TransBioLine
Translational Safety
Biomarker Pipeline

Innovative Medicines Initiative - TransBioLine
Drug-induced CNS Injury Work Package

Letter of Intent

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Release Date: February 25, 2020

CONFIDENTIAL

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Abbreviations

ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
BBB	Blood brain barrier
BMI	Body Mass Index
CICARO	Chemotherapy-induced Cognitive Alterations in Recruits With Ovarian and Breast Cancer
CNS	Central nervous system
COU	Context of use
CSF	Cerebral spinal fluid
CT	CAT scan
DINI	Drug-Induced CNS Injury
ELISA	Enzyme-linked immunosorbent assay
GCS	Glascow coma scale
HESI	Health and Environmental Sciences Institute
IMI	Innovative Medicines Initiative
FQP	Full qualification package
GFAP	Glial Fibrillary Acid Protein
LLOQ	Lower limit of quantification
LOD	Limit of detection
LOI	Letter of intent
MOA	Mechanism of Action
MRI	Magnetic Resonance Imaging
MS	Multiple sclerosis
NCE	New chemical entity
NFL	Neurofilament light chain
NHV	Normal healthy volunteers
PNS	Peripheral nervous system

pNFH	Phosphorylated neurofilament heavy chain
ROC	Receiver-operator curve
SAP	Statistical analysis plan
SIMOA	Single molecule array
Tau	Microtubule associated protein tau
TBI	Traumatic brain injury
TNS	Total neuropathy score
UCH-L1	Ubiquitin C-terminal hydrolase L1
ULOQ	Upper limit of quantification
ULN	Upper limit of normal

1 ADMINISTRATIVE INFORMATION

1.1 Submission Title:

Letter of Intent regarding the qualification of biomarkers of drug-induced CNS injury

1.2 Requesting Information:

Requesting Organization

Name: Innovative Medicines Initiative (IMI) TransBioLine
Drug-Induced CNS Injury (DINI) Work Package

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1.3 Submission Dates:

LOI submission date: February 25, 2020

2 INTRODUCTION

TransBioLine (Translational Safety Biomarker Pipeline) is a fully funded ~30 MM Euro IMI-2 project with a remit to qualify biomarkers for drug-induced injury of critical organ systems, including the nervous system. There are currently no easily accessible, sensitive and specific biomarkers for the detection of drug-induced CNS-injury in early clinical trials. This can put human volunteers in these trials at risk. It can also reduce drug development productivity by precluding practical, safe evaluation of potential breakthrough therapies in humans. Thus, there is a pressing need for fully qualified blood-based biomarkers of drug-induced CNS injury to enable better decisions in early clinical trials with a neurotoxicity risk. We plan to develop qualification data sets for five (5) serum-based biomarkers across three (3) CNS injury conditions; physical (traumatic brain injury), disease (multiple sclerosis) and drug-induced (chemobrain) injury conditions, and to demonstrate that one or more of these biomarkers (as single or composite scores) will support the proposed context of use. We hypothesize that CNS injury biomarkers that are sensitive across all three of these patient populations are the most likely candidates for the proposed context of use. We also propose studies in nonclinical models, but do not anticipate associated data as being necessary for a qualification decision.

3 DRUG DEVELOPMENT NEED STATEMENT

There are no sensitive and specific biomarkers for the detection of drug-induced CNS-injury. There is a pressing unmet medical need for such safety biomarkers to safeguard healthy volunteers and patients from serious, irreversible CNS injury during drug development.

Adverse neurological conditions are an undesirable side effect of many drugs (Demler, T. L., 2014). Upwards of 14% of new drug candidates were discontinued in preclinical development due to neurotoxicity in a representative pharmaceutical company (Cook, D. et al., 2014, Redfern, W.S. et al., 2010). A similar proportion (16%) of post-marketing withdrawals have been associated with neurotoxicity (Onakpoya, I. J. et al., 2016), suggesting that current preclinical bioassays do not detect a significant proportion of neurotoxic drugs during development (Walker, A. L. et al., 2018). Tragic unexpected fatal neurotoxicity can occur in Phase I clinical trials, as exemplified by the recent Bial incident (von Schaper, E., 2016). Although imaging (MRI, CT), neuropsychological testing and clinical examinations may be used to detect CNS injury, these tools are not particularly sensitive, nor are they cost-effective in Phase 1 clinical trial settings. Therefore, there is a pressing unmet medical need for sensitive, blood-based safety biomarkers to prevent healthy volunteers and patients from serious, irreversible CNS injury and to guide decision-making in early clinical trials (Roberts, R. A. et al., 2015). Moreover, the availability of such biomarkers would facilitate moving

potential breakthrough therapies safely into the clinic, thereby increasing drug development productivity.

