are planned by the TransBioLine team, could be modified as we generate data at the learning stage of the project. Note that decision on the selection of a biomarker for the confirmatory phase will be made based on the totality of the evidence based on the results of all the statistical procedures outlined below and biological/clinical relevance.

7.3.4.3 Confirmatory phase evaluation of DIVI biomarkers

In the confirmatory phase, selected biomarkers from the learning phase will be further substantiated using larger patient cohorts with the same diseases and conditions. In the vasculitides patients, the biomarkers will be compared between acute flare and remission/steady state to capture acute vascular injury above chronic quiescent state, as well as compared between acute flare to healthy volunteer reference range from the Healthy Volunteer study to capture acute vascular injury above normal state. Similar comparisons will be made for balloon angioplasty patients between pre- and post- mechanical injury and healthy volunteer reference range.

All statistical procedures described in the learning phase, correlation, statistical comparison and model- based classification are applicable also for the confirmatory phase. The statistical analysis outlined in the learning phase, will be applied on all biomarkers that are selected for the confirmatory phase. If needed the SAP for this confirmatory phase will be revised beforehand based on what is learned from the learning phase.

7.4 Benefits and risks:

Benefit:

Benefit to the patient is a critical driver in our considerations for clinical qualification of the biomarker of VI. The biomarkers are intended for a diverse patient population in disease type and spectrum of severity. However, the biomarkers may be especially important in patient populations having disease of lesser severity, wherein the risk:benefit of therapy is weighted toward benefit and thus, the potential to detect early, mild and reversible VI may reduce the perceived risk, assuming the biomarkers have a low risk for false negative response.

As such, the new biomarker(s) will provide a more sensitive and specific measure of DIVI, and thereby potentially provide an earlier indication of adverse events, providing researchers with greater confidence in dosing decisions.

This would result in more efficient, safer and potentially faster drug development with reduced frequency of abandoning the development of promising drugs with vascular safety signals in animal studies suspected to be of questionable human relevance or perhaps more importantly, because of a lack of detection possibility to safely approach or reach efficacious exposures.

Risk:

The anticipated overall risk in using these biomarkers to inform patient safety in clinical trials is considered low.

A false negative may occur when the biomarker levels observed following treatment by the drug candidate do not exceed a threshold established as “normal” in spite of DIVI and thus, there is false confidence to continue treatment or dose escalate, especially if conventional inflammation or functional markers are not informative. This possibility will be integrated into the evaluation

of the totality of information for each program and relative to the strength qualification data to establish conservative thresholds.

A false positive may occur when the biomarker changes observed following treatment by the drug candidate exceed a threshold established as "normal" in the absence of DIVI and thus, clinical trial progression may be halted inappropriately. This possibility would be addressed by assessing individual findings in the totality of the data to assign truism and causality but moreover, progression to the clinic from a nonclinical DIVI finding likely would not have occurred if the biomarkers were not available.

Risk Mitigation Strategy:

The following approaches could be used to help mitigate the perceived risk to patients.

• Conventional (inflammatory and organ injury biomarkers) can be used in conjunction with the new VI biomarkers to help inform an upper limit to mitigate false negative occurrences.

• The biological qualification of new biomarkers against a breadth of defined morphologic responses that capture multiple mechanisms of injury with large and small molecules and across various vasculitides provides the foundation for the new biomarkers to capture diverse pathologies associated with DIVI and thus mitigate false positive and negative occurrences.

• Demonstrate for each drug candidate that the new biomarkers provide detection assurances of vascular safety in animal studies anchored in histopathology that are being conducted with the same exact test agent (or a surrogate for large molecules) that is being proposed for investigation in a clinical trial would mitigate risk for unknown mechanisms of false positive or false negative biomarker responses.

• The initial focus of using the new biomarkers in healthy volunteers, and the exclusion of certain named underlying inflammatory diseases from clinical trial investigations, provides some basis for reducing potential for false positives, and also some understanding for follow-up of potential positive findings that may be suspected of not deriving from a true drug-induced vascular injury event.

7.5 Current knowledge gaps, limitations, and assumptions

The assumption is that patients with systemic or localized vasculitides can serve as a surrogate population to demonstrate the performance of the vascular injury biomarkers to detect drug-induced vascular injury.

The assumption is that the biomarkers will be used as a panel, either through individual or combined biostatistical approaches.

The assumption is that our current list will pass analytical validation.

8 SUPPORTING INFORMATION

8.1 Rationale for biomarker selection:

The panel will contain endothelial, smooth muscle, and inflammation biomarkers that will indicate vascular injury irrespective of mechanism or cause of injury. The specificity of certain biomarkers to endothelial cells or (vascular) smooth muscle cells, either in a resting or activated state, will lend specificity of the biomarker panel to VI. The inflammation biomarkers, of which several are associated with vascular components, are anticipated to augment sensitivity of the biomarker panel to VI. The selection of biomarkers in the panel encompasses the breadth of vascular response to injury in pathogenesis and in time, and thus lends itself to being agnostic of the mechanism of injury. Thus, the panel of biomarkers, in an individual or combined statistical approach, should provide a reliable, sensitive and specific means to detect VI.

