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**CONTRACTING ORGANIZATION:** University of California, San Francisco

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**14. ABSTRACT**

TRACK-TBI Precision Medicine utilizes the rich TRACK-TBI dataset to validate biomarkers of diffuse axonal injury (DAI), microvascular injury (MVI), and neuro-inflammation using advanced blood-based assay platforms and MRI sequences. The project will validate early and ultra-early blood-based and imaging biomarkers of DAI, MVI, and neuro-inflammation and will conduct a multicenter, double-blind, placebo-controlled exploratory clinical trial comparing the impact of cyclosporine A on blood-based and imaging biomarkers of DAI and neuro-inflammation in moderate/severe TBI patients.

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## TABLE OF CONTENTS

## 1. INTRODUCTION:

TRACK-TBI Precision Medicine utilizes the rich TRACK-TBI dataset to validate biomarkers of diffuse axonal injury (DAI), microvascular injury (MVI), and neuro-inflammation using advanced blood-based assay platforms and MRI sequences. The project will validate early and ultra-early blood-based and imaging biomarkers of DAI, MVI, and neuro-inflammation (Phase I and Phase II) and will conduct a multicenter, double-blind, placebo-controlled exploratory clinical trial comparing the impact of cyclosporine A on blood-based and imaging biomarkers of DAI and neuro-inflammation in moderate/severe TBI patients (Phase III).

## 2. KEYWORDS:

traumatic brain injury, concussion, blood-based biomarker, biofluid biomarker, neuroimaging biomarker, clinical outcome assessment (COA), Glial Fibrillary Acidic Protein (GFAP), clinical trial, diffuse axonal Injury, microvascular Injury, Neuroinflammation, brain magnetic resonance imaging (MRI), brain computed tomography (CT), precision medicine

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

**Overview of Research Plan**

- Phase 1-Base: Validate biomarkers of Diffuse Axonal Injury (DAI), Microvascular Injury (MVI), and Neuroinflammation using advanced blood-based assay platforms and MR imaging sequences in prospectively-collected data from existing TRACK-TBI subjects.
- Phase 2-Option I: Validate early and ultra-early blood-based and novel imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe TBI subjects.
- Phase 3-Option II: Conduct a multicenter double-blind, placebo-controlled exploratory clinical trial comparing the impact of Cyclosporine A (CsA) on imaging and blood-based biomarkers of DAI, MVI, and neuroinflammation in moderate-severe TBI patients admitted to the ICU.

**Phase I-Base (09/30/2018 – 09/29/2019)**

In Phase 1 (Aims 1.A-C), blood-based protein biomarkers will be measured in **existing** biospecimen samples; 2 sets of **existing** MRI scans on these same subjects will be analyzed in concert with serially collected multivariate outcome measures. These will be used to characterize specific mechanistic endophenotypes in individual patients, DAI, MVI, and neuroinflammation,

chosen for: (i) their importance to TBI outcome across the severity spectrum; (ii) existing pre-clinical and clinical data supporting the biomarkers' ability to identify these mechanistic endophenotypes; and (iii) the opportunity to translate candidate therapeutics against these mechanisms into targeted clinical trials.

**Phase II-Option 1 (09/30/2019 – 09/29/2021**

**Validate early and ultra-early blood-based and novel imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe subjects.**

In the existing TRACK-TBI study, MRIs were first obtained at 2-weeks after injury, and the initial blood sample was obtained within 24 hours after injury. Since it is likely that many effective TBI therapies will have to be started within hours or days post-injury, to obtain the necessary precision to design future clinical trials, we must have more granular information about the early evolution of the imaging and molecular biomarkers identified in Phase 1. Our Aim 2 study protocol will enroll a cohort of moderate to severe TBI subjects (N=50), stratified according to VA/DoD criteria for these injury severities through 5 TRACK-TBI network sites to obtain novel advanced neuroimaging and more frequent biomarker sampling. Subjects will be assessed according to the existing TRACK-TBI outcomes protocol over 3 months. In this cohort, we will validate blood-based biomarkers at early (<24h) and ultra-early time periods (~6h), and neuroimaging biomarkers at early (2w) and ultra-early (<48h) time periods, the selection of which will be informed by Phase 1 (Aim 1) findings. (See Appendix 1 – TRACK-TBI Precision Medicine Phase 2-Option I Clinical Protocol).

**Phase III-Option 2 (09/30/2021 – 09/29/2022)**

**Conduct a multicenter double-blind, placebo controlled exploratory clinical trial comparing the impact of e.g., Cyclosporine A (CsA) on blood-based and imaging biomarkers of DAI, MVI, and neuroinflammation in moderate-severe TBI patients admitted to the ICU.**

This early Phase IIa pilot will use biomarkers to select subjects with specific injury endophenotypes, as well as to assess whether the drug is engaging its molecular target and impacting the pathobiology of injury.

**What was accomplished under these goals?**

**IRB and HRPO Approval for Phase I of the study (09/30/2018 - 04/09/2019 (COMPLETED)**

UCSF received local IRB approval as well as HRPO approval for the "TRACK-TBI Precision Medicine - Pathomechanistic Classification of Traumatic Brain Injury: The Bridge to Targeted Therapies" protocol. This IRB approval encompasses biospecimen and imaging analyses and was shared with the University of Pennsylvania and University of Florida to use as a template. The IRBs at both the University of Pennsylvania and University of Florida determined that the study was not human subjects' research at their local institutions, and issued a review status of *exempt*. HRPO reviewed the protocols from UPenn and UF and issued determinations of "Research Not Involving Human Subjects."

**TOTAL PROTOCOLS:**
**Phase I Protocol (1 of 1 total):**
Protocol [HRPO Assigned Number]: E00485.1a
Title: TRACK-TBI Precision Medicine - Pathomechanistic Classification of Traumatic Brain Injury: The Bridge to Targeted Therapies

IRB and HRPO approval was obtained for the TRACK-TBI Precision Medicine - Pathomechanistic Classification of Traumatic Brain Injury: The Bridge to Targeted Therapies at the University of Pennsylvania, University of Florida, and University of California, San Francisco for Phase I of the study. See below for more information about IRB and HRPO status for Phase II of this study.

**Protocol Development Phase II Option I (09/30/2019 - 09/29/2020 ONGOING)**

Planning for Phase II Option I of the study began by creating and distributing a site selection questionnaire requesting information as to each sites' ability to conduct the planned study activities, with a particular focus on availability of MRI in the acute care environment (e.g., hospital, ICU, OR). Questionnaires were received from the sites and review showed that 5 of our Core sites had the necessary MRI equipment and study personnel to immediately implement the aim of collecting an MRI and blood-based biomarkers in the ultra-acute phase of brain injury. An additional 3-4 sites were identified that could potentially participate in the study with reasonable modifications to their current equipment and/or personnel resources. Development of the clinical protocol (Table 1) has been finalized and we plan to collect a slightly modified TRACK-TBI Phase I MRI, which includes the following sequences: 3D T1 MPRAGE/IR-SPGR, 3D T2* GRE, DTI, resting state fMRI, 3D T2-FLAIR CUBE/SPACE/VISTA, and ASL (in place of the final Phase I sequence of 3D T2 CUBE/SPACE/VISTA). These sequences will be collected at 0-48 hours, 2 weeks, and 3 months from injury. An MR physicist has been hired to oversee formal qualification of the MRI scanners at these sites, including phantom testing of each scanner with ADNI (structural MRI), BIRN (functional MRI) and NIST (diffusion MRI) phantoms to ensure that all scanners are calibrated properly and for cross-site standardization of the scanner images. A plan has been formulated for remote monitoring of the scanner qualification and monitoring process by the MR physicist, given the current travel restrictions due to the COVID19 pandemic. We remain on schedule to begin patient enrollment soon after COVID19 restrictions on in-person research activities, including MRI scanning, are lifted at each of the participating sites.

Table 1. Proposed Protocol Timeline

| Time Post-TBI | Modified TRACK-TBI Imaging Protocol | Blood Collection Biomarker Analysis | Outcome Assessment Battery |
|---|---|---|---|
| Day 1-2 | Clinical CT + Phase 1 MRI | X -Collected at 8h intervals | |
| Day 3-4 | Phase 1 MRI | X Collected at 12h intervals | |
| Day 5 | | x | |
| 2 Weeks | Phase 1 MRI | x | x |
| 6 Weeks | | | x |
| 3 Months | | | x |
| 6 months | Phase 1 MRI | x | x |

With imaging priorities of the protocol finalized, the working group then determined that blood sample collection should take place at <6h, 12h, 24h, 2d, 3d, 5d, 14d, 42d, and 90d, from the time of injury. At each time point, one 8-mL plasma blood tube and one 8 mL serum tube will be collected. DNA will be extracted from the plasma tube. This blood collection protocol will also allow for the analysis of predetermined biomarkers.

After timelines for blood sample collection and MRI scans were finalized, clinical outcome assessment leads Drs. Temkin, Giacino, and McCrea analyzed possible measures for

administration of the outcome assessment battery. They settled on an enrollment battery composed of 3 measures given to eligible participants at consent or by phone within 24 hours of injury:

1. Rivermead Post Concussion Symptoms Questionnaire (RPQ)
2. Brief Symptom Inventory 18 (BSI-18)
3. Standard Assessment of Concussion (SAC)

The total time to complete the enrollment battery is approximately 11 minutes.

Drawing on experience from the TRACK-TBI U01 study, the team has developed a Comprehensive Assessment Battery for the 2-week, 6-week, and 3-month follow ups that will take approximately 89-103 minutes to administer. Patients who are too impaired to tolerate the Comprehensive Assessment Battery will undergo assessment on the Abbreviated Assessment Battery (detailed in section 8.3 below), which consists of standardized measures of basic neurobehavioral (e.g., Coma Recovery Scale-Revised [CRSR]) and cognitive (e.g., Confusion Assessment Protocol [CAP]) function.

The full Comprehensive and Abbreviated battery is presented in Table 2 & 3 below. Table 4 shows the timeline of study procedures.

Table 2. Comprehensive Battery

| Domain | Subdomain | Instrument | Administration Time | Order of Administration In person (~90 min) |
|---|---|---|---|---|
| Screening | Screening | Assessment of Speech Intelligibility* | 2 min | 1, and as needed |
| | | Galveston Orientation and Amnesia Test (GOAT)* | 5 min | 2, and as needed |
| History | Participant Interview (or Informant Interview) | Interview to update occupational status; living situation; medical history (e.g., known neurologic, cognitive, psychiatric conditions)* | 10 min[#] | 3 |
| Daily/Global Function | Global Outcomes | Glasgow Outcome Scale Extended (GOSE)* | 15 min[#] | 5 |
| | | Functional Status Exam (FSE)* | 10 min[#] | 4 |
| Psychological Health/ Neurobehavioral Symptoms | Depression, Anxiety, Somatic | Brief Symptom Inventory-18 (BSI-18)* | 3 min | 13 |
| | TBI-Related Symptoms | Rivermead Post Concussion Symptoms Questionnaire (RPQ)* | 3 min | 11 |
| | Post-traumatic stress | PTSD Checklist for DSM-5 (PCL-5)* | 3 min | 12 |
| | Suicide | Columbia Suicide Severity Rating Scale Screening Version[+](C-SSRS)* | 5 min | As needed |
| | Life Quality (Brain) | Quality of Life after Brain Injury Overall Scale (QoLIBRI-OS)* | 3 min | 7 |
| Symptom Validity | Symptom Validity | Structured Inventory of Malingered Symptomatology (SIMS)* | 10 min | 6 |
| Cognitive Performance | Episodic Memory | Rey Auditory Verbal Learning Test (RAVLT) | 15 min | 8 (delay after BSI-18) |
| | Executive Function | Trail Making Test | 5 min | 9 |
| | Processing Speed | Wechsler Adult Intelligence Scale – 4th Edition Processing Speed Index (WAIS-IV) PSI | 4 min | 10 |
| Motor | Motor | Finger tapping or grip strength | 5 min | 14 |
| | | Short physical performance battery (SPPB) | 5 min | 15 |

Table 3. Abbreviated Assessment (AA) Battery (2 W, 6 W, 3 M) 60-67 minutes

| Domain | Subdomain | Instrument | Administration Time | Order of Administration In person (~72 min) |
|---|---|---|---|---|
| Screening | Screening | Assessment of Speech Intelligibility* | 2 min | 1, and as needed |
| | | Galveston Orientation and Amnesia Test* | 5 min | 2, and as needed |
| Consciousness and Basic Cognition | Confusion | Confusion Assessment Protocol (CAP) | 15 min | Determined by Flexible Outcome Battery Flow Chart (page 18 of TRACK-TBI Outcomes SOP V 10) |
| | Consciousness | Coma Recovery Scale Revised (CRS-R) | 15-30 min | |
| Daily/Global Function | Global Outcomes | Glasgow Outcome Scale Extended (GOSE)* | 15 min# | 3 |
| | | Disability Rating Scale (DRS)* | 10 min# | 4 |

*Measures that should be collected over the phone

Table 4. Study Procedures

| Study Procedures | Screening/ Enrollment | <6 hr | 12 hr | 24 hr | Day 2 | Day 3 | Day 5 | Day 14 | Day 42 | Day 90 |
|---|---|---|---|---|---|---|---|---|---|---|
| Screening and Eligibility | X | | | | | | | | | |
| Inclusion/Exclusion criteria | X | | | | | | | | | |
| Informed consent | X | | | | | | | | | |
| Eligibility CT reading | X | | | | | | | | | |
| Blood collected | | X | X | X | X | X | X | X | X | X |
| MRI scan | | | | (X) | | | | X | | X |
| *Clinical Data Collection | X | | | | | | | | | |
| TRACK-TBI Outcome Enrollment Battery | | | | X | | | | | | |
| TRACK-TBI Follow –up Outcome Battery | | | | | | | | X | X | X |

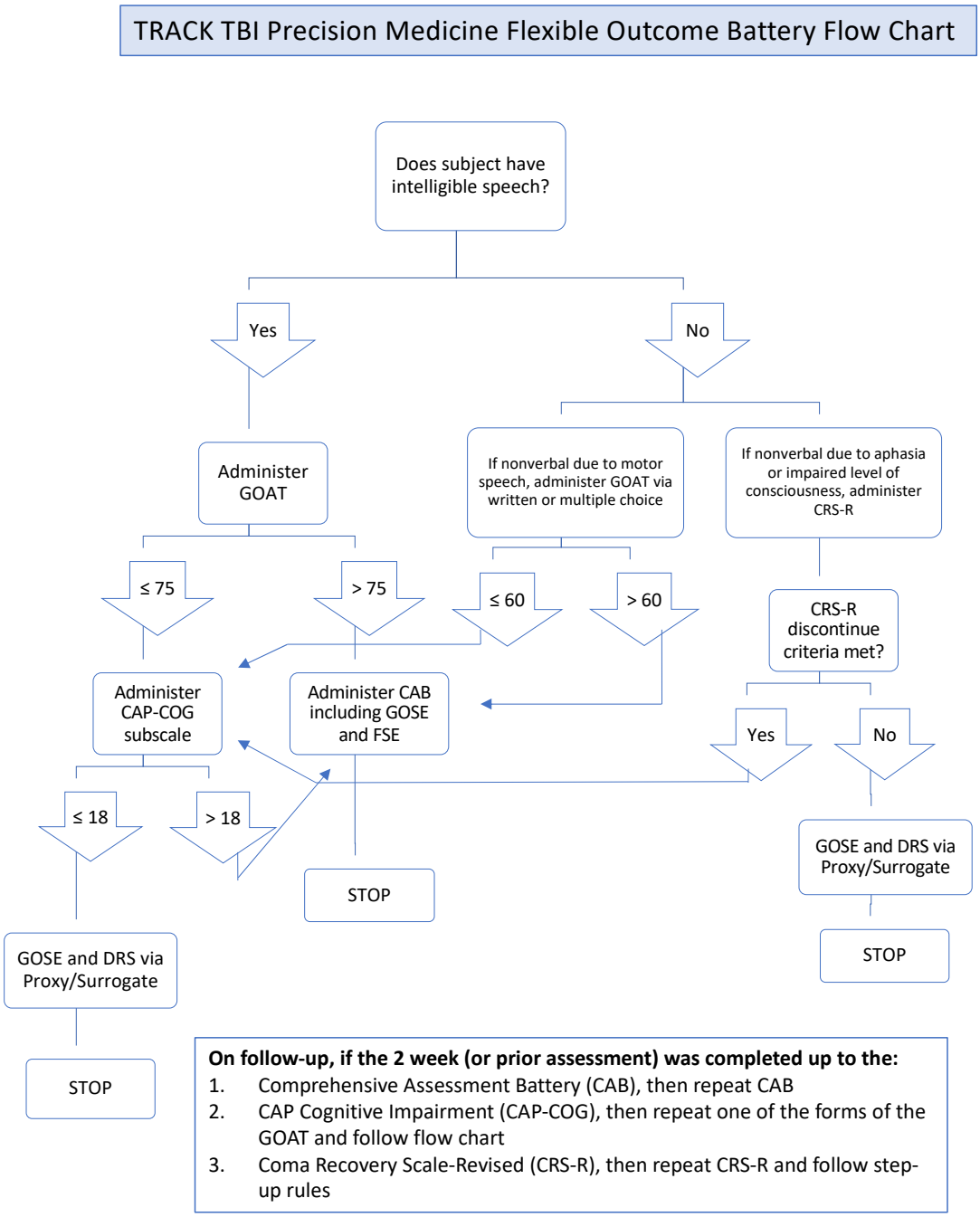
X = MRI will be collected on either Day 1 or Day 2
* Clinical Data will be collected daily until the subject is discharged from hospital