4 BIOMARKER INFORMATION AND INTERPRETATION

4.1 Biomarker name

We plan to evaluate five (5) nervous system-derived proteins commonly reported in the literature to be associated with CNS injury or disease (Marques, T. M. et al., 2019, Papa, L. et al., 2012a, Papa, L. et al., 2012b, Shekhar, S. et al., 2016, Singh, P. et al., 2011) for qualification as safety biomarkers of neurotoxicity in serum. The five molecular markers are described in Table 4.1.

Table 4-1. Individual CNS-injury biomarkers

Acronym	Name	HUGO ID	Description
GFAP	Glial fibrillary acid protein	HGNC:4235	An intermediate filament protein expressed by glial cells (astrocytes) of the CNS.
UCH-L1	Ubiquitin C-terminal hydrolase L1	HGNC:12513	A deubiquitinating enzyme that is highly expressed in neurons/axons of the CNS.
NFL	Neurofilament light chain	HGNC:7739	A ~70 kDa cytoskeletal intermediate filament protein that is expressed in neurons/axons.
pNFH	Phosphorylated neurofilament heavy chain	HGNC:7737	A phosphorylated, ~200 kDa cytoskeletal intermediate filament protein that is expressed in neurons/axons.
Tau	Microtubule associated protein tau	HGNC:6893	A microtubule-associated protein expressed in CNS neurons.

4.2 Analytical methods

We plan to measure NFL, pNFH, GFAP, UCH-L1 and Tau in human serum using commercially available, ultra-sensitive SIMOA digital ELISA assays available from Quanterix Corporation (Billerica, MA). A recent cross-company study demonstrated the sensitivity, accuracy, precision and reliability of this assay platform for detection of low-level proteins in blood (Chunyk, A. G. et al., 2017). Using the same reagents as a conventional ELISA, this method can measure proteins in a variety of different matrices (serum, plasma, cerebrospinal fluid, urine, cell extracts, etc.) at femtomolar

concentrations, offering a roughly 1000-fold improvement in sensitivity over conventional ELISAs. The technology makes use of arrays of femtoliter-sized reaction chambers, referred to as **single-molecule arrays** (SIMOA®).

4.3 Measurement units and limit(s) of detection

The SIMOA digital ELISA method has been used to measure proteins in a variety of different matrices, including serum, plasma, and cerebrospinal fluid at femtomolar concentrations. Commercially available assays on this platform, the multiplexed Human Neurology 4-Plex "A (NFL, Tau, GFAP & UCH-L1) and stand-alone pNFH assays, demonstrate LLOQs in the range of 0.5 to 10 pg/ml (see Attachment 11.1.1 and Attachment 11.1.2).

4.4 Biomarker interpretation and utility

At this time, it is challenging to predict exactly how our biomarkers will be applied and interpreted to support the proposed COU. Once we collect our data, we will need to determine if single or composite measures will be used and whether these measures will be applied to individuals or a cohort of individuals in a phase 1 clinical trial. Using the data sets developed from the NHV and CNS-injury patient populations proposed in our clinical validation plan (see section 7.3) we are confident that we will be able to define thresholds or cut-offs which would flag an increased risk of CNS injury. Ideally, such thresholds would be above an upper limit of normal in NHV, but still below levels associated with detectable CNS injury in our three patient populations.

To explore the feasibility of defining such thresholds we analyzed serum GFAP data simulated from the descriptive statistics reported for a control human cohort (N=176) (Papa, L. et al., 2012a). This analysis revealed that ~95% of serum GFAP levels within this simulated data set fall within a reference range with an ULN of 0.13 ng/mL. Mean levels of serum GFAP associated with mild TBI reported in this same paper were 0.531 ng/mL (GCS 15 cohort) or 1.13 ng/mL (GCS 14 cohort). Additionally, generation of ROC curves from modeled data revealed good-to-very good discriminatory ability with AUCs of 0.87 (GCS 15 cohort, N=77) and 0.91 (GCS 14 cohort, N=18). This preliminary analysis of published data suggests that we will be able to identify cut-offs for one or more of our proposed biomarkers with good predictivity of CNS injury risk.