8.1.1 Endothelial biomarkers:

• Angiopoietin 2 (ANGPT2) – Renders the endothelial barrier responsive to pro-inflammatory cytokines. In the absence of angiogenic inducers, such as VEGF, ANGPT2-mediated loosening of cell-matrix contacts may induce endothelial cell apoptosis with consequent vascular regression. In concert with VEGF, it may facilitate endothelial cell migration and proliferation, thus serving as a permissive angiogenic signal.

• P-selectin (SELP) – Mediates, in addition to intercellular adhesion molecule 1 and E-selectin, the interaction of activated endothelial cells or platelets with leukocytes (rolling and adhesion).

• Thrombomodulin (THBD) – Endothelial cell receptor and cofactor for thrombin, initiating an essential anticoagulant pathway via factor V and VIIIa.

• Vascular adhesion protein 1 (VCAM1) – Expressed on both large and small blood vessels only after the endothelial cells are stimulated by cytokines; mediates the adhesion and signal transduction of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium.

8.1.2 Smooth muscle biomarkers:

• Caldesmon (CALD1) – Role in contractility through regulation of the actomyosin interactions in smooth muscle and non-muscle cells (fibroblasts), acting as a potential bridge between myosin and actin filaments.

• Calponin 1 (CNN1) – Role in contractility through regulation and modulation of the actomyosin interactions in smooth muscle.

• Smoothelin isoform b variant (SMTNb) – Role in contractility through modulation of contractile properties of vascular smooth muscle cells in association with alpha actin.

8.1.3 Inflammation biomarkers:

• C-reactive protein (CRP) – Acute phase protein associated with host defense, promoting agglutination, bacterial capsular swelling, phagocytosis and complement fixation. Can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells.

• Neutrophil gelatinase associated lipocalin (NGAL) – Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development. In innate immunity, it sequesters iron to limit bacterial growth.

• Interleukin 6 (IL6) – Acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine; osteoblasts secrete IL-6 to stimulate osteoclast formation. Vascular smooth muscle cells and leukocytes produce IL-6 as a potent inducer of the acute phase response. Also plays a role in leukocyte differentiation.

• Interleukin 8 (IL8) – Attracts neutrophils, basophils and T lymphocytes; involved in neutrophil activation (phagocytosis); and a potent promoter of angiogenesis.

• Growth regulated alpha protein (GroA) – Attracts neutrophils and exerts autocrine effect on endothelial cells in angiogenesis and arteriogenesis. Also has a role in spinal cord development by inhibiting the migration of oligodendrocyte precursors as well as in wound healing and tumorigenesis.

• C-X-C motif chemokine 10 (CXL10) - Attracts monocytes, T-lymphocytes, NK cells, and dendritic cells, and promotes T cell adhesion to endothelial cells in response to activated endothelial cells, monocytes and fibroblasts.

• Matrix metalloproteinase 3 (MMP3) – Degrades fibronectin, laminin, gelatins of type I, III, IV, and V; collagens III, IV, X, and IX, and cartilage proteoglycans. Activates procollagenase. In addition, MMP3 can also activate other MMPs such as MMP1, MMP7, MMP9, MMP13, MMP14 and MMP15, rendering it a critical part of a tissue-specific acute response to remodeling stimuli.

• Pentraxin-related protein (PTX3) – Plays a role in the regulation of innate resistance to pathogens, inflammatory reactions and possibly clearance of self-components in response to secretion from endothelial cells and activated leukocytes. Also a role in female fertility

• Metalloproteinase inhibitor 1 (TIMP1) – Inhibits and irreversibly inactivates matrix metalloproteinases, including MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13 and MMP16, to promote extracellular matrix proliferation (i.e., tissue remodeling). Also functions as a growth factor that regulates cell differentiation, migration and cell death and activates cellular signaling cascades via CD63 and ITGB1, which promotes cell survival, reorganization of the actin cytoskeleton, cell adhesion, spreading and migration, as well as VEGFA signaling and the adhesion of leukocytes onto endothelial cells via regulation of SELP trafficking.