Biospecimen and Neuroimaging Standard Operating Procedures (SOPs) have been developed and finalized by Core leads. (Appendix 2 &3)

The Outcomes Core team reviewed and confirmed the 'TRACK TBI-II Flexible Outcome Battery Flow Chart' (Fig. 1) and description of new outcome measures; the Structured Inventory of Malingered Symptomatology™ (SIMS™), the Standardized Assessment of Concussion (SAC) and the short physical performance battery (SPPB), for the protocol. For testing motor function, the team decided to keep only 'Finger Tapping Test (FTT) and omit 'grip strength' test. The rationale being that we have SPPB measure (Short Physical Performance Battery). Drs. Temkin, McCrea, and Giacino had a teleconference on 6/26/2020 to discuss and finalize the participant interview. The participant interview designed for Precision Medicine is an adaptation of previous TRACK-TBI 3-month follow-up participant interview with the additions of a neurologic screen for Epilepsy as well as questions pertaining to the impact of the COVID-19 pandemic. The Covid-19 survey questions will assess the impact COVID-19 had or is having on the participant and/or someone close to the participant. Participant interview will be administered at 2-week, 6-week, and 3-month

follow up. Under consideration is the conversion of some aspects of the Outcome Battery to telephonic capture, in view of the ongoing pandemic. Our concern is to make the in-person visits as short and “low-touch” as is responsible and feasible. Measures that should be collected over the phone (as decided by the Outcomes core team) are marked by an asterisk in Table 2.

Fig. 1. Flexible Outcome Battery Flow Chart



Since the team has decided to keep participant enrollment to fifty (n=50), Dr. Sonia Jain revised the statistical analysis plan section of the protocol.

The Biostatistics Core played an active role in developing the statistical considerations section for the study's master protocol. The design was reviewed and discussions were conducted with the study team. The statistical considerations section was finalized at the end of Year 2.

With the finalization of the Clinical Protocol and supporting study Standard Operating Procedures for the TRACK-TBI Cores, the TRACK-TBI Administrative Core has submitted a finalized clinical protocol and informed consent form for Phase II of this study for pre-review to HRPO, which will be followed by the IRB submission process at UCSF. Upon local IRB approval, the other 4 study sites will be added to the IRB protocol with UCSF as the central IRB for this study using the SMART IRB platform. All sites will obtain formal HRPO approval prior to beginning enrollment.

**Phase II Protocol (1 of 1 total):**
Protocol [HRPO Initial Contact Log No.] E00485.2a
Title: TRACK-TBI Precision Medicine - Pathomechanistic Classification of Traumatic Brain Injury: The Bridge to Targeted Therapies
HRPO pre-approval submitted on 27 October for Phase II of this study.

## Biofluid Biomarker Accomplishments (09/30/2019 - 09/29/2020 ONGOING)

Over the course of Year 2, analyses were completed of 3,920 biospecimens for GFAP, UCHL1, S100, and NSE. This completes the entire TRACK-TBI cohort at all time points and now allows us to examine these biomarkers serially to define their natural history after TBI. We are now using this data to establish time points for biospecimen collection for the Phase II Option I Clinical Protocol. We have also analyzed the diagnostic and prognostic utility of these biomarker measured over time (see below).

We have also analyzed biospecimens for 425 of 550 TRACK-TBI Phase I MRI subjects for neurofilament light (NFL), Tau, IL6, IL10, and TNF. These series of biomarkers are expected to be informative of specific TBI endophenotypes such as diffuse axonal injury (DAI) and inflammation/neuroinflammation, with the goal of determining whether such biomarkers will be useful for incorporation into clinical trials of drugs targeting these specific injury mechanisms. These studies confirm the preliminary results from samples obtained during the TRACK-TBI Pilot Study, and due to the much larger sample size, provide the level of precision and rigor that is needed for incorporation into the design of clinical trials.

**Biomarkers of Diffuse Axonal Injury.** We have found that neurofilament light chain (NF-L) is elevated in patients with TBI, particularly those with trauma-related abnormalities in standard CT or MRI (**Figure 2a, b**). We also find, as others have reported in smaller studies, that NF-L levels increase over the first several days after TBI, a pattern which is quite distinct from GFAP, UCH-L1, and other widely studied TBI biomarkers (NSE, S100).

Fig. 2a. Biomarkers of diffuse axonal injury

Fig. 2a.

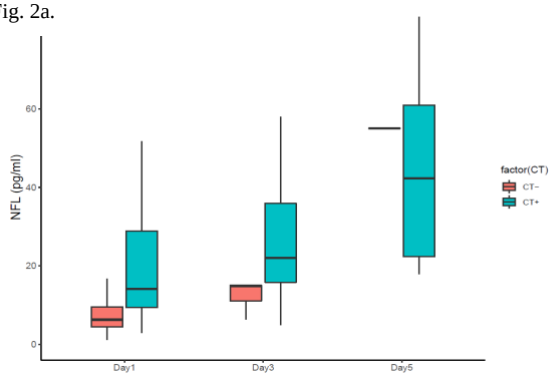
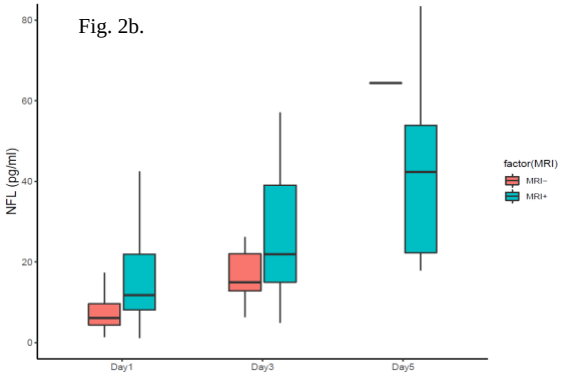



Fig. 2b.




Thus, it appears that NFL is measuring a distinct endophenotype, which we believe is diffuse axonal injury (DAI). This hypothesis will now be tested by correlating the NF-L elevations with MRI evidence of DAI noted on the 2-week MRI scans. Preliminary data shown in Figure 1 indicates that NF-L is elevated in subjects with DAI (MRI+) versus subject with no DAI (MRI-). If confirmed in the entire Phase I MRI cohort, these findings will show promise for NFL as a prognostic, predictive, and potentially pharmacodynamic biomarker of therapies targeting DAI, such as cyclosporine A (CsA). NFL is also potentially critical for informing the design of adaptive clinical trials aimed at optimizing the dose, timing, and duration of therapies aimed at DAI.

**Biomarkers of Neuroinflammation.** We have also confirmed our prior findings, based on data from the TRACK-TBI Pilot Study, that cytokines such as IL-10, IL-6, and TNF are elevated after TBI, in a pattern distinct from other brain injury biomarkers (GFAP, UCH-L1, and NfL). Unlike other TBI biomarkers, the relationship with trauma-related imaging abnormalities is weak, indicating that inflammation after TBI is orthogonal to macroscopically-visible lesions, and that it also reflects systemic inflammation. We expect that with the larger and more granular sample size available through these studies we will confirm our earlier findings that these inflammatory cytokines will provide prognostic information which will be orthogonal to the information obtained from neurodegenerative biomarkers (GFAP, tau) and will improve multivariate models of outcome prediction. We anticipate that these cytokines will be useful in early-phase clinical trials of anti-inflammatory therapies.

At the University of Florida analytic site, Dr. Wang continues to analyze emerging blood-based protein biomarkers for additional inflammatory targets including 10 cytokines, 10 proinflammatory cytokines, and 10 chemokines. Preliminary data for these analyses were presented in the previous Quarterly Report. Analyses have also been expanded for potential markers of microvascular injury (MVI). Several of these markers were presented in the previous report and now include an additional 7 angiogenesis markers and 4 new vascular injury markers.

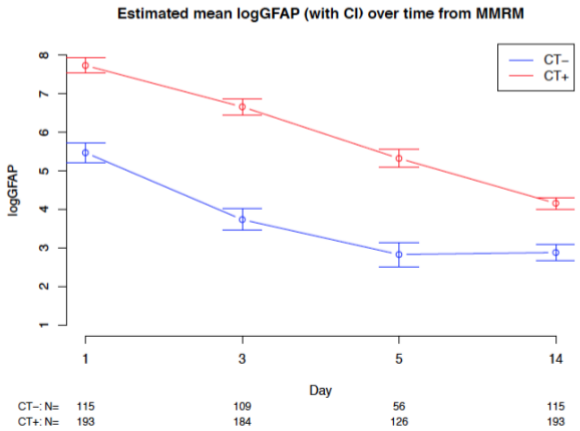
We have also obtained data for biomarkers of microvascular injury, using the MSD S-Plex array. These include fully validated marketed assays, as well as novel assays for which validation is under way. We provided biospecimens from 159 participants from the TRACK-TBI Pilot Study for analysis. We assayed Angiopoetin-1 (Ang-1), Angiopoetin-2 (Ang-2), basic fibroblast growth factor (bFGF), E-selectin, Flt-1, ICAM-1, ICAM-3 MCP-1, Platelet Derived Growth Factor Receptor beta (PDGFRb), PIGF, P-Selectin, Serum Amyloid A, Thrombomodulin, Tie-2, VACM-1, VEGF-C, VEGF-C, and von Willebrand Factor (vWF). Of

these, ANg-1, bFGF, ICAM-3, MCP-1, SAA, Tie-2, VEGF-C, and vWF robustly discriminated between TBI patients with abnormal CT scans from those with normal CT scans, suggesting that they are attractive candidates as biomarkers of TBI severity. Analyzing their relationship to outcome at 3 and 6 months after injury, we found that Ang-1, Ang-2, ICAM-1, SAA, Thrombomodulin, Tie-2 VCAM-1, and vWF discriminated between patients with favorable outcome from those with unfavorable outcomes.

**Diagnostic Utility of Serial Biomarkers**

Fig. 3. Two Week Serial Collection of GFAP

| | CTneg | CTpos | Contrast | S.E. | CI.lower | CI.upper | Pvalue |
|---|---|---|---|---|---|---|---|
| Day1 | 5.472 (5.209,5.735) | 7.737 (7.535,7.939) | 2.265 | 0.17 | 1.931 | 2.598 | <0.001 |
| Day3 | 3.734 (3.454,4.013) | 6.663 (6.448,6.878) | 2.929 | 0.18 | 2.575 | 3.283 | <0.001 |
| Day5 | 2.827 (2.513,3.142) | 5.323 (5.094,5.553) | 2.496 | 0.199 | 2.106 | 2.886 | <0.001 |
| Week2 | 2.879 (2.679,3.08) | 4.159 (4.005,4.313) | 1.28 | 0.13 | 1.026 | 1.534 | <0.001 |




Blood levels of glial fibrillary acidic protein (GFAP) collected within 12 hours of suspected traumatic brain injury (TBI) have been FDA approved to aid in determining the need for a brain CT scan in subjects within 12 hours of injury. We performed an analysis to determine whether sufficient evidence exists for expanding a biomarker context of use beyond 12h post-injury with *serial analyses* of these promising biomarkers (≤24h, 3-5 days and 2 weeks following a TBI) (Fig. 3). Cohorts were dichotomized by initial brain imaging results (CT+/CT-). Longitudinal models were performed to compare log-transformed biomarker levels between cohorts, adjusting for age and gender. 308 individuals were enrolled within 24h of injury, admitted to the hospital (91 ward and 217 ICU admission). TBI participants were adult (mean age 42±17.2), 72% male, and 82% Caucasian. Initial Glasgow Coma Scale (GCS) score was across the spectrum of injury (GCS 3-8, 18%; 9-12, 8%; and 13-15, 74%). Blood samples were analyzed for GFAP in a blinded fashion at: initial presentation (within 24h), acute hospitalization (days 3-5) and 2-week follow-up. As previously reported GFAP was diagnostic for TBI (CT+/CT-) at the initial timepoint (<24 hour) and maintained diagnostic discrimination at both 3-5 day timepoint and 2-weeks post injury ($p$< 0.001). We conclude that GFAP levels are useful for aiding in decision making regarding evaluation with head CT beyond 12h and up to 5 days following injury.

Progressing through Year 2, we further expanded the examination of a panel of neuroinflammation, vascular injury/modification, and additional neuroinjury biomarkers. This is in addition to GFAP, UCH-L1, NFL (and Tau) that we have previously assayed using the TRACK-TBI Pilot acute samples set (≤ 24 h post-injury).

The Meso Scale Diagnostics (MSD) 40-Plex Human Biomarker Kit was utilized at the University of Florida Program for Neurotrauma, Neuroproteomics & Biomarker Research analytic site led by Dr. Kevin Wang to conduct assays in multiplex panels:

- Proinflammatory
- Cytokine
- Chemokine
- Angiogenesis
- Vascular injury

Procedures were followed as described in these kits to quantitatively determine concentrations of analytes in human serum/plasma.

The first panel includes cytokine markers involved in the neuroinflammation process. Several proinflammatory markers were recently highlighted to be of potential interest in TBI research. Those of particular interest include IL-6, IL-1b, IL-10, IL-16, and IL-15 as these markers are elevated in TBI cases versus healthy controls based on our preliminary results previously reported in quarterly reports.

The second panel includes chemokine markers that are involved in both the neuroinflammation and vascular injury process. These markers are released during chemotaxis of immune cells to the brain vasculature system, potentially leading to macrophage and lymphocyte infiltration to the brain. Of interest are Eotaxin-3 and MCP-1, as these markers are elevated in TBI cases versus healthy controls based on our preliminary results previously reported in quarterly reports.

The third panel includes markers involved in the vascular injury process. These markers are released as a result of vascular damage and modification to the blood-brain barrier. Of interest are ICAM-1, VCAM-1, and C-Fibronectin as these markers are elevated in TBI cases versus healthy controls based on our preliminary results previously reported in quarterly reports.

The fourth panel includes markers involved in angiogenesis. These markers are released following injury, as blood vasculature is regenerated after the damaging injury. These markers include VEGF-A, bfGF, VEGF-C, and VEGF-D.

The fifth panel includes markers that highlight the post-TBI acute phase response. These markers are released by the liver in the acute phase of TBI. Notable markers are HMGB1 (Tecan assay), SAA, and C-RP.

The sixth panel is a set of known neurobiomarkers which includes a new marker pNF-H (Quanterix SIMOA assay) that we introduced. pNF-H and NF-likely appear in the setting of diffuse axonal injury. UCH-L1 and GFAP, which likely reflect on contusion injury type, are released as a result of acute brain cell (neurons and astroglia) injury or necrosis. The FDA has approved a TBI biomarker as an early diagnostic of pathoanatomic lesions among TBI patients with initial GCS 13-15.

We are interested to see if these additional biomarkers can aid in the prognosis of TBI patients.

Imaging and GOSE Characteristics of TRACK-TBI Pilot Subjects with plasma samples are presented in Table 5. Descriptions of the analyses follow Table 5.