5 CONTEXT OF USE STATEMENT

A blood-based safety biomarker or biomarker panel to aid in the detection of acute drug-induced CNS injury risk in phase 1 trials in healthy volunteers when there is an a priori concern that a drug may cause CNS injury in humans.

Our proposed COU is focused on:

1.&Blood-based biomarkers to enable minimally invasive sample acquisition procedure in trial volunteers

2. Safety biomarkers of neurotoxicity to address a critical safety concern with an unmet medical need, i.e. acute drug-induced CNS injury risk (drug development need)

3. Improved decision-making in phase 1 clinical trials, e.g. decisions to stop dosing, to modify dosing regimen, or to conduct additional testing/monitoring with established modalities before dose escalation, etc. (clinical use decision tree, Figure 7-1)

4. Normal healthy volunteers, a narrow target population to enable evaluations against a normal background variation, and the population anticipated for first in human studies

5. Drug candidates with an *a priori* concern for CNS injury, e.g. candidates with a known class effect or a concern characterized in preclinical toxicity studies.

6 ANALYTICAL CONSIDERATIONS

6.1 Pre-Analytical Considerations

Quality control of pre-analytical variables is a key factor in generating consistent and accurate experimental data. We thus plan to develop and implement a rigorous pre-analytical sample collection, processing and shipping protocol. Assay validation will reveal, if the stability of the five analytes is impacted by pre-analytical factors.

6.2 Analytical Considerations

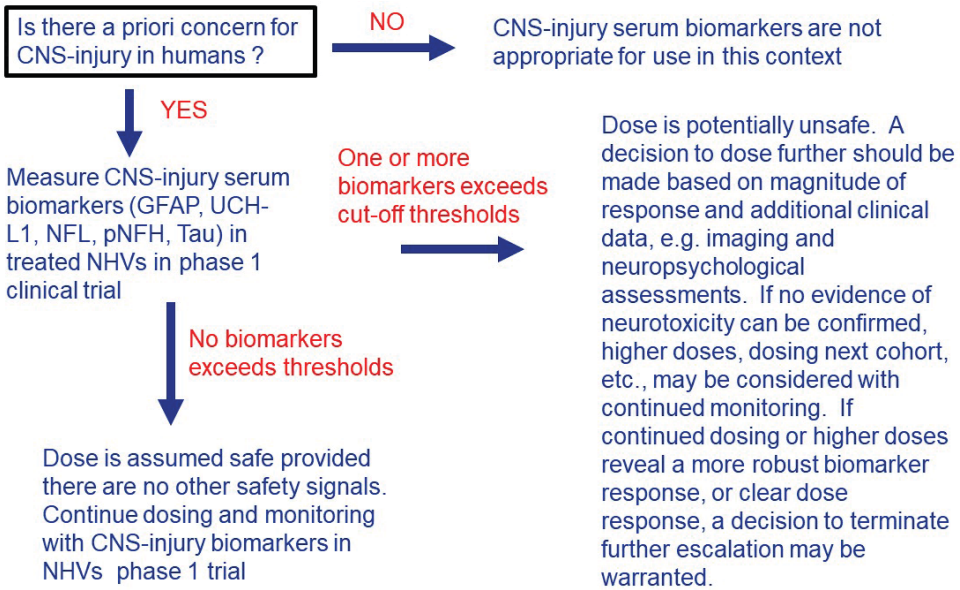
The concentration of proposed biomarkers will be measured using two assay kits available from Quanterix; the Neurology 4-Plex assay, which is available as a research grade assay and the pNFH assay, which is available as a “Discovery kit”. These assays have been evaluated by Quanterix for validation parameters like LOD, LLOQ, dilutional linearity, precision and endogenous levels in plasma, serum and CSF. Manufacturer data sheets summarizing those results are attached as Attachment 11.1.1 (neurology 4-Plex) and Attachment 11.1.2 (pNFH). We plan to expand the evaluated parameters and conduct fit-for-purpose validation for both assay kits. This validation will be performed using serum samples from patients with different CNS diseases and will address the following parameters: standard curve performance, intra-run and inter-run precision, assay dynamic range/assay limits (ULOQ, LLOQ), parallelism, reproducibility of endogenous analyte and bench-top, freeze-thaw and long-term stability. Very limited validation will be performed also for CSF. Run acceptance criteria will be established to ensure high quality data. Detailed validation plans for Neurology 4-Plex and Simoa® pNF-heavy Discovery Kit are attached as Attachment 11.1.3 (neurology 4-Plex) and Attachment 11.1.4 (pNFH).