8.1.4 Circulating microRNAs:

• MicroRNAs are small non-coding RNAs of approximately 22 nucleotides length that modulate post-transcriptional gene expression. Out of approximately 2000 known human microRNAs, several microRNAs are known to exhibit highly tissue-specific transcription patterns. This includes microRNAs that are specifically transcribed in vascular and microvascular endothelial cells (e.g. hsa-miR-126-3p), as well as vascular smooth muscle cells (hsa-miR-143, hsa-miR-145) (Jamaluddin et al. 2011; Zampetaki et al. 2012; Mikaelian et al. 2013; Kondkar & Abu-Amero 2015). Active or passive release of microRNAs from cells, specifically upon tissue injury, enables minimal-invasive detection of such microRNAs in the circulation. In addition, several miRNAs have been associated with vascular injury in humans and preclinical species (Jamaluddin et al. 2011; Thomas et al. 2012; Mikaelian et al. 2013; Keirstad et al. 2015; Gareri et al. 2016; Bijkerk et al. 2017; Chen et al. 2018). Hence, we hypothesize that circulating microRNAs might serve as sensitive and specific biomarkers for VI. We will apply next-generation sequencing as non-targeted analytical platform to select VI microRNA biomarker candidates in the learning phase.

8.2 Existing and Planned Studies

- Exploratory nonclinical data has been generated by PSTC that supported receipt of the clinical Letter of Support correlating the biomarker response to histopathology (rodent). This data was summarized in the submission package for the Letter of Support.
- A second “confirmatory” set of seven rodent vascular toxicity studies and 1 rat balloon angioplasty study is being completed this year by the PSTC and will be published. This includes six studies using small and large vascular toxicants across different mechanisms of toxicity in which biomarker performance is evaluated against the same histomorphologic outcomes. A similar, but more consistent study design and the same pathology lexicon was used as in the aforementioned “learning” set of rodent studies that is summarized in SAFE-T’s submission that resulted in the Letter of Support.
- Exploratory clinical data has been generated by IMI SAFE-T correlating the biomarker response to standard diagnostic determinants of vasculitides with shared histomorphologic outcomes as that evaluated in aforementioned rodent data. This data is summarized in the submission package for the Letter of Support. In addition, published data from clinical trials using similar proteins as exploratory diagnostic and efficacy biomarkers further supports the inference to correlate biomarker response to shared histomorphologic outcomes (Monach 2014, Monach et al. 2013).

9 PREVIOUS QUALIFICATION INTERACTIONS AND OTHER APPROVALS

On November 7, 2016, FDA issued a Letter of Support (LoS) to the Safer and Faster Evidence-based Consortium (SAFE-T) and the Predictive Safety Testing Consortium (PSTC) for the further study and development of safety biomarkers for monitoring drug-induced vascular injury (DIVI) in early clinical drug development.

The SAFE-T consortium was funded by the EU's Innovative Medicines Initiative (IMI). IMI funded projects have a limited lifetime, which is typically five years. SAFE-T was funded from 2009 to 2014, and then was granted a one-year extension up to June 2015 to complete the regulatory submission process.

After the formal end of SAFE-T, the consortium received Letters of Support from FDA and EMA for a subset of new safety biomarkers for drug-induced vascular injury; none of the markers had achieved regulatory qualification at that time.

Towards the end of SAFE-T, plans were made for a follow-up project, aiming at completion of qualification of those biomarkers for which SAFE-T received Letters of Support, expanding the project scope towards additional toxicity areas, as well as the investigation of new biomarkers for safety detection.

The proposed plan was submitted by an industry consortium and approved by IMI; the corresponding IMI call for applications was published in November 2017. IMI selected a consortium from among competing applicant consortia in April 2018, and the winning consortium joined the already existing industry consortium under the name TransBioLine, the Translational Safety Biomarker Pipeline.

On January 14, 2019, in response to an FDA request, the TransBioLine DIVI Work Package submitted the Status Update for the Legacy Biomarker Qualification Project DDTBMQ000037 (Drug-Induced Vascular Injury Biomarkers). On May 17, 2019, an FDA De-brief Meeting was held to discuss issues raised during the review of the Status Update submission.

10 REFERENCES

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11 ATTACHMENTS

11.1 List of publications

Bendjama K, Guinaud S Aras G, Arber N, Badimon L, Bratfalean D, Brott D, David M, Doessegger L, Firat H, Gallas JF, Gautier JC, Hoffmann P, Kraus S, Padro T, Saadoun D, Szczesny P, Thomann P, Vilahur G, Lawton, M, Cacoub P. 2014. Translation strategy for the qualification of drug-induced vascular injury biomarkers. Toxicol Pathol 42(4): 658-671.

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11.2 Long-term objectives

Not applicable at this time

11.3 Other supporting information (***Optional*** **– *not for public posting*)**

The following optional confidential information is being submitted as optional attachments and should not be publicly posted.

11.3.1 RT-qPCR Assay Validation Plan

11.3.2 LC-MSMS Assay Validation Plan

11.3.3 LC-MSMS Assay Analytical Method Description

11.3.4 Validation of the Quantitative Analysis of IP-10 in Serum Samples using the Chemokine Panel 1 Kit from Meso Scale Discovery

11.3.5 Statistical approaches for Learning Phase