Table 5. Imaging and GOSE Characteristics of TRACK-TBI Pilot Subjects with Plasma Samples

| **Admission Head CT** | | **N = 163** | |
|---|---|---|---|
| | No lesion (-) | 87 | (53.4%) |
| | Intracranial lesion only (+) | 70 | (42.9%) |
| | Extracranial lesion only (+) | 1 | (0.6%) |
| | Intracranial and extracranial lesion (+) | 5 | (3.1%) |
| **Outcome (6-month)** | | **N = 121** | |
| | GOSE = 1 | 8 | (6.6%) |
| | GOSE = 2 | 0 | (0.0%) |
| | GOSE = 3 | 6 | (4.9%) |
| | GOSE = 4 | 5 | (4.2%) |
| | GOSE = 5 | 15 | (12.4%) |
| | GOSE = 6 | 23 | (19.0%) |
| | GOSE = 7 | 35 | (28.9%) |
| | GOSE = 8 | 29 | (24.0%) |

## Correlation of acute post-injury plasma levels of several related markers

Fig. 4. Acute (<24h) post-TBI levels of two candidate vascular injury markers (ICAM-1, VCAM-1) display a significant linear correlation with each other (R2 = 0.3351, *)

Fig. 4. VCAM-1 and ICAM1 correlation in acute TBI samples

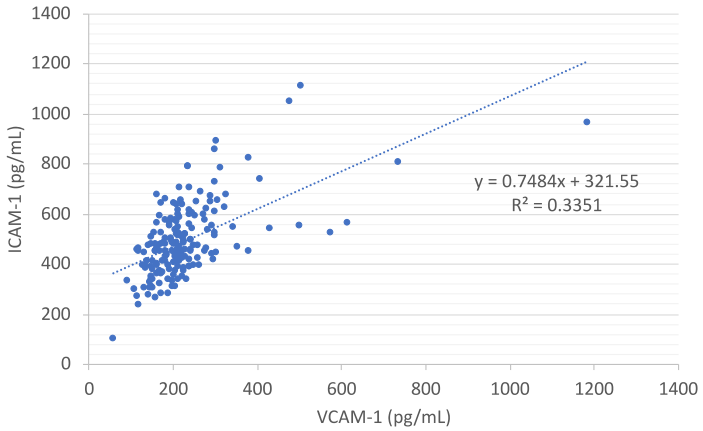
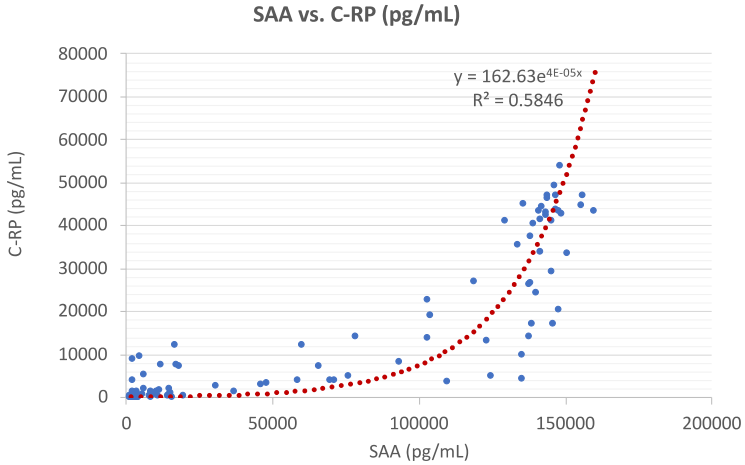



Fig. 5. Acute (<24h) post-TBI levels of two acute phase response proteins (serum amyloid-A /SAA and C-RP displays a significant non-linear correlation with each other (R2 = 0.585; **). These data suggest that acute phase response protein release into plasma seems to be a ubiquitous post-TBI systemic response.

Fig. 5. SAA vs. C-RP levels correlation with acute TBI samples

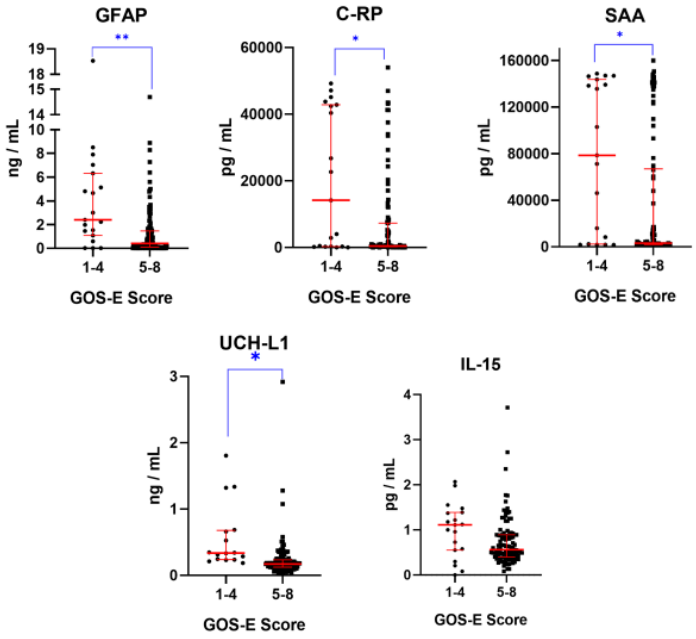



## Relationship of selected biomarkers to patient outcome (GOSE at 6 months)

GFAP, CRP, and SAA levels were found at significantly higher concentrations in those with unfavorable outcome (GOSE 1-4vsGOSE 5-8). IL-15 was found to distinguish full recovery (GOSE 8) versus incomplete recovery (GOSE<8) at a p-value of <0.05 but was not able to distinguish unfavorable outcome (GOSE 1-4vsGOSE 5-8). UCH-L1 was found to demonstrate an opposite effect.

Fig. 6 shows several biomarkers in differentiating GOSE scores 1-4 (unfavorable outcome) from GOSE. GFAP demonstrated statistical significance to $p < 0.01$, while C-RP, UCH-L1, and SAA demonstrated significance to $p < 0.05$. GFAP demonstrated statistical significance to $p < 0.01$, while C-RP, UCH-L1, and SAA demonstrated significance to $p < 0.05$.

Fig. 6. Individual biomarker function in predicting poor recovery (GOSE-E 1-4) vs. GOS-E 5-8)




On ROC curve analysis (Fig. 7.), 3 of the 5 biomarkers, each observed alone, were only modestly predictive (AUC < 0.70) of a poor recovery (GOSE 1-4). GFAP was adequately predictive of a poor recovery (AUC 0.74), while UCH-L1 alone demonstrated strong ability to predict poor outcomes (AUC 0.85).

Fig. 7. Receiver-Operator Curves for several biomarkers in predicting poor outcome

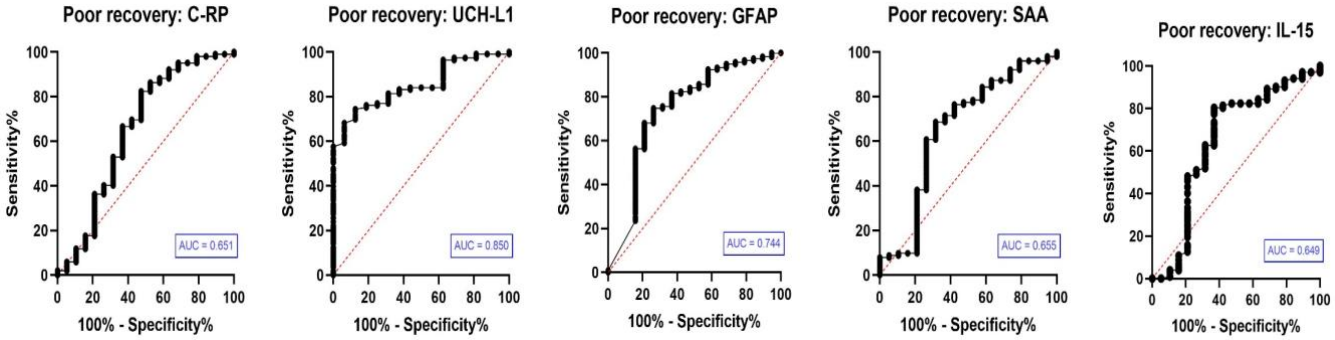


Fig. 8 Biomarkers differentiating GOS-E 7-8 (good outcomes) from GOS-E scores 1-6 (poor to moderate outcomes), GFAP, C-RP, SAA, and IL-15 demonstrated statistical significance with a p-value of < 0.01.

Fig. 8. Individual biomarker function in predicting good recovery (GOS-E 7-8) vs. GOS-E 1-6)

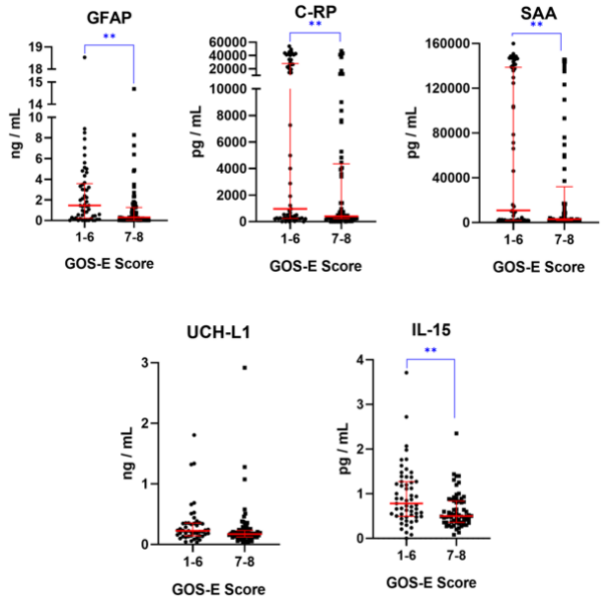
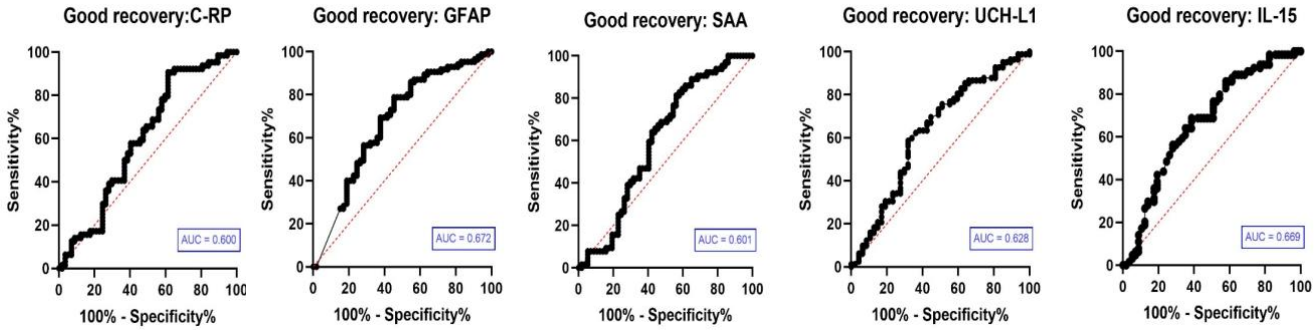



Fig. 9 shows the receiver-operating characteristics (ROC) curve of the selected biomarkers in predicting good patient outcome (GOSE 7-8) vs. those with moderate-poor outcome (GOSE 1-6). We found that none of the markers are good prognostic markers in that regard (with AUC < 0.7). Among the best are GFAP and IL-15.

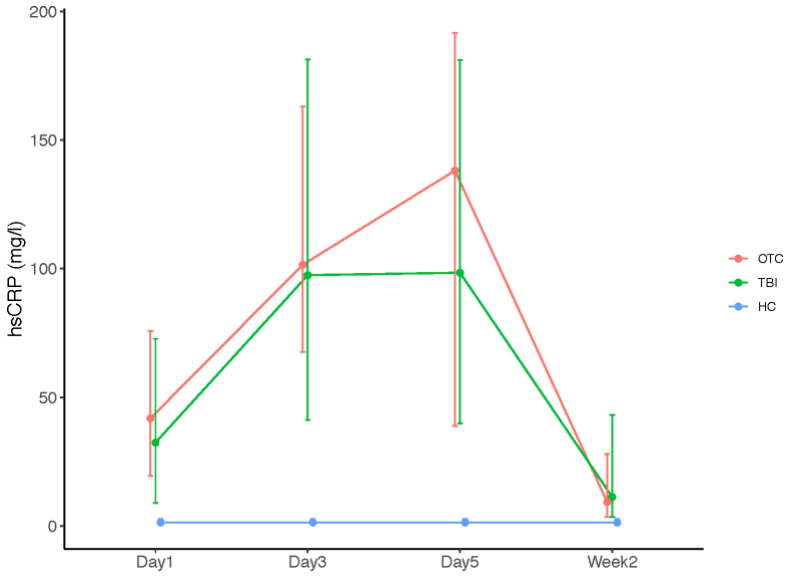
Fig. 9. Receiver-Operator Curves for individual biomarker performance in predicting good recovery (GOS-E 7-8) vs. (GOS-E 1-6)




Our data show that hsCRP rises in TBI and OTC and is increased in CT+ vs. CT- cases. Day 1 hsCRP was higher in TBI subjects compared with healthy controls (HC) (median [IQR]) (9.091 [2.11-30.932] vs. 1.34 [0.642-2.785] mg/L, p<0.0001). hsCRP values rose over the first 5 days in

both TBI and Orthopedic Trauma Controls (OTC). In those with serial samples (Day 1 and 2 Weeks, as well as Day 3 and/or Day 5), there was no significant difference in hsCRP between TBI and OTC at any time point (**Fig. 10.**), suggesting that OTC were well-matched to TBI subjects for systemic injury severity. Among patients with mild TBI (mTBI, GCS 13-15), a slight trend toward decreased hsCRP compared with OTC was observed at all time points, which did not reach significance.

Fig. 10. Receiver-Operator Curves for biomarkers alone in predicting good recovery (GOS-E 7-8) vs. (GOS-E 1-6)




In TBI patients with Day 1 samples, median hsCRP was higher in CT+ cases compared with CT- cases on Day 1 (20.415 [5.979-54.244] vs. 4.233 [1.327-17.479] mg/L, p<0.0001). Within CT- cases, Day 1 hsCRP was higher among MRI+ cases compared with MRI- cases (3.905 [1.84-16.219] vs. 2.94 [0.8-11.349] mg/L, p=0.0075). In those with serial samples, hsCRP remained significantly higher in CT+ vs. CT- cases at all time points, increasing from Day 1 to 5 in CT+ cases and plateauing between Days 3 and 5 in CT- cases.

As future clinical trials may require enrolling subjects within time windows shorter than 24 hours, we investigated hsCRP elevation by blood draw time intervals from 0-6h, 7-12h, 13-18h, and 19-25 hours postinjury. hsCRP increased temporally over the first 24 hours in both CT- and CT+ TBI subjects, and hsCRP was significantly higher in CT+ cases at all time points.

**CRP rises with increasing overall Injury Severity Score**

In subjects with recorded ISS and Day 1 samples, Day 1 hsCRP increased with ISS (**Figure 11a**), with similar findings seen in mTBI subjects. In subjects with serial samples, median hsCRP increased with ISS at all time points (**Fig. 11a,b**). In mTBI subjects with serial samples, hsCRP increased with ISS at all time points but reached significance only on Days 1, 3, and 5.

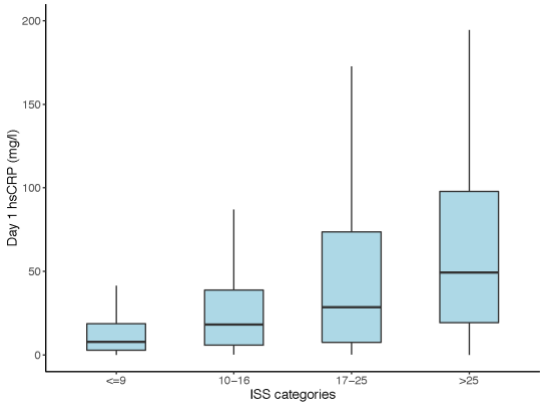



Fig. 11a.

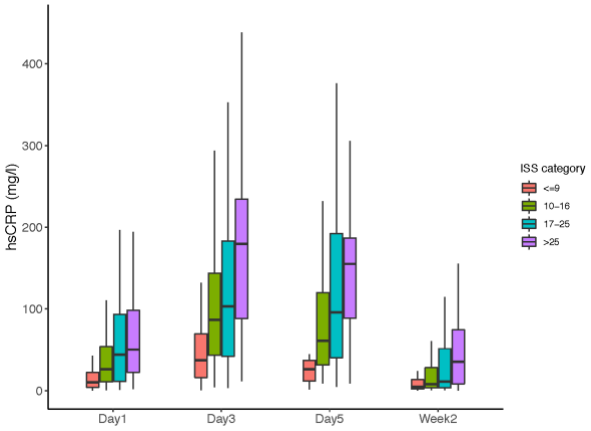



Fig. 11b.