7 CLINICAL CONSIDERATIONS

7.1 Use in Drug Development

During drug development a sponsor may identify a new therapy that could significantly improve patient's lives, but it may carry a risk for CNS injury. Without a biomarker to enable safe evaluation in the clinic, it is unlikely that such a drug could ever be tested in humans. For new drug candidates with an *a priori* signal of concern for CNS injury (e.g. a preclinical histologic change or behavioral effect, a potential class effect, etc.), a sponsor may have a compelling argument for advancing this drug into the clinic safely (e.g. evidence that a proposed MOA should not translate to humans, a large therapeutic index, species sensitivity differences, etc.). In this instance, the proposed novel serum biomarkers would be monitored in treated NHVs in phase 1 trials for levels exceeding predetermined thresholds or cutoffs associated with increased risk of CNS injury. If these thresholds are exceeded, a weight of evidence approach would be used for further dosing decisions (Figure 7.1). This may include evaluating additional clinical data, as well as conducting follow-up imaging (CT, MRI, functional) and/or neuropsychological testing. If no evidence of neurotoxicity can be confirmed, higher doses, dosing next cohort, etc., may be considered with continued monitoring. If the magnitude of the biomarker change approaches levels associated with detectable brain injury identified in the human qualification data sets, i.e. those defined in our 3 proposed CNS-injury populations a decision to stop further dosing may be warranted.

Figure 7-1. Decision tree for clinical use of CNS-injury biomarkers



7.2 Patient population or drug development setting

As described in the COU, the human population in which we propose to use these biomarkers will be a NHV population of phase 1 clinical trials. This population will be screened using standard inclusion and exclusion criteria for phase 1 NHVs. Our proposed criteria are as follows:

Inclusion criteria:

- Healthy female or male subjects, who, at the time of screening, are ≥18 years of age. Healthy is defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination including blood pressure, pulse rate measurement, and clinical laboratory tests.
- Body mass index (BMI) of 17.5 to 30.5 kg/m2; and a total body weight >50 kg (110 lbs).
- Evidence of a personally signed and dated informed consent document indicating that the subject has been informed of all pertinent aspects of the study.
- Subjects who are willing and able to comply with scheduled visits, laboratory tests, and other study procedures.

Exclusion Criteria:

- Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease.
- Treatment with an investigational drug within 30 days or 5 half-lives proceeding the first study period whichever is longer.
- Subjects with abnormalities in clinical laboratory tests at screening.
- Other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavior or laboratory abnormality that may increase the risk associated with study participation or may interfere with the interpretation of study results.
- Pregnancy or breastfeeding

7.3 Clinical validation

The first step of our plan will be to understand the relationship between CSF and serum for our five protein biomarkers to support using serum as a readily accessible fluid compartment in subsequent NHV and patient studies. We will then characterize our proposed biomarkers in serum samples from an untreated NHV population consistent with the typical makeup of volunteers recruited in phase 1 clinical trials. These NHV data sets will allow us to characterize normal background variability of our biomarkers

in order to determine standard reference ranges, upper limits of normal, cut-offs of concern, etc. Next, we will characterize the responses of our biomarkers in TBI and MS patients, populations that represent injury to the two major types of CNS cells (neuronal and glial). These data sets will allow us to define thresholds associated with injury to these cell types using current "gold standard" assessments, e.g. imaging and/or neuropsychological assessments. Finally, we will evaluate the thresholds defined above to detect CNS-injury risk in a drug-induced CNS-injury model, namely patients with gynecological cancer undergoing chemotherapy. The clinical cohorts, samples and assays that will support this strategy are indicated in Table 7-1.

Table 7-1. Proposed clinical cohorts and samples

Objectives	Assays	Cohorts	Patient / Sample #
CSF/serum correlations Analyse correlations between serum and CSF measurements	GFAP, UCH-L1, NFL, pNFH, Tau	MS, TBI	60 patients 120 samples (1 CSF and 1 serum sample per patient)
Reference Ranges Establish reference ranges in serum samples from healthy volunteers	GFAP, UCH-L1, NFL, pNFH, Tau	Normal healthy volunteers (NHV)	280 volunteers 300 samples (10 HV x 3 longitudinal, plus 270 HVs x single time point)
CNS physical injury exploratory data set Analyse biomarkers in sera of TBI patients	GFAP, UCH-L1, NFL, pNFH, Tau	Traumatic brain injury (TBI)	100 patients 100 samples (1 sample per patient)
CNS disease injury exploratory data set Analyse biomarkers in sera of MS patients	GFAP, UCH-L1, NFL, pNFH, Tau	Multiple sclerosis (MS)	100 patients 200 samples (2 longitudinal samples per patient)
Drug-induced CNS injury confirmatory data set Analyse biomarkers in sera of oncology patients with chemobrain	GFAP, UCH-L1, NFL, pNFH, Tau	Chemotherapy induced brain injury (chemobrain)	100 patients 200 samples (2 longitudinal samples per patient)

7.3.1 Comparison of Serum and CSF

We will determine levels of the five protein biomarkers in paired serum and cerebrospinal fluid (CSF) samples from 30 patients with MS and 30 patients with TBI to analyze the relationship between serum and CSF measurements. This will provide data confirming that the serum compartment reflects CSF changes in our proposed biomarkers and that serum can be used as readily accessible fluid compartment as a surrogate for CSF in the patient studies indicated below.

7.3.2 Establishment of Reference Ranges

We will determine GFAP, NFL, pNFH, Tau, and UCH-L1 in serum samples collected from normal healthy volunteers with no known CNS disease or injury, to obtain between subject reference ranges in normal healthy controls (N=270) and to assess intra-individual variability (N=10 x 3 timepoints) of the five biomarkers. We will also

analyze the influence of age, sex and ethnicity on the five biomarkers. A preliminary statistical analysis of published serum GFAP, UCH-L1, NFL, Tau and pNFH levels in controls (Marques, T. M. et al., 2019, Papa, L. et al., 2012a, Papa, L. et al., 2012b, Shekhar, S. et al., 2016, Singh, P. et al., 2011) indicates that with a sample size of 100 there is an 80% chance that we will detect anticipated increases (Cohen's D of 0.25) at a 5% significance level. Thus, serum samples from up to 280 NHVs should enable the use of subpopulations with matched demographic data for comparisons to patient data.

7.3.3 Evaluation of Injury/Disease States

7.3.3.1 Exploratory Phase

Traumatic Brain Injury (TBI)

We will quantify the levels of the five protein biomarkers in n=100 serum samples withdrawn within 12 hours of injury from patients diagnosed with mild/moderate TBI (Glasgow Coma Scale score of 9-15). We will use statistical models on measures from this patient population along with reference ranges from healthy volunteers to define cut-off thresholds that are predictive of TBI for each biomarker, or combinations of biomarkers, in serum (see the statistical analysis plan (SAP) in Attachment 11.1.5). Models may also be developed that further stratify patients based on GCS and imaging scores obtained from all patients. Given the extensive literature and recent FDA approval of serum GFAP and UCH-L1 for use with TBI, our TBI data sets will enable us to benchmark our assays and results against this published work.

Multiple Sclerosis (MS)

We will leverage an ongoing longitudinal prospective cohort (Berlin CIS-Cohort) (ClinicalTrials.gov, 2011) of >150 patients with a clinically isolated syndrome (CIS, the first clinical manifestation of MS) or early relapsing-remitting MS, which started recruitment in 2011. Patients included in the cohort undergo a baseline and yearly follow-up examinations, including clinical evaluation, sampling of biospecimens, cerebral MRI, and neuropsychological testing. We will measure the five biomarkers in n=100 baseline and n=100 one-year follow-up serum samples. Similar to TBI patients, we will use statistical assessment on measures from this patient population along with those from our healthy volunteers to enable us to define cut-off thresholds predictive of CIS/MS for each biomarker, or combinations of biomarkers, in serum. The availability of atrophy data from cerebral MRIs and results of neuropsychological testing after a follow-up of two years will enable us to evaluate the five protein biomarkers against these established measures of neurotoxicity. We will benchmark our results against previous analyses of NFL in serum as a marker for neurodegeneration in CIS/MS and will also be able to clarify the role of the other four protein biomarkers in this context.