**Fig. 11. Relationship between hsCRP and ISS at (A) Day 1 and (B) Day 1, 3, 5, and 2 Weeks**
Boxplots indicate median and 25$^{th}$-75$^{th}$ percentile (interquartile range, IQR) of hsCRP values. Upper whisker indicates the smaller value of: the maximum value or 75$^{th}$ percentile +1.5*IQR, and lower whisker indicates the larger value of: the minimum value or 25$^{th}$ percentile -1.5*IQR. ISS total score was separated into four score categories: ≤9, 10-16, 17-25, and >25. (A) Among all TBI patients with available Day 1 hsCRP samples, Day 1 hsCRP rises with increasing ISS total score. (B) Among patients with serial hsCRP samples (Day 1 and 2 Weeks, and Day 3 and/or Day 5), hsCRP rises with increasing ISS total score at all time points.

**CRP is a prognostic biomarker for predicting death/severe disability (GOSE <5) vs. moderate disability/good recovery (GOSE ≥5)**
In TBI patients with serial samples, hsCRP level at each of the 4 time points was significantly elevated in subjects with death/severe disability (GOSE <5) compared to those with moderate disability/good recovery (GOSE ≥5) (**Figure 12a**). The AUC of hsCRP for discriminating 6-month disability was highest at 2 weeks (AUC=0.892) The same analysis was performed in mTBI subjects and revealed similar findings with 2-week hsCRP (AUC=0.928).

Fig. 12a.

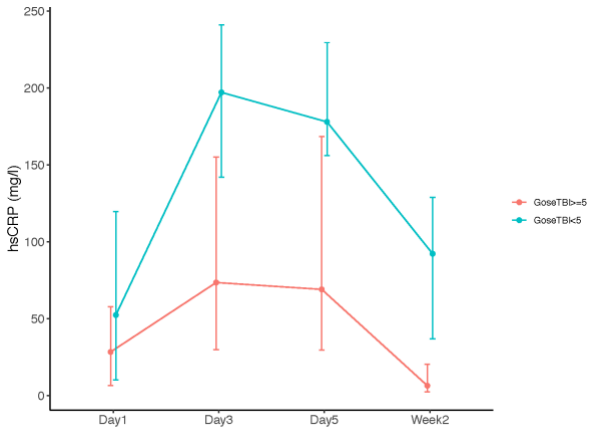



Fig. 12b.

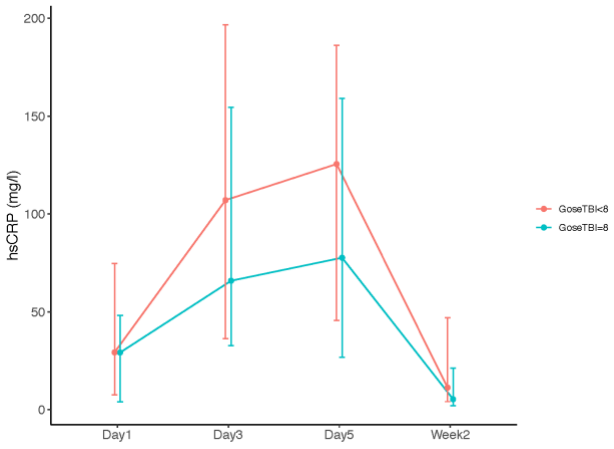



Fig. 12. hsCRP and Outcome after TBI: (A) GOSE ≥5 vs. <5 (B) GOSE =8 vs. <8
Line plots indicate median and 25$^{th}$-75$^{th}$ percentile. (A) In patients with serial hsCRP samples (Day 1 and 2 Weeks, and Day 3 and/or Day 5), hsCRP level was compared between patients with

unfavorable outcome (GOSE <5, indicating death/severe disability) and favorable outcome (GOSE ≥5). Patients with favorable outcome had significantly higher hsCRP level at all time points compared to patients with unfavorable outcome. (B) In patients with serial hsCRP samples, hsCRP level was compared between patients with complete recovery (GOSE =8) and incomplete recovery (GOSE <8). Patients with incomplete recovery had significantly higher hsCRP level at the 2-Week time point compared with patients with complete recovery, but were not significantly different at any other time point. Median hsCRP differed between subjects who experienced complete recovery (GOSE =8) from those with incomplete recovery (GOSE <8) at only the 2-Week time assessment (**Fig. 12b**). The AUC of hsCRP for discriminating complete recovery at 6 months increased with time after injury but was poor at all time points, similar to findings in the mTBI subjects with 2-Week hsCRP (AUC=0.547).

Notably, among TBI subjects, combining 2-week hsCRP (AUC=0.892) and 2-Week GFAP (AUC=0.890) improved discrimination of 6-month GOSE <5 vs. GOSE ≥5 to AUC=0.939 (95%CI: 0.900-0.978), higher than either marker individually (Table 6).

Table 6. Combined predictive performance of acute measurement of hsCRP and GFAP on GOSE (<5 vs ≥5) at 6 months following traumatic brain injury.

| | **AUC (95% CI)** |
|---|---|
| **Day 1** | |
| GFAP | 0.768 (0.662-0.875) |
| hsCRP | 0.640 (0.521-0.760) |
| GFAP + hsCRP | 0.765 (0.655-0.875) |
| **Day 3** | |
| GFAP | 0.873 (0.798-0.949) |
| hsCRP | 0.800 (0.729-0.871) |
| GFAP + hsCRP | 0.902 (0.846-0.959) |
| **Day 5** | |
| GFAP | 0.900 (0.827-0.972) |
| hsCRP | 0.777 (0.691-0.862) |
| GFAP + hsCRP | 0.911 (0.850-0.971) |
| **2-Week** | |
| GFAP | 0.890 (0.823-0.956) |
| hsCRP | 0.892 (0.839-0.944) |
| GFAP + hsCRP | 0.939 (0.900-0.978) |

**MSD 40-Plex and Additional Biomarker Analyses**
Dr. Wang has continued to analyze plasma samples with the MSD 40-plex assay that is tailored for inflammatory and vascular biomarkers that include chemokines, cytokines, proinflammatory cytokines, angiogenesis markers, and vascular Injury markers. He is also using dedicated assays for HMGB1 (Shinotest), pNF-H (Quanterix) and C-fibronectin (Enzo). A description of the biomarkers and their related endotype (e.g. neuroinflammation) are shown in the Table 9.

Table 7. TBI Precision Biomarkers

| Biomarkers | # markers screened / platform | Key markers | TBI Endotype | Mechanism of biomarker production / release |
|---|---|---|---|---|
| Inflammatory cytokines | 20 - MSD Quanterix 3-plex | **IL-1b,** IL-4, **IL-6,** IL-7, IL-8, **IL-10 IL-16, IL-17A,** IFN-γ [TNF-a,IL-1b, IL-6] | Neuroinflammation | Immune-cells released |
| Chemotactic cytokines | 9 / MSD | **MCP-1,** MCP4, **MIP-1b**, **MDC Exotaxin,** Exotaxin-3 | Neuroinflammation / Vascular injury | Chemotaxis of immune cells to brain vasculature |
| Acute-phase proteins / alarmin | 3 / MSD / Shinotest | **HMGB1/C-RP/ SAA** (Serum amyloid A) | Post-TBI acute phase response | Liver - released |
| Vascular Injury/ modulation Panel | 10 / MSD / Enzo | **VGEF-A, VGEF-C,** sFLT-1 **ICAM-1, VCAM-1,** C-Fibronectin | Vascular injury | Vascular damage and BBB modification |

Initial analyses have demonstrated an increase in inflammatory and vascular biomarkers as well as acute phase proteins following TBI. We are now examining the association of these biomarkers with CT and MRI abnormalities.

While additional results are pending, the team has spent time over the past quarter analyzing data, writing and submitting manuscripts for publication. These manuscripts include:

Xu L, et al, **"High-sensitivity C-Reactive Protein is a Prognostic Biomarker of 6-month Disability After Traumatic Brain Injury: Results from the TRACK-TBI Study."** In Press. Journal of Neurotrauma. Main results from this study suggest that serum high-sensitivity CRP measured within 2 weeks of TBI is a prognostic biomarker for disability 6 months later. hsCRP may have utility as a biomarker of target engagement for anti-inflammatory therapies.

Okonkwo D, et al, **"Point-of-Care Blood Biomarker Testing of GFAP versus S100B for prediction of traumatic brain injuries: a TRACK-TBI study."** Journal of Neurotrauma. 2020 Sep 14. Online ahead of print.

Wang K, et al **"Diagnostic performance of new point-of-care UCH-L1 assay in distinguishing CT- and MR-detected abnormalities in patients with traumatic brain injury: a TRACK-TBI prospective multicenter cohort study."** Under review. EBioMedicine

Korley F, et al **"Comparative Prognostic Performance of Brain Injury Biomarkers Measured During the First 2 Weeks Following Traumatic Brain Injury: A TRACK-TBI."** Under Review. Neurotrauma Reports

**Neuroimaging Biomarkers (09/30/2019 - 09/29/2020 ONGOING)**

The neuroimaging team has completed coding of Common Data Elements (CDE) findings on the TRACK-TBI 3 Tesla (3T) MRI scans, to identify microbleeds on 3D T2* weighted susceptibility imaging sequences that represent microvascular injury (MVI). Diffusion tensor imaging (DTI) and advanced diffusion analyses have also been completed to allow for assessment of DAI and neuroinflammation.

**Diffusion Tensor Imaging (DTI)**

Pre-processing and post-processing of the diffusion tensor imaging (DTI) sequences from the entire TRACK- TBI Phase 1 MRI TBI patient cohort (n=550 participants) that have both 2-week and 6-month post-injury scans, as well as a cohort of orthopedic trauma control patients that did not sustain TBI (n=83) who also underwent DTI at 2 weeks and 6 months after injury have been completed. The data have undergone statistical analysis to generate definitive results on how brain white matter microstructure evolves after TBI, which is considered to be the most sensitive standard imaging biomarker of diffuse axonal injury (DAI). A final analysis of 389 TBI (GCS 13-15) patients age $\geq$17 years from this cohort that passed quality control for DTI shows that global white matter fractional anisotropy (FA), the most commonly used measure of microstructural integrity, declined from 2-weeks to 6-months post-injury with a Cohen's d effect size of 0.17 ($p<0.001$). Regional white matter FA in many candidate tracts also showed longitudinal reductions in all tracts with effect sizes ranging from 0.10 to over 0.25 ($p<0.05$ for all tracts), with the greatest effects seen in the corpus callosum, internal capsule, corona radiata, thalamic radiations and association tracts such as the superior longitudinal fasciculus. In contradistinction, the orthopedic trauma control subjects did not show any decline in global white matter FA, or in individual white matter tracts, during that time interval. Instead, there was a non-significant trend toward increasing FA in the control participants.

This is the first definitive demonstration of long-term white matter degeneration due to ***DAI*** from TBI that is reproducible across 13 MRI scanners at 11 different patient enrollment sites, with standardized DTI acquisition, post processing and analysis procedures. This result provides clarity to the DTI of TBI literature, which has shown different and often conflicting results across many single-center studies using non-standardized methodology. We have also established that the major change in diffusivity from 2 weeks to 6 months after injury in global brain white matter, as well as in most white matter tracts, is a decline in axial diffusivity (AD). Elevations of radial diffusivity (RD) are also seen in certain tracts. This also resolves a current controversy in the DTI of TBI literature, in which cross-sectional studies of chronic mTBI patients compared to matched controls have suggested that the major change is in RD rather than AD. Further statistical analysis is ongoing to relate these DTI of TBI findings to the clinical, cognitive and behavioral outcomes of these patients.

In addition to completing the pre-processing and post-processing of the longitudinal DTI sequences from the entire TRACK- TBI Phase 1 MRI TBI patient cohort (n > 500 participants) that was completed early in year 2, the Imaging Core has also completed the same for the entire Phase 1 cohort of acute orthopedic trauma control patients (n > 100) that did not sustain TBI, who also underwent DTI at 2 weeks and 6 months after injury. The Core has also completed pre- and post-processing of 2-week and 6-month DTI of most of the Phase 1 demographically matched ("friend") control cohort (n > 100) that did not sustain acute injury of any sort.

The data have undergone statistical analysis to generate definitive results on how brain white matter microstructure evolves after TBI, which is considered to be the most sensitive standard imaging biomarker of diffuse axonal injury. Definitive results were generated on how microstructural white matter injury evolves over time from 2 weeks to 6 months after injury due to mild TBI, reproducible across 11 different medical centers, which is a first for the biomedical literature on DTI of TBI. The team now has definitive novel findings that microstructural white matter integrity on DTI at 2 weeks post-injury affects recovery at 6 months following mTBI, after accounting for the major demographic and clinical injury factors as well as for CT scan evidence of brain injury. These results are also reproducible across 11 different medical centers across the USA. Specifically, quantitative DTI white matter metrics at 2 weeks can help predict whether patients will achieve full recovery (GOSE score of 8) at 6 months post-mTBI. We are currently in the process of preparing two manuscripts reporting these results, a scientific report for the longitudinal microstructural white matter evolution of mTBI and a clinical report focused on the prediction of long-term mTBI outcome from semi-acute DTI assessment.

Pre-processing and post-processing of the longitudinal diffusion tensor imaging (DTI) sequences acquired at 2 weeks and at 6 months post-injury was completed from 43 additional mTBI patients that were added to the TRACK-TBI Phase 1 MRI TBI cohort during the current quarter, bringing the total size of the cohort to n=592 patients. In addition to the preprocessing and postprocessing of longitudinal DTI from the entire Phase 1 MRI cohort of acute orthopedic trauma control patients (n > 100) that did not sustain TBI that was completed in the past quarter, during this quarter the Core has also completed pre- and post-processing of 2-week and 6-month DTI of the entire Phase 1 demographically matched ("friend") control cohort (n > 100) that did not sustain acute injury of any sort.

With the final serial DTI data from all complete Phase 1 MRI cohorts now in hand, the definitive statistical analysis is in process that will represent the largest DTI study of TBI to date and the first to produce reproducible cross-sectional and longitudinal findings over all 11 of the patient enrollment sites across the USA. We aim to submit a manuscript describing the results prior to the end of the July-September 2020 quarter for publication in a peer-reviewed journal.

The definitive statistical analysis has been completed for the largest DTI study of mild TBI (mTBI) to date and the first to produce reproducible cross-sectional and longitudinal findings over all 11 of the initial TRACK-TBI patient enrollment sites across the USA. This Phase 1 TRACK-TBI cohort, after exclusions based on age range, data quality and other factors, includes approximately ~400 mTBI patients as well as ~100 orthopedic trauma controls and ~100 demographically matched "friend" controls, both at 2 week and 6 month time point post-injury. The results show robust and statistically significant alterations of white matter microstructure in the mTBI group versus both control groups at 2 weeks post-mTBI, some of which persist at 6 months post-mTBI. We also find statistically significant changes of white matter microstructure in the mTBI group longitudinally across the 2 week and 6 month time points, reflective of time-varying pathology such as long-term

axonal degeneration. These DTI findings are all reproducible across the 11 sites of the Phase 1 TRACK-TBI study. These results are presently being correlated to demographic factors, injury severity as well as symptomatic, clinical and cognitive outcome variables to investigate DTI as a robust prognostic biomarker for mTBI in a large multicenter study.

**Advanced Diffusion Imaging**

Advanced diffusion MRI processing using the Neurite Orientation Dispersion and Density Imaging (NODDI) has been completed for the 2-week and 6-month scans of a pilot group of n=40 Phase 1 mild TBI patients, showing evidence of white matter vasogenic edema suggesting ***neuroinflammation*** that evolves over time. The results also show loss of white matter axonal density over time that is more sensitive than DTI metrics and thought to represent the sequela of ***DAI.*** These preliminary results have been published in bioRxiv and the definitive manuscript is currently under review for publication in a peer-reviewed journal.

Advanced diffusion MRI processing using the Neurite Orientation Dispersion and Density Imaging (NODDI) has been completed for the 2-week and 6-month scans of a pilot group of n=40 Phase 1 mild TBI patients, showing evidence of white matter vasogenic edema suggesting neuroinflammation that evolves over time. The results also show loss of white matter axonal density over time that is more sensitive than DTI metrics and is thought to represent the sequela of DAI***.*** These preliminary results have been published in bioRxiv and the definitive manuscript has been revised and resubmitted for publication in a high-impact peer-reviewed journal after receiving a very favorable initial round of reviews.