7.3.3.2 Confirmatory Phase

The cut-off thresholds defined in the TBI and MS patient populations will be evaluated in 50 patients receiving chemotherapy. Patients will also be evaluated for associated changes in neuropsychiatric or peripheral neuropathy scores. The ability of these biomarkers to detect chemotherapy-induced CNS injury (chemobrain) will be determined.

Drug-induced CNS injury (Chemobrain)

We will make use of an ongoing longitudinal prospective cohort from the study entitled: Chemotherapy-induced Cognitive Alterations in Recruits With Ovarian and Breast Cancer (CICARO) (ClinicalTrials.gov, 2017) that started recruitment in 2016. In this study, neurotoxic effects of chemotherapy in patients with mammary or ovarian carcinoma are being assessed. For both, patients with mammary carcinoma and patients with ovarian carcinoma, the study has a treatment and a control arm. Patients with histologically confirmed mammary carcinoma are treated either with combination chemotherapy, including paclitaxel, epirubicine and cyclophosphamide (chemotherapy), or with antihormonal therapy (control). Patients with histologically confirmed ovarian carcinoma receive treatment with paclitaxel, carboplatin and optionally bevacizumab (chemotherapy). Patients with benign gynaecological tumors serve as controls. All patients and controls undergo clinical examinations, neuropsychological testing, total neuropathy score (TNS), and collection of biospecimens before and 5 months after start of chemotherapy. We will measure the five protein biomarkers in n=50 patients before and 5 months after start of chemotherapy. Likewise, we will determine the 5 biomarkers in n=50 patients from the control groups at baseline and month 5. We will also compare the biomarker levels in the patient populations with reference ranges obtained in healthy controls. This study design will permit us to evaluate whether serum levels of one or more of the five biomarkers associated with CNS injury in our exploratory studies in TBI and MS patients will be predictive of chemotherapy induced brain injury (chemobrain) as previously suggested for pNFH (Natori, A. et al., 2015, Petzold, A. et al., 2010). Furthermore, we will analyze associations of the five biomarkers with results from neuropsychological testing and TNS to benchmark them against these established markers of neurotoxicity.

7.4 Benefits and risks

During drug development a sponsor may identify a breakthrough therapy that could significantly improve patient's lives. However, if that new therapy carries a risk for CNS injury and no biomarker exists to enable safe evaluation in the clinic, it is unlikely to ever be tested in humans. A clear benefit for serum-based biomarkers of CNS injury is thus to improve drug development efficiency by enabling safe clinical evaluation of new chemical entities. The risks associated with the use of the proposed biomarkers in a clinical setting include the limits of any biomarker, perhaps most importantly the

potential for false negative biomarker results. During development and evaluation of the proposed serum biomarkers of CNS injury we will optimize the predictivity of our assay in order to minimize these risks. However, such risks are associated with any diagnostic test and can be mitigated by using clearly defined decision criteria (see decision tree in Figure 7.1).

7.5 Current knowledge gaps, limitations, and assumptions

We chose the five (5) proposed protein markers because these biomarkers have been studied extensively in human nervous system diseases and injuries. We chose the proposed patient populations (TBI, MS and chemobrain) because they represent diverse CNS injury conditions, ranging from physical injury to CNS disease to chemical-induced injury, and they represent injury to the two major types of CNS cells (neuronal and glial). Additionally, TBI patients offer us the added advantage of being able to benchmark our biomarker responses against the extensive published literature available for our proposed biomarkers and TBI. We hypothesize that markers that are sensitive across all 3 of these patient populations are most likely to be generally applicable for our COU. However, we recognize that we are assuming that key underlying pathophysiological processes within our surrogate patient populations, i.e. traumatic brain injury and neuroinflammatory disease patients, are also present within a drug-induced CNS injury population. We hypothesize that cellular damage is a common process across all 3 populations and that injury of neural and glial cells will be detectable using one or more of our 5 proposed serum-based biomarkers. Supporting information is outlined in Section 8 below.