During the current quarter, additional analyses were performed and revisions were made to peer review comments on our report of Neurite Orientation Dispersion and Density Imaging (NODDI) has been completed of the 2-week and 6-month scans of a pilot group of n=40 Phase 1 mild TBI patients and a replication group n=40 Phase 1 mild TBI patients, compared to the orthopedic trauma controls and friend controls. These showed evidence of white matter vasogenic edema, suggesting neuroinflammation that evolves over time. These novel results also show loss of white matter axonal density over time that is more sensitive than DTI metrics and is thought to represent the sequela of DAI***.*** These results have now been accepted for publication and the paper is currently in press at the high-impact journal *Science Advances.*

Our report of Neurite Orientation Dispersion and Density Imaging (NODDI) of the 2-week and 6-month scans of a single-center pilot group of n=40 Phase 1 mild TBI patients and a single-center replication group n=40 Phase 2 (“HDFT”) mild TBI patients, compared to the orthopedic trauma controls and friend controls, was published in the high-impact journal *Science Advances.* These novel results demonstrated early white matter vasogenic edema, suggesting neuroinflammation, that evolves over time. These findings also showed loss of white matter axonal density over time that is more sensitive than DTI metrics and is thought to represent the sequela of DAI***.***

**Structural Imaging**

A manuscript has been prepared that reports the prognostic significance of abnormal findings on early brain imaging after traumatic brain injury (TBI), on one of the largest TBI study populations with granular long-term outcome measures to one year. This manuscript is based on 755 patients presenting to 11 U.S. Level 1 trauma centers for suspicion of acute TBI, and who underwent both day-of-injury head computed tomography (CT) and brain MRI at 2 weeks postinjury, with high follow-up rates (91% at Week 2, 83% at Month 3, 80% at Month 6, and 74% at Month 12). Three

U.S. board-certified neuroradiologists evaluated 2-week brain MRI exams for structural abnormalities using the NIH TBI Imaging Common Data Elements (CDE). We applied multivariable ordinal logistic regression to determine the relevance of the brain MRI findings to global functional outcome, as measured by the Glasgow Outcome Scale – Extended (GOS-E) at 2 weeks, 3 months, 6 months and 1 year. Brain contusion on MRI was a statistically significant predictor of GOSE at 2 weeks and at 3 months, even after controlling for intracranial injury on CT, age, GCS, gender, years of education, psychiatric history, and history of prior TBI. Both brain contusion and presence of ≥4 microhemorrhages on MRI were statistically significant predictors of lower-moderate or worse disability (GOSE ≤5) at 1 year, even after controlling for intracranial injury on CT, age, GCS, gender, years of education, psychiatric history, and history of prior TBI. This confirms the utility of MRI as a diagnostic and prognostic imaging biomarker for clinical trials.

In the prior quarter, the Imaging Core completed longitudinal Freesurfer 6.0 analysis of global and regional brain volume changes from 2 weeks to 6 months after injury in the 3D T1-weighted structural sequence of the TRACK-TBI Phase 1 MRI TBI cohort (n > 500 patients) and this has undergone statistical analysis to generate definitive results on how brain volume changes after TBI. In this quarter, the team has completed longitudinal Freesurfer 6.0 analysis of global and regional brain volume changes from 2 weeks to 6 months after injury in the 3D T1-weighted structural sequence of the TRACK-TBI Phase 1 MRI cohort of acute orthopedic injury control subjects who did not sustain acute TBI (n > 100 patients).

Also, in this quarter, the team has completed statistical analysis of the 2-week Phase 1 MRI volumetric data that shows that regional brain volumes at this semi-acute stage are associated with symptomatic outcome at 6-months after mTBI as measured by the Rivermead Post Concussion Symptoms Questionnaire, reproducible across 11 medical centers. Two of these brain volume measures, those of the insula and posterior corpus callosum, were predictive of long-term symptomatic outcome even after controlling for the major demographic and clinical injury severity variables as well as CT scan evidence of brain injury. This novel finding has been favorably reviewed for publication in a high-impact peer-reviewed journal and is currently being revised for resubmission. Additional active analyses are being performed to evaluate the effect of longitudinal volumetric changes of MRI from 2-weeks to 6-months after injury on recovery from TBI, and its association with imaging findings on conventional CT and MRI.

**Structural MRI**

Freesurfer 6.0 global and regional brain volumetric analysis of the longitudinal 3D structural T1-weighted sequences acquired at 2 weeks and at 6 months post-injury is in process from 43 additional mTBI patients that were added to the TRACK-TBI Phase 1 MRI TBI cohort during the current quarter, bringing the total size of the cohort to n=592 patients. To our knowledge, this represents the largest serial volumetric analysis of TBI patients ever conducted to date. In addition to the Freesurfer analysis of serial 3D T1-weighted imaging from the entire Phase 1 MRI cohort of acute orthopedic trauma control patients (n > 100) that did not sustain TBI that was completed in the past quarter, during this quarter the Core is also completing Freesurfer volumetric analysis of 2-week and 6-month 3D T1-weihted imaging of the entire Phase 1 demographically matched (“friend”) control cohort (n > 100) that did not sustain acute injury of any sort.

These additional data are being added to revisions requested by peer reviewers to our original manuscript submission reporting global and regional brain volume changes that are predictive of long-term symptomatic outcome (“persistent post-concussive syndrome”) after mTBI that are independent of demographic, clinical and conventional CT and MR factors.

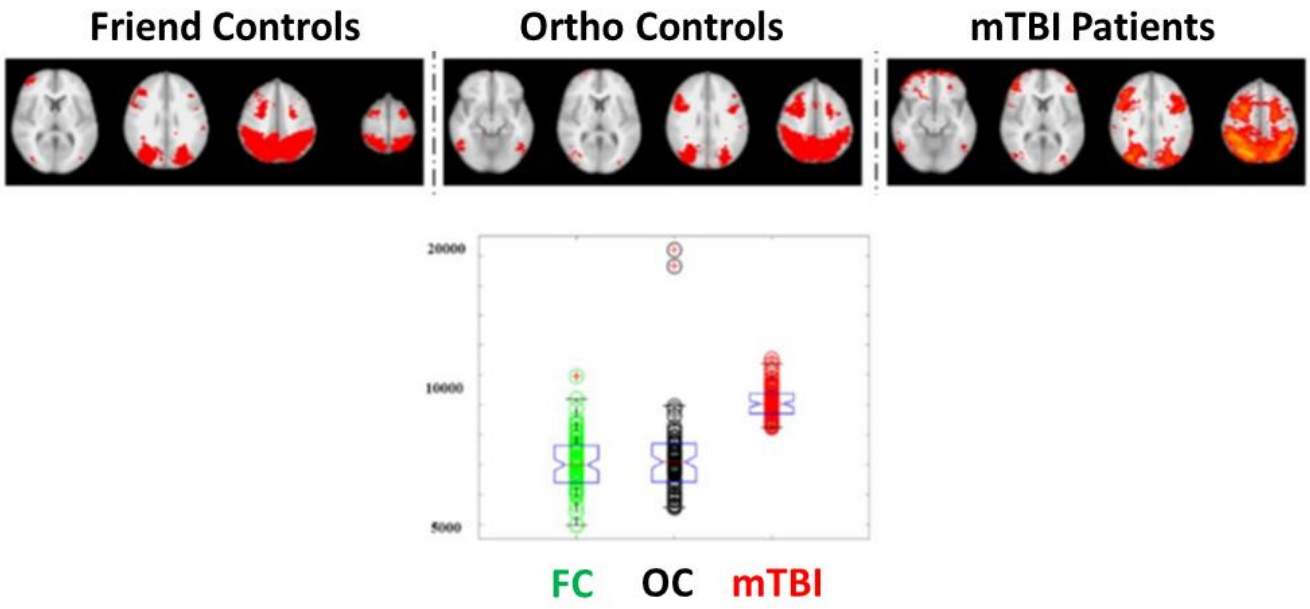
We have completed the Freesurfer 6.0 global and regional brain volumetric analysis of the longitudinal 3D structural T1-weighted sequences acquired at 2 weeks and at 6 months post-injury for the TRACK-TBI Phase 1 MRI cohort (~500 patients) during the current quarter. We have also completed longitudinal Freesurfer 6.0 analysis of orthopedic trauma controls (~100 subjects) for the Phase 1 MRI cohort. We are now conducting Freesurfer processing of the 3T MRI scans of the ~300 friend control subjects who are demographically matched to the Phase 1 MRI cohort of TBI patients. To our knowledge, this represents the largest serial volumetric analysis of TBI patients and controls ever conducted to date. The initial statistical analysis shows a small but statistically significant reduction of global and selected regional brain volumes between the 2 week and 6 month time points, which is indicative of a macrostructural effect of brain injury even in this predominantly mild TBI cohort.

**Functional MRI (fMRI)**

In Year 2, we applied a novel and very powerful new deep learning-based analysis of resting state fMRI that we have developed known as deep matrix fitting (DMF). A key advantage of DMF compared with other unsupervised fMRI analysis techniques, including traditional ones such as independent component analysis (ICA) or newer methods such as sparse dictionary learning (SDL) or deep belief networks, is a lesser constraint from spatial independence, which means that DMF can easily identify extensively overlapped functional brain regions that traditional techniques cannot. DMF is purely data-driven and can automatically leverage dictionary size, and number of layers, without requiring any manual parameter tuning to decompose the fMRI signal matrix. Finally, DMF is a linear deep model and is therefore highly efficient computationally and can be run solely on central processing units (CPUs) without requiring the specialized graphics processing units (GPUs) or tensor processing units (TPUs) required by traditional deep learning methods. Figure 1 shows an example of DMF analysis of resting state fMRI data collected across 5 different TRACK-TBI sites employing a mix of 3T GE, Siemens and Philips MR scanners. The observed effect size of the increased functional connectivity of the DAN is greater than that previously reported using traditional ICA of fMRI, despite the additional variation introduced by inter-site MR scanner hardware and software differences. These results represent the first alterations in human brain functional connectivity networks due to mTBI that are shown to be robustly reproducible across centers. A manuscript reporting these exciting initial results is currently in preparation for submission to a high-impact peer-reviewed scientific journal.

We have continued to apply a novel and very powerful new deep learning based analysis of resting state fMRI that we have developed known as deep matrix fitting (Deep MF). A key advantage of Deep MF compared with other unsupervised fMRI analysis techniques, including traditional ones such as independent component analysis (ICA) or newer methods such as sparse dictionary learning (SDL) or deep belief networks, is a lesser constraint from spatial independence, which means that DMF can easily identify extensively overlapped functional brain regions that traditional techniques cannot. DMF is purely data-driven and can automatically leverage dictionary size, and number of layers, without requiring any manual parameter tuning to decompose the fMRI signal matrix. Finally, DMF is a linear deep model and is therefore highly efficient computationally and can be run solely on central processing units (CPUs) without requiring the specialized graphics processing units (GPUs) or tensor processing units (TPUs) required by traditional deep learning methods. This fMRI analysis has been conducted on mTBI patients, orthopedic trauma controls and friend controls of both Phase 1 and Phase 2 TRACK-TBI MRI cohorts. A research manuscript is currently under preparation reporting this innovative new fMRI analysis method and its initial application to mTBI patients vs control subjects.

Fig. 13. Deep Matrix Fitting of Resting State fMRI Reveals Elevated Functional Connectivity of the Dorsal Attention Network 2 Weeks after mTBI (p<0.00001 for mTBI vs FC or OC). FC: Friend Controls (n=84); OC: Orthopedic Trauma Controls (n=70); mTBI: Mild TBI Patients (n=100).




**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

Nothing to report

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

**Dr. Wang** gave multiple presentations to the scientific community that served to disseminate results of this biofluid biomarker work:

Invited Speaker**:** *Leveraging TBI biomarker development experience for developing biomarkers for TBI – induced visual impairment.* VA Field Based Biomarkers meeting on TBI. (Virtual) June 24-26, 2020

Keynote Speaker: *Blood-based brain injury biomarkers as theranostic tool to accelerate drug development*. Markets-and-Markets Virtual Conference: Liquid Biopsy. May 20-21, 2020.

Invited Speaker: *TBI biomarkers: Past, Present, and Future*. Markets-and-Markets Research Companion Biomarker Conference. San Diego, CA, February 7, 2020.

**Dr. Diaz-Arrastia** gave multiple presentations to the scientific community that served to disseminate results of this biofluid biomarker work:

Invited Speaker: *Endophenotypes of traumatic brain injury: What we need to know for the next generation of clinical trials.* Broward Health Medical Center, Ft. Lauderdale, FL. February 6, 2020.

Invited Speaker: *Imaging and Molecular Biomarkers: Measuring TBI endophenotypes*. National Association of Brain Injury Specialists, New Orleans, LA. February 26, 2020

Invited Speaker: *Dementia after Traumatic Brain Injury: What is the Pathology.* National Association of Brain Injury Specialists, New Orleans, LA. February 27, 2020.

Invited Speaker: *Endophenotypes of traumatic brain injury: What we need to know for the next generation of clinical trials.* Institute for Neuroscience, University of Texas Rio Grande Valley. Harlingen, TX. Via Zoom. October 6, 2020.

**Dr. Manley** gave multiple presentations to the neurotrauma community that served to disseminate the goals of TRACK-TBI Precision Medicine:

Invited Speaker: *TRACK-TBI: Early Deliverables and Future Directions. ;* Joyce Massey Traumatic Brain Injury Summit, Ann Arbor, Michigan, October 18, 2019

Keynote Speaker: *Transforming Scientific Knowledge in TBI into Meaningful Clinical Practice* National Association of Brain Injury Specialists. New Orleans, LA. February 27, 2020.

Invited Panelist: *Advances in Rapid Detection of Traumatic Brain Injury.* General Dynamics Information Technology in Support of the Defense and Veterans Brain Injury Center. 2020.

**What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period, the TRACK-TBI Precision Medicine Phase II protocol will be submitted to HRPO for pre-review, followed by submission through the local IRB process at UCSF after incorporating any feedback from HRPO following the pre-review process. Upon IRB approval, the other 4 study sites sign on to the IRB protocol with UCSF as the central IRB for this study using the SMART IRB platform. All sites will submit to HRPO for formal approval prior to beginning enrollment.

In parallel with the IRB and HRPO approval process, the TRACK-TBI Administrative Core will oversee the creation and implementation of the electronic data capture (EDC) system that will be used for this study. The Admin Core will work directly with QuesGen Systems Inc., the same company who has created the databases used for TRACK-TBI studies over the last 7+ years, to create the EDC for this study. The TRACK-TBI Precision Medicine EDC will leverage the previous TRACK-TBI databases to build on the current infrastructure. The EDC and accompanying case report forms (CRFs) for this study will be finalized by the time IRB and HRPO approval are obtained by the sites. Enrollment into Phase II of the study will be able to begin as soon as all approvals are in place.

The Blood-based/Biofluid Biomarker team plans to complete the following analyses:

- A subset of TRACK-TBI (U01) study TBI patients with different injury severity, orthopedic injury controls and friend controls that we have acute and 2 week samples for will be selected as further verification of the following markers selected from our Phase I study:

- **Acute brain injury**: UCHL1/GFAP
- **Neurodegeneration**: Tau, P-Tau
- **Delayed axonal injury**: NF-L, neuroinflammation: e.g. IL-6, IL-10, IL-15, IL-16, MCP-1, MCP-4
- **Acute phase markers:** C-RP, SAA, HMGB-1
- **Vascular injury**: e.g. CAM-1, VCAM-1, c-fibronectin, VEGF-A.

- This dataset will allow us to then conduct validation studies with the TRACK TBI-II patient cohort with longitudinal samples as stated above.

The Neuroimaging team plans to complete the following analyses in order to achieve the stated goals of the project: (Revise)

- Correlate DTI findings to demographic factors, injury severity as well as symptomatic, clinical and cognitive outcome variables to investigate DTI as a robust prognostic biomarker for mTBI
- Extend the NODDI analysis to the remainder of the Phase 2 HDFT MRI patients across all participating TRACK-TBI centers in order to validate these single-center results in a large multicenter study
- Perform additional multivariate statistical analyses to account for the effects of demographic factors and injury severity on these global and regional volumetric measurements, as well as their correlation with symptomatic, clinical and cognitive outcome variables
- Perform an independent replication of the fMRI results on a validation cohort derived from the Phase 1 and Phase 2 TRACK-TBI MRI cohorts.