8 SUPPORTING INFORMATION

8.1 Association of biological processes with proposed biomarkers

There are many causes of brain injury including physical trauma, disease processes or chemical insult. There are also many subsequent processes and pathways that may lead to nervous system dysfunction, including neuroinflammation, oxidative stress, and mitochondrial dysfunction (Kanthasamy, A. et al., 2010). However, an outcome common to all these processes and pathways is cellular damage. Cellular damage, if sufficiently severe, may lead to cell death or uncompensated loss of function. We hypothesize that biomarkers sensitive to cellular damage within the CNS will enable detection of drug-induced neurotoxicity before it becomes irreversible or impacts normal function. A biomarker that is specific to a particular region of the brain or a particular disease stage or injury process is not necessary for our proposed COU. Rather, for our proposed COU, the biomarker(s) only need to be sensitive and specific indicators of injury to cells in the CNS.

Cell damage is typically associated with a change in membrane permeability. The plasma membrane of injured or dying cells lose their barrier function and will leak intracellular proteins into the surrounding environment (Kristensen, S. R., 1994). For example, damaged hepatocytes leak intracellular enzymes into the systemic circulation and concentrations of these enzymes (e.g. ALT, AST) in the blood are used as markers of hepatocyte injury (Kamiike, W. et al., 1989, Pratt, D. S. Kaplan, M. M., 2000, Reichling, J. J. Kaplan, M. M., 1988, Sherman, K. E., 1991). Thus, extracellular detection of proteins unique or enriched in a particular cell type can be used to detect organ specific cell injury or death. As indicated earlier, leakage of proteins expressed in the CNS to the systemic circulation, including the 5 we have proposed, are reported as potential biomarkers of CNS injury and disease (Marques, T. M. et al., 2019, Papa, L. et al., 2012a, Papa, L. et al., 2012b, Shekhar, S. et al., 2016, Singh, P. et al., 2011). Notably CNS-derived proteins will also need to cross the blood brain barrier (BBB) in order to be detected in the peripheral circulation. Changes in passive or active transport across the BBB will impact the level of circulating biomarkers. Thus, brain-derived proteins may also be used as indicators of damage to the BBB (Kawata, K. et al., 2016).

All five of the proteins we propose to evaluate (GFAP, UCH-L1, NFL, pNFH, Tau) derive primarily from nervous system tissues. GFAP is an intermediate filament found primarily in astrocytes of the CNS (Yang, Z. Wang, K. K., 2015). UCH-L1 is a deubiquitinating enzyme that is highly expressed in neurons/axons of the CNS (Wilson, P. O. et al., 1988). Both GFAP and UCH-L1 have been extensively studied as biomarkers of traumatic brain injury (Bazarian, J. J. et al., 2018, Takala, R. S. et al., 2016). In fact, serum-based measures of these two proteins were recently approved by the FDA for use with triaging patients with TBI for CT scans. NFL and NFH are both cytoskeletal intermediate filaments expressed in neurons/axons (Yuan, A. et al., 2012). Neurofilament proteins have been studied as biomarkers of axonal injury, degeneration and loss (Petzold, A., 2005, Shaw, G. et al., 2005). Tau is a major microtubule associated protein expressed in the CNS (Morris, M. et al., 2011), which has been studied extensively as a biomarker of neurodegenerative diseases (Schraen-Maschke, S. et al., 2008). Given that all five of these proteins are highly expressed within cells of the CNS and that all 5 have been studied extensively as potential biomarkers of cellular damage associated with CNS injury and/or disease, we hypothesize that these 5 proteins are excellent candidates for systemic biomarkers of drug-induced neurotoxicity. However, it is important to keep in mind that they are not expressed exclusively within the CNS. Some are expressed within the PNS and even non-nervous tissues. Thus, like most biomarkers monitored within the systemic circulation, specificity to CNS injury within the proposed COU will need to be confirmed. To analyze the influence of two important systemic organ injury on the 5 biomarkers we could measure the 5 biomarkers in samples from patients with kidney injury or liver injury, but without CNS disease.