Additionally, the Neuroimaging team intends to submit the following manuscripts:

- Longitudinal Freesurfer volumes in TBI
- Inter-rater reliability of 3T MRI CDEs
- Inter-rater reliability of CT CDEs
- Longitudinal DTI changes of white matter microstructure in mTBI

## *4.* IMPACT:

**What was the impact on the development of the principal discipline(s) of the project?**

We intend to deliver on critical military and public health knowledge gaps and needs: objective classification of TBI based on what we term mechanistic endophenotypes, e.g., diffuse axonal injury (DAI), microvascular injury (MVI), and neuroinflammation. An endophenotype is an internal phenotype discoverable by biochemical, physiological, radiological, pathological, or other techniques. Endophenotypes are quantitative, continuous variables, unlike a phenotype which is usually a binary, categorical variable. These mechanistic endophenotypes, defined by imaging and blood-based biomarkers, will direct targeted treatments based on mechanism, providing the tools needed for successful execution of precision medicine clinical trials. To achieve the goal of

precision medicine in TBI, it is necessary to identify subgroups of TBI patients that will respond to a targeted therapy. To achieve this, our proposed approach is the combined use of MR neuroimaging sequences and biofluid biomarker panels to aid in the identification and monitoring of major TBI subphenotypes.

**What was the impact on other disciplines?**

*NOTHING TO REPORT*

**What was the impact on technology transfer?**

*NOTHING TO REPORT*

**What was the impact on society beyond science and technology?**

*NOTHING TO REPORT*

## 5. CHANGES/PROBLEMS:

*NOTHING TO REPORT*

**Changes in approach and reasons for change**

**Actual or anticipated problems or delays and actions or plans to resolve them**

We have tried to mediate and mitigate any anticipated issues with enrollment related to the continuing COVID-19 pandemic, and currently all sites are permitted to enroll. Of course, this is a dynamic situation, and we have designed our protocol to capture all required data as efficiently and in as “low touch” a way as possible, notwithstanding that our blood-based biomarker and neuroimaging collection require in-person recall of participants.

**Changes that had a significant impact on expenditures**

*NOTHING TO REPORT*

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

**Significant changes in use or care of human subjects**
N/A

**Significant changes in use or care of vertebrate animals**
N/A

**Significant changes in use of biohazards and/or select agents**
N/A

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

  **Journal publications.**
  Palacios EM, Owen J, Yuh EL, Wang M, Vassar MJ, Ferguson AR, Diaz-Arrastia R, Giacino J, Okonkwo DO, Robertson CS, Stein MB, Temkin NR, Jain S, McCrea M, Mac Donald C, Levin HS, Manley GT, Mukherjee P. **The evolution of white matter changes after mild traumatic brain injury: A DTI and NODDI study.** Sci Adv. 2020 Aug 7;6(32) 2020 Aug.
  Okonkwo DO, Puffer RC, Puccio AM, Yuh EL, Yue JK, Diaz-Arrastia R, Korley FK, Wang KW, Sun X, Taylor SR, Mukherjee P, Markowitz AJ, Jain S, Manley GT and the TRACK-TBI Investigators. **Point-of-Care Blood Biomarker Testing of GFAP versus S100B for prediction of traumatic brain injuries: a TRACK-TBI study**. Journal of Neurotrauma. 2020 Sep 14. Online ahead of print.
  Xu L, Yue JK, Korley F, Puccio AM, Yuh EL, Sun X, Rabinowitz M, Vassar M, Taylor SR, Winkler EA, Puffer RC, Deng H, McCrea M, Stein MB, Robertson C, Levin H, Dikmen S, Temkin N, Giacino JT, Mukherjee P, Wang K, Okonkwo DO, Markowitz A, Jain S, Manley GT, Diaz-Arrastia R, and the TRACK-TBI Investigators. **High-sensitivity C-Reactive Protein is a Prognostic Biomarker of 6-month Disability After Traumatic Brain Injury: Results from the TRACK-TBI Study**. In Press. Journal of Neurotrauma.

- **Books or other non-periodical, one-time publications.**
  *NOTHING TO REPORT*

**Other publications, conference papers and presentations**.

N/A

- **Website(s) or other Internet site(s)**

A dedicated page to TRACK-TBI Precision Medicine is located on the main TRACK-TBI NET Website: https://tracktbinet.ucsf.edu/precision-medicine

- **Technologies or techniques**

N/A

- **Inventions, patent applications, and/or licenses**

N/A

- **Other Products**

N/A

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### What individuals have worked on the project?

| Personnel Name | Institution | Role | Total Calendar Month Worked 9/30/19-9/29/20 | Contribution |
|---|---|---|---|---|
| Manley, Geoffrey | UCSF | Principal Investigator | 1.20 | Contact PI |
| Mukherjee, Pratik | UCSF | Co-Investigator | 0.36 | Dr. Mukherjee oversees all aspects of the neuroimaging for the Specific Aims. |
| Yuh, Esther | UCSF | Co-Investigator | 0.60 | Dr. Yuh provides imaging biomarker expertise |
| Palacios, Eval | UCSF | Specialist | 3.24 | Eva Palacios performs the advanced neuroimaging analyses in support of accomplishing the Specific Aims and reports to Dr. Mukherjee. |
| Vu, An Thanh "Joseph" | UCSF | MR physicist | 1.20 | Dr. Vu supervises MRI site qualification and QA monitoring |
| Wren-Jarvis, Jamie | UCSF | Staff Research Associate | 2.76 | Jamie performs structural MR image pre-processing, post-processing and analysis |
| Kurlander, Danielle | UCSF | Staff Research Associate | 3.48 | Danielle performs structural MR image pre-processing, post-processing and analysis |
| Nguyen, NhuNhu | UCSF | Clinical Resrearch Coordinator | 5.52 | NhuNhu Nguyen coordinates the work of the other Clinical Research Coordinators (CRCs) and Staff Research Associates (SRAs) for accomplishing the neuroimaging aspects of the Specific Aims and reports to Dr. Mukherjee. |
| Bourla, Ioanna | UCSF | Clinical Resrearch Coordinator | 3.24 | Ioanna Bourla performs data and metadata cleaning, curation and other forms of data management for the neuroimaging analyses. |
| Zargham, Sina | UCSF | Staff Research Associate | 11.40 | Sina Zargham performs data and metadata entry for the neuroimaging analyses. |
| Wang, Kevin | UF | Co-Investigator | 1.56 | Project coordination (internal, external with UCSF overall PI Dr. G. Manley and other project members), data interpretation |
| Yang, Zhihui | UF | Co-Investigator | 0.67 | Biomarker assay, data graphing, presentation, interpretation, and report preparation |

| | | | | |
|---|---|---|---|---|
| McCrea, Michael | MCW | Co-Investigator | 1.04 | Provide clinical outcomes expertise |
| Okonkwo, David | UPMC | Co-Investigator | 0.60 | Dr. Okonkwo provides clinical trial expertise |
| Puccio, Ava | UPMC | Co-Investigator | 0.60 | Dr. Puccio provides biospecimen collection coordination |
| Sharpless, Jane | UPMC | Regulatory Coordinator | 0.15 | Ms. Sharpless provides regulatory consultation |
| Billigen, Julia | UPMC | Technician | 0.62 | Ms. Billigen provides programmatic support |
| McIntyre, Peyton | UPMC | Technician | 2.25 | Ms. McIntyre provides programmatic support |
| Giacino, Joseph | SRH | Co-Investigator | 1.20 | Dr. Giacino provided expertise on clinical outcome assessment measures that will be used to identify, validate, and test the blood-based biomarkers in this study. |
| Temkin, Nancy | UW | Co-Investigator | 0.36 | Dr. Temkin has worked on analyzing data relevant to the study and participating in calls/meetings |
| Robertson, Claudia | BCM | Co-Investigator | 0.60 | Dr. Robertson provides clinical trial expertise |
| Diaz-Arrastia, Ramon | Penn | Co-Investigator | 0.60 | Dr. Diaz-Arrastia supervises the conduct of the study at the University of Pennsylvania, and oversees the work of Dr. Lynch as well as Mr. Morrison and Ms. Silverman. He has worked closely with TRACK-TBI leadership in coordinating biomarker assays and data analysis. |
| Lynch, Cillian | Penn | Research Investigator | 6.00 | Dr. Lynch manages all the blood biomarker related aspects of this project at the University of Pennsylvania, including maintaining the repository of blood samples, running biomarker assays, and under the supervision of Dr. Diaz-Arrastia, performing quality controls and analyzing the data. |
| Morrison, Justin | Penn | Clinical Resrearch Associate | 1.20 | Mr. Morrison is primarily responsible for maintaining the research database, working closely with Drs. Diaz-Arrastia and Lynch. |
| Silverman, Erika | Penn | Clinical Resrearch Coordinator | 1.20 | Ms. Silverman is primarily responsible for the regulatory and financial aspects of this project, including IRB submissions, subcontracts, managing necessary purchases. |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

The following PIs have had changes in their support.

**MCCREA, MICHAEL**

**ACTIVE**

| | | |
|---|---|---|
| W81XWH-14-2-0176 (Manley) | 10/1/14 - 9/30/20 | 0.6 Cal Month |
| **Sponsor:** | CDMRP (sub w. UCSF) | |
| **Title:** | TBI Endpoints Development (TED) | |
| **Major Goals:** | The goal of this project is to validate candidate endpoints and improve clinical trial design to inform and accelerate FDA approval of diagnostic tools and therapeutic agents for TBI. | |
| **Overlap:** | No scientific or budgetary overlap. | |

| | | |
|---|---|---|
| --- (Meehan) | 10/31/17 - 10/30/20 | 1.7 Cal Month |
| **Sponsor:** | NFL (Sub w. BCH) | |
| **Title:** | Neurologic Function across the Lifespan: A Prospective, LONGitudinal, and Translational Study for Former National Football League Players: NFL-LONG | |
| **Major Goals:** | The overarching goal of this project is to assess the association between concussion, sub-concussive exposure, cerebral tau, and clinical outcomes. | |

| | | |
|---|---|---|
| W81XWH-15-9-001 (Manley) | 9/14/18 – 11/30/23 | 1.2 Cal Month |
| **Sponsor:** | MTEC (Sub w. UCSF) | |
| **Title:** | Transforming Research and Clinical Knowledge in Traumatic Brain Injury Network (TRACK-TBI NET) | |
| **Major Goals:** | The goal of this project is to leverage the TRACK-TBI and TED infrastructure and establish the TRACK-TBI NETWORK (TRACK-TBI NET), an innovative Phase 2 TBI adaptive clinical trials network that delivers on DoD and NIH recommendations. | |

| | | |
|---|---|---|
| HU0001-18-2-0008 (Pasquina) | 7/1/19 - 6/30/22 | 1.9 Cal Month |
| **Sponsor:** | UHSUS (Subaward with IU) | |
| **Title:** | Service Academy Longitudinal TBI Outcomes Study (SALTOS) | |
| **Major Goals:** | This project proposes to leverage the existing NCAA-DOD Concussion Assessment Research and Education (CARE) consortium research efforts at four U.S. Military Service Academies (MSAs) to examine in more detail the acute, intermediate, and long-term effects associated with concussion and/or repetitive head impact exposure on the health and military performance of Service Academy (SA) cadets/midshipmen. | |

W81XWH-18-2-0047 (McAllister, Broglio, McCrea) 9/1/18-8/31/21 3.2 Cal Month

**Sponsor:** USAMRMC & NCAA (Sub w. IU)

**Title:** Cumulative and Persistent Intermediate Effects of Concussion and Head Impact Exposure in CARE Consortium Military Service Academy Members and NCAA Athletes (CARE 2.0)

**Major Goals:** The goal of this proposal is to answer three critical questions related to the intermediate-term effects of concussion and/or repetitive head impact exposure in MSA cadets and NCAA student-athletes.

W81XWH-17-1-0019 (Bartsch) 11/01/18 – 10/31/20 0.4 Cal Month

**Sponsor:** DOD (Sub w. Prevent Biometrics)

**Title:** Human Head Impact Dose Concussion Risk Functions and Sensor-Based Military-Specific Environmental Monitoring System (Prevent)

**Major Goals:** The goal is to expand on data from the Head Impact Measurement Core of the CARE Consortium collection for other contact sports and military training activities, perform additional repetitive head impact exposure (RHIE) characterization in concussed football athletes, and perform repeated clinical assessments in non-concussed military personnel, cadets, and civilian athletes to characterize the dose relationship for clinical changes in non-concussed individuals and injury risk functions for the onset of concussion.

1R01NS110600-01 (Wang) 4/1/19 - 3/31/24 0.6 Cal Month

**Sponsor:** NIH/NINDS

**Title:** Longitudinal Evaluation of Changes in Brain Structure & Function after Exposure to Subconcussive Impacts

**Major Goals:** The goal of this research is to combine biomechanical and neuroimaging metrics to identify key pathophysiological processes underlying brain alterations due to repetitive subconcussive impacts and develop in vivo measurements of the effects of recurrent impacts during contact sports.

6 U01CE002944-01-02 (Thomas) 9/30/18-9/29/22 0.2 Cal Month

**Sponsor:** Centers for Disease Control (CDC)

**Title:** Active Injury Management (AIM) after Pediatric Concussion

**Major Goals:** The goal of this project is to determine the benefit of prescribed low intensity physical activity, behavioral management, or both, versus standard initial (24-48 hrs) rest in acute mTBI patients presenting to the pediatric Emergency Department.

**PENDING**

0005 (Puccio) 11/1/19 – 2/28/21 0.12 Cal Month

**Sponsor:** Chuck Noll Foundation (Sub w. Pittsburgh) (Fixed Pricing)

| | |
|---|---|
| **Title:** | Biomarker Panel for Inflammation and Tau in Concussed Athletes (Tau Sweat Test) |
| **Major Goals:** | The goal of this project is to further develop our understanding of the pathophysiologic mechanism underlying progressive neurodegeneration following multiple concussive episodes and repetitive head impact (subconcussive) exposure by examining sweat patch technology analyses. |

**OVERLAP**

No overlap with any of the listed grants

**Diaz-Arrastia, Ramon - Other Support**

**ACTIVE**

Title: **Brain Oxygen Optimization in Severe TBI—Phase 3 (BOOST-3)** (Diaz-Arrastia, Protocol PI)
Time Commitment: 2.4 calendar months
Supporting Agency: NIH/NINDS U01 NS099046
Program Official: Scott Janis (janiss@ninds.nih.gov)
Performance Period: 8/1/2018 – 7/31/2023
Level of Funding:
Project Goals: BOOST-Phase 3 is designed to obtain definitive data regarding the clinical efficacy of a treatment protocol based on$PbtO_2$ monitoring.
Specific aims: We propose one primary and several secondary hypotheses:
*Primary Hypothesis:* The prescribed treatment protocol, based on $PbtO_2$ monitoring, results in improved neurologic outcome measured by the Glasgow Outcome Scale-Extended (GOS-E) 6 months after injury.
*Secondary Hypotheses:*

- The prescribed treatment protocol, based on $PbtO_2$ monitoring, results in improved neurologic outcome 6 months after injury based on functional, cognitive, and behavioral assessments.
- Safety hypotheses: Adverse events associated with $PbtO_2$ directed therapy are low.
- The prescribed treatment protocol, based on $PbtO_2$ monitoring, results in improved survival at discharge
- The prescribed treatment protocol, based on $PbtO_2$ monitoring, results in shortened time to follow commands (Glasgow Coma Scale Motor Score).
- The prescribed treatment protocol, based on $PbtO_2$ monitoring, results in reduction of total hypoxia exposure, measured by the area under the curve $PbtO_2$ below 20 mm Hg
- Total hypoxia exposure is correlated with worse neurological outcome as measured with the GOS-E.
- Total hypoxia time is correlated with worse neurological outcome as measured by a composite outcome measure based on functional, cognitive, and behavioral assessments.