8.2 Nonclinical plans and considerations

We plan to study nonclinical models of nervous system injury to provide supportive information for the qualification of biomarkers of nervous system injury for a phase 1 clinical trial setting. Proposed biomarkers are conserved across species and analytical assays for rodents are available for most of the biomarkers. Evaluation of biomarkers in nonclinical species will provide direct link to histopathological changes and greatly expand the amount of treatments that can be interrogated since we are practically limited in the number of human patient populations we can evaluate. Additionally, studies can be designed to study biomarker response to increasing dose and dosing duration and monitor recovery where possible. Nonclinical data would also provide supportive evidence that the proposed biomarkers are likely to be general markers of drug-induced CNS injury and not just specific to the patient populations we are proposing. However, we do not envision that any of our nonclinical data would form a primary basis for the final qualification decision and our proposed COU.

To achieve the maximum number and diversity of nonclinical studies used, we plan to pursue interactions with the IMI Neuroderisk group as well as the HESI neurotoxicity biomarkers subcommittee. We plan to perform additional rodent studies with diverse mechanisms of neurotoxicity and targets (linkage to certain brain cell type or PNS/CNS localization of injury).

Proposed biomarkers have been studied in nonclinical models, even though not to the extent of the publications in humans. pNFH has been shown to be increased in rat serum of traumatic axonal injury (Yang, Z. et al., 2019) as well as in mouse model of ALS in $SOD1^{G93A}$ mice (Lu, C. H. et al., 2012). NF-L was increased in plasma of chemotherapy induced peripheral neuropathy in rat (Meregalli, C. et al., 2018) as well as in R6/2 mouse model of Huntington disease (Soylu-Kucharz, R. et al., 2017) and mouse models of proteopathic neurodegenerative diseases (Bacioglu, M. et al., 2016). NFL was also increased in CSF and blood in inducible transgenic CamKII-TetOp25 mouse model of neurodegeneration (Brureau, A. et al., 2017). Similarly to human, increased levels of GFAP after TBI have been shown in rat CSF (DeDominicis, K. E. et al., 2018) and in rat plasma and CSF (Huang, X. J. et al., 2015). UCH-L1 increases after TBI were also observed in rat plasma and CSF (Huang, X. J. et al., 2015). Tau in CSF and serum was increased in rat model of traumatic spinal cord injury (Tang, Y. et al., 2019) and in rodents following controlled cortical impact (Rubenstein, R. et al., 2015).

Table 8-1. Planned studies in nonclinical species to support understanding of biomarker response to DINI

Toxicant / animal	Mechanism	Nervous system damage
5-FU / mouse	Inhibits the enzyme thymidylate synthase blocking the thymidine formation required for DNA synthesis	Model of delayed damage to myelinated tracts of the CNS Associated with altered transcriptional regulation in oligodendrocytes and extensive myelin pathology.
Cuprizone / rat	Chelator of copper. Reduces the mitochondrial activity and metabolism of oligodendrocytes with activation of oxidative stress and ROS production. These events trigger microglial/macrophage recruitment and, subsequently, the secretion of proinflammatory cytokines, which ultimately promote oligodendrocyte death or inhibit differentiation.	Established animal model of MS. Induces severe oligodendrocyte damage with concomitant microglial activation and severe astrocytosis. Potential for regeneration (remyelination).
2-Chloroproprionic Acid / rat	Depletes glutathione (liver and brain)	Necrosis to the granule cell layer of the rat cerebellum and Purkinje cells
Doxorubicin/Epirubicin / rat	Interferes with the function of DNA (deoxyribonucleic acid) topoisomerase II	Causes cognitive dysfunction in patients. Does not cross the BBB. In rat causes lesions in PNS – dorsal root ganglia and trigeminal ganglia. Not extensively studied.
IMI Neuroderisk studies where biomarker results could be shared: **Vincristine, Acrylamide** and **Paclitaxel**		

9 PREVIOUS QUALIFICATION INTERACTIONS AND OTHER APPROVALS (IF APPLICABLE)

Pre-LOI Meeting held on October 29, 2019 with CDER Biomarker Qualification Program team.

10 REFERENCES

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