Overlap: None

Title: **Biomarkers in Brain Oxygen Optimization in Severe TBI Trial (Bio-BOOST)** (Diaz-Arrastia, PI)
Time Commitment: 0.9 calendar months
Supporting Agency: DOD/USAMRMC BA170613 W811XWH1910829
Program Officer: Christie Vu, PhD
Performance Period: 4/1/2018 – 3/30/2023

Level of Funding:
Project Goals: The recently funded BOOST-3 (Brain Oxygen Optimization in Severe TBI Phase 3) trial. BOOST-3 offers a unique opportunity to study and validate biomarkers and therefore accelerate our understanding of the pathophysiology of severe TBI, and promote the development of effective interventions. Capitalizing on the infrastructure and the rich study population for BOOST-3, we propose conducting an ancillary biomarker study, Bio-BOOST. Bio-BOOST will profile longitudinal changes in target molecular biomarkers measured in blood and cerebrospinal fluid (CSF), to identify unique molecular signatures that classify severe TBI with improved precision. Given that BOOST-3 will study severe TBI only, Bio-BOOST will fill an important gap in the field, since ongoing biomarker collections efforts through the Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) and TBI Endpoints Development (TED) efforts are primarily focused on mild TBI.
Specific aims: Our primary objective is to quantify the effect of brain tissue hypoxia exposure on brain injury using fluid-based biomarkers of brain injury. We hypothesize that total brain tissue hypoxia exposure within 48 hours of randomization (defined as the hours of $PbtO_2$ < 20 mmHg and the area over the curve (hours * mm Hg) (Fig 6) during the first 48 hours of injury is independently associated with higher peak levels of biomarkers of astrocytic (GFAP) and axonal (UCH-L1, Total Tau and NFL) injury, after adjusting for age, gender, and time between injury and randomization. We will also explore the association between lower cutoffs for brain tissue hypoxia ($PbtO_2$ < 15 mmHg and $PbtO_2$ < 10 mmHg) and brain injury.
Our secondary objectives are:

- To determine the effect of total cerebral hypoperfusion exposure (defined as the depth and duration of cerebral perfusion pressure (CPP) < 60 mmHg within 48 hours of randomization, quantified using the AUC methodology) on peak levels of GFAP, UCH-L1, Total Tau and NFL.
- To determine whether a prescribed treatment protocol based on $PbtO_2$ monitoring results in a decrease in blood and CSF levels of GFAP, UCH-L1, Total Tau and NFL.
- To determine whether in severe TBI patients, the initial CSF and blood levels of brain injury biomarkers (GFAP, UCH-L1, Total Tau and NFL) are associated with unfavorable functional outcome as measured by the Glasgow Outcome Scale Extended (GOSE) 6 months after injury.
- To determine whether the rate of increase in brain injury biomarker levels during the first 24 hours of randomization are associated with unfavorable functional outcome.
- To determine the time-point at which GFAP, UCH-L1, Total Tau and NFL levels provide the best discriminative ability for TBI outcome.
- To create a biorepository at the NINDS funded BioSpecimen Exchange for Neurological Disorders (BioSEND) of longitudinal serum, plasma, CSF, RNA and DNA samples of severe TBI patients for validating novel brain injury biomarkers. These samples will be available for future research on TBI biomarkers.

Overlap: None

Title: **Clinical validation of serum neurofilament light as a biomarker of traumatic axonal injury.** (Diaz-Arrastia, PI)
Time Commitment: 1.2 Calendar Months
Supporting Agency: NIH/NINDS U01 NS114140
Program Official: Mary Ann Pellymounter (mary.pelleymounter@nih.gov)
Performance Period: 12/1/2019 – 11/30/2024
Level of Funding: overall
Project Goals: To measure serum NfL in the TRACK-TBI and TRACK-TBI LONG cohorts, assess the trajectory of serum NfL in relation to clinical outcomes and MRI measures of traumatic axonal

injury. To develop age and gender specific Reference Intervals for NfL in a population-based cohort of community-dwelling individuals.
Overlap: None

Title: **TRACK-TBI Epileptogenesis Project.** R. Diaz-Arrastia, PI
Time Commitment: 0.6 calendar months
Supporting Agency: DoD/USAMRMC EP180013 W81XWH-19-1-0861
Program Official: Anthony Pacifico, PhD (anthony.m.pacifico.civ@mail.mil)
Performance Period: 6/1/2019 – 5/30/2023
Level of Funding: overall
Project Goals: *Specific aim 1: Extend follow-up period of TRACK-TBI participants from 1 to 5 years.* The extensive clinical, imaging, and biomarker data that has already been collected in these subjects will be leveraged to identify risk factors, co-morbidities, and prognostic biomarkers of PTE. *Specific aim 2: Extend follow-up period of the TRACK-TBI affiliated studies SD-2, from 6 months to 2 years.* TRACK-TBI EPI will extend follow-up of these severe TBI patients through 2 years after injury, identifying over 75% of those who eventually will develop PTE.
*Specific aim 3: To conduct specialist epileptologist evaluation for all TBI patients who screen positive for PTE.* Participants who answer yes to screening questions for PTE will be invited for in-person evaluations by expert epileptologist at each sites. Epilepsy Clinic visits will include an outpatient EEG. We will also assess functional and neuropsychological outcome at 6, 12, 24, and 60 months after injury. A subset of patients from each parent study who do not screen positive for PTE, matched by age, gender, and injury characteristics, will also be invited for an in-person evaluation.
*Specific aim 4: To measure candidate blood biomarkers* to determine if they are prognostic for epileptogenesis. We will used existing blood samples collected from current participants in TRACK-TBI. We will measure specific molecular biomarkers of neural injury and neuroinflammation/autoimmunity (35-36) using highly sensitive multiplexed immunoassays. Serum samples at 1d, 14d, 6, 12 months after injury will be used.
Overlap: None

Title: **Characterizing Concussion using Brain-Derived Exosomes.** D. Issadore, PI
Time Commitment: 0.3 calendar months
Supporting Agency: DoD/USAMRMC W81XWH1920002
Program Official: Florence D'Orazi, PhD (Florence.dldorazi.ctr@mail.mil)
Performance Period: 5/1/2019 – 4/30/2022
Level of Funding: overall
Project Goals: ***Phase 1***

a. *Next Generation Technology Development:* We will develop an improved nanomagnetic sorting platform aided by finite element models, to better design and fabricate our chip to enhance manufacturability and performance. We will validate our approach using exosomes derived from cultured cell lines and healthy volunteer plasma and serum, and benchmark performance to gold standard technology (e.g. ultracentrifuge, nanoparticle tracking analysis).

b. *In Vitro Biomarker Selection:* We will identify a set of surface markers to specifically isolate exosomes from multiple cell types from the brain to enable the exosome based diagnostic to capture an increasingly comprehensive picture of the injured and recovering brain.

c. *Pilot Clinical Evaluation:* We will isolate multiple exosome subpopulations from injured patients and healthy controls using the ExoTENPO on existing samples from $N = 20$ injured subjects and $N = 20$ controls. We will also sequence exosomes isolated from $N = 40$ banked serum samples from a porcine injury model, to compare this model to our clinical data and validate it for further use in our study.

***Phase 2***

d. *Porcine Model Study:* We will profile injury and recovery states in a pig injury model using our chip and next generation sequencing. We will measure the exosomal RNA signature of healthy animals and animals with mTBI. Based on this analysis, we will generate a panel of exosomal markers that can predict the endophenotypic state of an injured and recovering brain, which will subsequently be validated using user-blinded samples to avoid the effects of overfitting.
e. *Next Generation Technology Development:* We will incorporate the ExoTENPO exosome isolation with cell-phone based droplet PCR, including droplet production, thermal cycling, and droplet readout.

***Phase 3***

f. *Exosome Biomarker Characterization of Endophenotypes in Clinical Samples:* We will measure N = 100 prospectively collected samples from those with TBI, by combining prospective samples collected expressly for this study with archival samples collected by co-investigators on this study.
g. *Next Generation Technology Evaluation:* Our mobile exosome diagnostic will be used in clinical settings and compared directly to a variety of emerging gold standard technologies, including Quanterix.

Overlap: None

Title: **Transforming Research and Clinical Knowledge in TBI Clinical Trials Precision Medicine Pathomechanistic Classification of Traumatic Brain Injury (TRACK-TBI Precision Medicine)** Diaz-Arrastia Co-PI (with Geoff Manley, MD (UCSF)) DM180187
Time Commitment: 1.2 calendar months
Supporting Agency: Department of Defense/USAMRMC W81XWH-18-2-0042
Performance Period: 10/1/2018 – 9/30/2021
Overall Level of Funding:
Project Goals: To characterize magnetic resonance imaging (MRI) and blood-based biomarker features of traumatic brain injury (TBI) to inform the design of a next-generation precision medicine TBI exploratory clinical trial and evaluate the use of serum biomarkers and MRI for the diagnosis of TBI.
The specific aims are to (1) validate biomarkers of diffuse axonal injury (DAI), microvascular injury (MVI), and neuroinflammation using advanced blood-based assay platforms and MRI sequences in prospectively collected data from existing Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) subjects;
(2) validate early and ultra-early blood-based and imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate/severe TRACK-TBI subjects; and
(3) conduct a multicenter, double-blind, placebo-controlled exploratory clinical trial comparing the impact of cyclosporine A (CsA) on blood-based and imaging biomarkers of DAI and neuroinflammation in moderate/severe TBI patients admitted to the ICU. The hypotheses to be tested are that (1) particular blood-based biomarkers will correlate with select MRI biomarkers (phase 1) that can be validated as predictive and pharmacodynamic biomarkers of DAI, MVI, and neuroinflammation (phase 2) and that (2) CsA (phase 3) will affect these biomarkers, directing treatment for TBI based on well-defined mechanistic endophenotypes. An exploratory clinical trial will be conducted.
Overlap: NoneDM180187

Title: **Transforming Research and Clinical Knowledge in TBI Clinical Trials Network (TRACK-TBI NET)**
Time Commitment: 1.8 calendar months

Supporting Agency: Department of Defense/Medical Technology Enterprise Consortium
W81XWH-15-9-001
Performance Period: 9/1/2018 – 8/31/2023
Overall Level of Funding:
Project goals: We propose to leverage the TRACK-TBI and TED infrastructure and experience, in partnership with globally recognized CRO ICON, to establish the TRACK-TBI NETWORK (TRACK-TBI NET), an innovative Phase 2 TBI adaptive clinical trials network that delivers on DoD and NIH recommendations. We propose a 5-year (4 years of enrollment), Phase 2 multi-arm, multi-stage (MAMS) adaptive platform design for multi-site, randomized, controlled clinical trials for patients with moderate to severe TBI. An adaptive trial permits design modifications to be made after subjects have been enrolled and some responses have been observed, without compromising the validity of the scientific method and trial integrity. In this flexible, precision medicine approach to TBI Phase 2 trials, numerous hypotheses may be addressed, from dose-finding to selection of a therapeutic intervention for a confirmatory Phase 3 trial. The study cohort will first be enriched for TBI using objective imaging and blood-based biomarkers and then, based on the drug/drugs selected, stratified into cohorts based on pathoanatomic features (e.g., presence/absence of contusion) and pathophysiologic features (e.g., presence/absence of neuroinflammation).
Overlap: None

Title: **Characterize the Extent, Distribution, and Range of Pathologies Contributing to TREND (CONNECT-TBI).** (D. Smith, Univ. of Pennsylvania, Contact PI)
Time Commitment: 0.6 calendar months
Supporting Agency: NINDS U54NS115322
Program Official: Patrick Bellgowan (PatrickFrostBellgowan@nih.gov)
Performance Period: 9/1/2019 – 8/31/2024
Level of Funding:
Project Goals: This multi-center application addresses “TBI-Related Neurodegeneration” (TReND) utilizing multiple TBI and neurodegenerative disease (ND) human brain archives. The goals are to develop a comprehensive tissue resource and virtual archive to characterize various unique phenotypes of TReND in comparison to other NDs.
Overlap: None

Title: **The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx)** (A. Toga, USC, PI)
Time Commitment: 0.05 calendar month
Supporting Agency: NINDS U54NS100064
Program Official: Randall Stewart (StewartR@ninds.nih.gov)
Performance Period: 1/15/2017 – 11/30/2021
Level of Funding:
Project Goals: EpiBioS4Rx is designed to facilitate the development of antiepileptogenic therapies by removing barriers and promoting large-scale collaborative research efforts by multidisciplinary teams of basic and clinical neuroscientists with access to extensive patient populations, well-defined and rigidly standardized animal models, and cutting-edge analytic methodology. We focus our proposal on antiepileptogenesis in post- traumatic epilepsy (PTE) following traumatic brain injury (TBI), as this condition offers the best opportunity to determine the time of onset of the epileptogenic process in patients.
Overlap: None

Title: **Penn Center for Excellence in Neuroscience Clinical Trials (Penn NeuroNEXT)** (Diaz-Arrastia, PI)
Time Commitment: 1.2 calendar months

Supporting Agency: NIH/NINDS U24 NS107199
Program Official: Janice Cordell (cordellj@ninds.nih.gov)
Performance Period: 9/1/2018 – 8/31/2023
Level of Funding:
Project Goals: The goal of the proposed Penn Center for Excellence in Neuroscience Clinical Trials (Penn NeuroNEXT) is to bring together and optimize the contributions of a large group of experienced investigators focused in the clinical neurosciences at the University of Pennsylvania Perelman School of Medicine (UPennSOM), across four major teaching hospitals: the Hospital of the University of Pennsylvania (HUP), the Children's Hospital of Philadelphia (CHOP), Pennsylvania Hospital (PAH) and Penn Presbyterian Medical Center (PPMC). UPennSOM has long been a leading Center for clinical research in the neurosciences, and internationally recognized leaders in neurology, neurosurgery, neuroradiology, and psychiatry have a strong track record of collaboration, evidenced by existing multiple program projects, collaborative agreements, successful participation in federally and industry-sponsored multi-center clinical trials, and a large number of investigator-initiated studies. The expertise of Penn NeuroNEXT investigators covers the lifespan, and they have been leading contributors to our understanding of and development of innovative therapies for neurological disorders from infancy through old age. The four hospitals collaborating in Penn NeuroNEXT compromise the largest health system in the Philadelphia area, the nation's fifth largest city which anchors a metropolitan area of over 6 million people, which does not currently have a NeuroNEXT site. Additionally, UPennSOM has long been one of the leading Centers for training talented clinical neuroscientists, with large residencies and fellowship programs that routinely attract very promising young physician scientists, a large number of whom go on to highly productive academic careers.
Specific aims:
*Specific Aim 1:* To be an outstanding enrolling site for NeuroNEXT trials. Penn NeuroNEXT will participate in a minimum of 4 NeuroNEXT clinical trials over the next 5 years. We intend to meet or exceed recruitment and retention goals. We will ensure a high level of data quality in the context of specific protocols and regulatory standards.
*Specific Aim 2:* To optimize efficiency of clinical research in neurosciences at UPennSOM.
*Specific Aim 3:* To promote career enhancement of early stage clinical investigators. The University of Pennsylvania and our four hospitals are recognized as leading training centers in the clinical neurosciences, and we have been particularly successful in attracting talented young people interested in obtaining the necessary experience for launching academic careers.
Overlap: None

Title: **Pennsylvania Consortium on TBI (PACT)."** (D. Smith, Univ. of Pennsylvania, PI)
Time Commitment: 0.3 calendar months
Supporting Agency: Pennsylvania Department of Health
Performance Period: 1/1/2018 – 12/31/2020
Overall Level of Funding (3 years):
Project Goals: The goal of this project is to develop novel diagnostic techniques for acute and chronic neuropathologies of moderate to severe TBI.
Specific aims:
*Specific Aim 1:* Develop novel diagnostic techniques and approaches for acute and chronic *mild TBI/concussion* in humans that will predict which individuals will have poor outcomes.
*Specific Aim 2:* Develop novel diagnostic techniques for acute and chronic moderate-severe TBI in humans that are predictive of outcome.
*Specific Aim 3:* Preclinical analysis of novel diagnostic techniques to identify acute and chronic neuropathologies after TBI that can be extrapolated to Aims 1 and 2.
*Specific Aim 4:* Community outreach and education for TBI. This will include expanding our grade school

demonstrations and public seminars as well as enhancing our strong commitment to education of underrepresented minorities in our well-established TBI research training program.
Overlap: None

Title: **Transforming Research and Clinical Knowledge in Traumatic Brain Injury-Longitudinal (TRACK-TBI LONG)** (G. Manley, UCSF, PI)
Time Commitment: 0.05 calendar months
Supporting Agency: National Football League (NFL)
Program Officer: Nancy Smith-Shaw (nancy.smithshaw@onemind.org) One Mind, 120 Lakeside Avenue, Ste. 200, Seattle, WA 98122
Performance Period: 07/01/2018 – 6/30/2021
Overall level of funding (3 years) Direct Cost:
Project Goals: TRACK-TBI LONG will extend TRACK-TBI's current 1-year follow-up for 3 additional years, adding data ranging from 2-7 years post-injury. These data will validate endpoints for acute and chronic diagnosis, and advance knowledge of the epidemiology, risk factors, and pathology of TBI's long-term sequelae.
*Specific Aims:*
1) To extend examination of existing injured and control TRACK-TBI participants;
2) to characterize the long-term trajectory of imaging biomarkers in brain-injured and control subjects;
3) to characterize the long-term trajectories of neurocognitive/psychological function and their relationship to plasma-CSF proteomic markers related to inflammation and neurodegeneration, and imaging;
4) apply traditional and novel neuroimaging-guided and quantitative neuropathologic approaches to donated brains to interrogate TBI-associated structural and biological changes correlated with antemortem phenotyping.
OVERLAP: none

**Pending:**

Title: **Dose-Optimization Study of Sildenafil for the Treatment for Chronic Traumatic CereboVascular Injury (TCVI)** (R. Diaz-Arrastia, PI)
Time Commitment: 1.2 calendar months
Supporting Agency: NIH/NINDS
Program Officer: Patrick Bellgowan (PatrickFrostBellgowan@nih.gov)
Performance Period: 9/1/2020 – 8/31/2025
Overall level of funding (5 years):
Project Goals: This proposal builds on our recently completed pilot trial, which demonstrated that single dose administration of sildenafil citrate (50 mg orally) was effective in reversing CVR deficits in the chronic stage after TBI. We will determine the optimal dose of sildenafil to improve microvascular function using a range of sildenafil doses (20, 40, 80 mg), doses which are commonly used in the treatment of primary pulmonary hypertension, and also assess safety and tolerability of these doses.
*Aim 1 (Primary Aim- Pharmacodynamic):* To determine the optimal PDE5 inhibitor dose to improve microvascular function (ΔCVR measures) after a single PDE5 inhibitor dose.
*Hypothesis 1: Single dose administration of sildenafil in chronic TBI patients will improve CVR in a dose dependent fashion.*
*Aim 2 (Primary Aim- Safety and Tolerability):* To assess the safety and tolerability of a range of PDE5 inhibitor doses in 100 chronic TBI subjects.
Hypothesis 2: Chronic 4-week daily administration of PDE5 inhibitors in chronic TBI patients will be safe and well tolerated at all doses.

*Aim 3 (Secondary/Exploratory Aim):* To measure the effect of chronic (4-week) PDE-5 inhibitor administration at 3 different doses on TBI symptom self-report and functional outcome measures. *Hypothesis 3: Chronic sildenafil administration will result in improvement in post-concussive symptoms and overall clinical assessments in a dose-dependent fashion.*
**Overlap: None**

**Giacino, Joseph T – New Awards**

90DPCP0008 (Prime PI: Giacino) 10/1/20-9/30/25 0.60 calendar months
National Institute on Disability, Independent Living, and Rehabilitation Research
*Participatory Community Integration for TBI Survivors: Development of a Computer Adaptive Test*
The aim of this project is to develop a cutting edge computerized adaptive test that efficiently measures community participation of traumatic brain injury (TBI) survivors to help with needs assessment, connecting individuals with resources, and improving participation in meaningful life roles.
Role: Project Director

DP2 New Innovator Award (PI: Edlow) 8/15/19 – 6/30/24 0.60 calendar months
NIH Director's Office

*A Connectome-Based Clinical Trial Platform to Promote Early Recovery of Consciousness after Traumatic Coma*
The goal of this project is to develop and implement a clinical trial platform for personalized, targeted therapy aimed at restoring consciousness in patients admitted to the intensive care unit for traumatic coma.
Role: Mentor

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Nothing to Report*

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:**

## 9. APPENDICES:

Appendix 1


# TRACK-TBI Precision Medicine

# Phase 2-Option I Clinical Protocol

## November 1, 2020

## Version 1

The goal of TRACK-TBI Precision Medicine **Phase 2 Option 1** is to validate early and ultra-early blood-based and novel imaging biomarkers of diffuse axonal injury (DAI), microvascular injury (MVI), and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe TRACK-TBI subjects.

TRACK-TBI Precision Medicine is funded by the Department Of Defense Grant (W81XWH-18-2-0042).

## Table of Contents

## 1. ORIGINAL TRACK-TBI PRECISION MEDICINE GRANT SUBMISSION BACKGROUND AND STUDY OVERVIEW

Effective treatment of traumatic brain injury (TBI) remains one of the greatest unmet needs in public health. According to a 2006 publication, each year in the United States, at least 1.7 million people suffer TBI; it is a contributing factor in a third of all injury-related US deaths. An estimated 3.2 to 5.3 million people live with the long-term physical, cognitive, and psychological health disabilities of TBI, with annual direct and indirect costs estimated at over $60 billion.[1] More recent publications have reported an increase in these numbers.[2] Recent efforts have increased our understanding of the pathophysiology of TBI; however, these advances have failed to translate into a single successful clinical trial or treatment.[2] These failures are largely attributable to the fact that TBI classification approaches are blunt and have not changed in more than 3 decades. TBI patients are divided into the crude categories of "mild", "moderate", and "severe", using the Glasgow Coma Scale (GCS)[3], and functional outcome is measured using the equally crude Glasgow Outcome Scale-Extended (GOSE).[4] These symptoms-based categories do not permit mechanistic targeting for clinical trials. Clinical research has also been underpowered, hampered by lack of data standardization, and with limited multidisciplinary collaboration. Workshops coordinated by the National Institute of Neurological Disorders and Stroke (NINDS), Department of Defense (DOD), and the National Institute on Disability, Independent Living, and Rehabilitation Research (NIDILRR) since 2007 identified the urgent need for improved TBI classification using more accurate diagnostic and outcome tools (beyond the GCS and GOSE), along with a standardized approach to data collection. A multidisciplinary effort was launched to develop TBI Common Data Elements (TBI-CDEs). Domains included clinical data, imaging, biospecimens, and outcomes.[5-8]

In 2009, the multicenter Transforming Research and Clinical Knowledge in Traumatic Brain Injury Consortium was implemented to characterize the clinical, magnetic resonance imaging (MRI), and blood-based biomarker features of TBI to inform design of next-generation precision medicine clinical trials in TBI. Over the past 10+ years, TRACK-TBI has been supported by NINDS, DoD, Department of Energy (DoE), the National Football League, and other philanthropic and industry partners. TRACK-TBI has enrolled >3000 TBI subjects across the injury spectrum, and controls, at 18 US Level 1 Trauma Centers. Our effort has established the world's largest collection of TBI imaging studies and biospecimens and our study results are already being adopted into clinical research and bedside practice. The TRACK-TBI Consortium is now primed to deliver on critical military and public health knowledge gaps and needs: objective classification of TBI based on what we term "mechanistic" endophenotypes, e.g., diffuse axonal injury (DAI), microvascular injury (MVI), and neuroinflammation. An endophenotype is an internal phenotype discoverable by biochemical, physiological, radiological, pathological, or other techniques, which is intermediate between a complex phenotype and the presumptive genetic or environmental contribution to a disease.[9] Endophenotypes are quantitative, continuous variables, unlike a phenotype which is usually a binary, categorical variable. These mechanistic endophenotypes, defined by imaging and blood-based biomarkers, will direct targeted treatments based on mechanism, providing the tools needed for successful execution of precision medicine clinical trials. To achieve the goal of precision medicine in TBI, it is necessary to identify subgroups of TBI patients that will respond to a targeted therapy.

## 2. TRACK-TBI PRECISION MEDICINE RATIONALE, SPECIFIC AIMS, AND OBJECTIVES FROM THE ORIGINAL GRANT SUBMISSION

### 2.1 Rationale

In Phase I of this study, we will assess putative blood-based and neuroimaging biomarkers for DAI, MVI, and neuroinflammation (**Table 1**). Fluid biomarkers complement imaging markers and may provide important tools for precision medicine clinical trials. In Phase 2 of this study, we will collect acute data (early and ultra-early i.e., hours-days following injury), to validate the utility of these biomarkers identified during Phase I in defining TBI mechanistic endophenotypes for use in clinical trials.

***Table 1**. Proposed TBI Biomarkers and their Relationship to TBI Mechanistic Endophenotypes*

| Mechanistic Endophenotype | Blood-Based Biomarkers | Neuroimaging Biomarkers |
|---|---|---|
| Axonal injury | pNF-H, NF-L, Tau, P-Tau | MRI (DW, DTI, rs-fMRI) |
| Microvascular injury | vWF, c-fibronectin, ICAM-1, VCAM-1 | MRI (SWI; ASL perfusion) |
| Neuroinflammation | IL-6, IL-10, IL-17, TNFa, HMGB-1 AutoAb[GFAP]-IgM, IgG | MRI (free water imaging) - (FISO) from NODDI |
| *Neuronal Injury* | *UCH-L1* | *(as benchmark in Aims 2-3)* |
| *Astroglial Injury* | *GFAP* | *(as benchmark in Aims 2-3)* |

**2.2 Specific Aims**

- SPECIFIC AIM 1: To validate biomarkers of Diffuse Axonal Injury (DAI), Microvascular Injury (MVI), and Neuroinflammation using advanced blood-based assay platforms and imaging sequences.
  *Study activities conducted in support of this Aim are reviewed under separate IRB protocols:*
  - *University of California, San Francisco IRB # 18-26754*
  - *University of Florida IRB # IRB201802961*
  - *University of Pennsylvania IRB # 832422*

- SPECIFIC AIM 2: To validate early and ultra-early blood-based and novel imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe subjects.
  *Study activities conducted in support of this Aim are reviewed under this IRB protocol.*

- SPECIFIC AIM 3: Conduct a multicenter double-blind, placebo controlled exploratory clinical trial comparing the impact of Cyclosporine A (CsA) on blood-based and imaging biomarkers of DAI, MVI, and neuroinflammation in moderate-severe TBI patients admitted to the ICU.
  *Study activities conducted in support of this Aim will be reviewed under a separate IRB protocol.*

**2.3 Study Objectives**

**Phase 1-Base (Year 1): Validate biomarkers of Diffuse Axonal Injury (DAI), Microvascular Injury (MVI), and Neuroinflammation using advanced blood-based assay platforms and imaging sequences.**

- Phase I-Base analyses are reviewed under a separate IRB protocol.

**Phase 2-Option I (Year 2): Validate early and ultra-early blood-based and novel imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe TRACK-TBI subjects.**

- In Phase 2-Option 1 (study activities reviewed under this protocol), we will enroll a cohort of moderate to severe TBI subjects (N=50), stratified according to VA/DoD criteria for these injury severities through the existing TRACK-TBI network sites to obtain novel advanced neuroimaging and more frequent biomarker sampling. Subjects will be assessed according to the existing TRACK-TBI outcomes protocol over 3 months.

**Phase 3-Option II (Year 3): Conduct a multicenter double-blind, placebo controlled exploratory clinical trial comparing the impact of Cyclosporine A (CsA) on blood-based and imaging biomarkers of DAI, MVI, and neuroinflammation in moderate-severe TBI patients admitted to the ICU.**

- Phase 3-Option II will be reviewed under a separate IRB protocol.

## 3 STUDY DESIGN

### 3.1 Participating Sites

1. University of California, San Francisco (UCSF)
    a. PI Geoffrey Manley
2. University of Pittsburgh (UPitt)
    a. PI David Okonkwo
3. University of Pennsylvania (UPenn)
    a. PI Ramon Diaz-Arrastia
4. Medical College of Wisconsin (MCW)
    a. PI Michael McCrea
5. University of Utah
    a. PI Ramesh Grandhi

### 3.2 Participants

Subjects will be recruited among patients with TBI admitted to the emergency department, trauma, or neurosurgical services at 5 participating TRACK-TBI Clinical Sites. Each site has a system for identification and early notification of potential patients who qualify for the study. The early notification system will result in timely arrival of the study coordinator or other trained study personnel, who will evaluate a participant's eligibility. Individuals notifying study personnel of potential patients may include ambulance coordinating system personnel, neurosurgery residents, emergency room physicians, or other hospital personnel who are likely to see brain injured patients shortly after their arrival.

Once notified, study personnel will review the potential patient's information and screen the patient according to the study inclusion and exclusion criteria (Table 3). Upon determining that the patient is potentially eligible for the study, consent will be obtained from the patient or the legally authorized representative (LAR) according to local IRB guidelines. If the patient is determined to have capacity to provide his or her own consent, the patient will be asked to provide informed consent. For participants whose LAR originally gave consent, an informed consent will be obtained from the participant once they have the capacity to do so.

The target enrollment for this study is 50 participants.

### 3.3 Study Procedures

***Table 2****. Study Procedures*

| *Study Procedures* | *Screening/ Enrollment* | *<6 hr* | *12 hr* | *24 hr* | *Day 2* | *Day 3* | *Day 5* | *Day 14* | *Day 42* | *Day 90* |
|---|---|---|---|---|---|---|---|---|---|---|
| Screening and Eligibility | X | | | | | | | | | |
| Inclusion/Exclusion criteria | X | | | | | | | | | |
| Informed consent | X | | | | | | | | | |
| Eligibility CT reading | X | | | | | | | | | |
| Blood collected | | X | X | X | X | X | X | X | X | X |
| MRI scan | | | | (X) | | | | X | | X |
| *Clinical Data Collection | X | | | | | | | | | |
| TRACK-TBI Outcome Enrollment Battery | | | | X | | | | | | |
| TRACK-TBI Follow –up Outcome Battery | | | | | | | | X | X | X |

(X)—MRI will be collected on either Day 1 or Day 2

* Clinical Data will be collected daily until the subject is discharged from hospital

### 3.4 Milestone Plan

Our target follow-up rate for the 3-month duration of the study is 80%. The Clinical Core will monitor this rate at the overall study level as well as the individual site level. Women and minorities will be included.

## 4 SUBJECT ELIGIBILITY

### 4.1. Assessment of Eligibility

We will enroll adult patients (age 18-65y inclusive) presenting to the Emergency Department (ED) with a history of acute TBI as per American Congress of Rehabilitation Medicine (**ACRM) Criteria**, in which the patient has sustained a traumatically induced* physiological disruption of brain function, as manifested by ≥ one of the following:

- Any period of loss of consciousness (LOC)
- Any loss of memory for events (e.g., amnesia) immediately before or after the accident (PTA)
- Any alteration of consciousness/mental state at the time of the accident (feeling dazed, disoriented, and/or confused) (AOC)
- Focal neurologic deficits that may or may not be permanent

* Traumatically induced includes the head being struck, the head striking an object, or the brain undergoing an acceleration/deceleration movement (e.g., whiplash) without direct external trauma to the head.

<u>Screening questions for LOC</u>

Did you have a period of time after the event when you were completely unconscious? That means you had no ability to think, speak, or move and were completely unaware of the world around you.

<u>Screening questions for PTA</u>

Was there a period of time after the injury for which you have no memory? If so, how long did it take for your memory to return to normal or become consistent (e.g., who you saw, conversations, what you ate, etc. (Walk them through the post-injury events if and as necessary).

<u>Screening questions for AOC</u>

Right after the event, did you feel dazed or confused or in a fog? Did you have trouble knowing where you were or what happened to you? Did you keep asking the same question over and over? Did you insist you could do things that you could or should not do?

<u>Suggested prioritization for AOC and LOC:</u>

1st- EMS run report
2nd- Witness report
3rd- ED records (if positive)
4th- Participant

In general:

- **AOC**: should be recorded as "positive" if present in any of these sources. Only record AOC as negative if available sources (especially the participant) state it did not occur.
- **LOC:** Subject's recall is unreliable unless the subject explicitly states that a witness told informed the subject that the subject was knocked out cold.
- **PTA:** Use the participant report for mild cases. Use EMS or hospital medical records for more severe cases.

### 4.2 Inclusion/Exclusion Criteria

**Table 3** summarizes study inclusion and exclusion criteria. These criteria are informed by the experience of the TRACK-TBI U01 study, and are designed to mirror criteria appropriate for future trials of neuroprotective or neurorestorative therapies. These clinical and imaging criteria were identified through the TRACK-TBI U01 study, and include select subjects with TBI who are at high risk for incomplete recovery (GOSE < 8) out to 6 months after injury. The criteria also exclude participants with very severe TBI who stand a low chance of survival.

***Table 3**. Enrollment Inclusion and Exclusion Criteria*

| Criteria | Data Source | Comments |
|---|---|---|
| **Inclusion Criteria** | | |
| 1. Age 18 – 65y inclusive | Chart, Interview | |
| 2. History or evidence of TBI, according to DoD-VA criteria | Chart, Interview | |
| 3. GCS 3 – 15 after resuscitation in the ED | Chart | |
| 4. Cranial CT with evidence of trauma-related abnormality (i.e., CT positive except for isolated epidural hematoma (EDH)) | Chart | Based on radiologist's read |
| 5. Ability to undergo MRI within 48 hours of injury | Chart, Interview | |
| 6. Ability to obtain informed consent from participant or LAR within 6 hours of injury | Interview | |
| 7. Fluency in English or Spanish | Interview | Based on test battery and available personnel |
| **Exclusion Criteria** | | |
| 1. Unstable respiratory or hemodynamic status | Chart | |
| 2. Evidence of penetrating brain injury | Chart | |
| 3. Isolated EDH as only trauma-related CT abnormality | Chart | Favorable natural history |
| 4. Systemic traumatic injury that would preclude participation in study, which is expected to result in long-term disability not related to TBI | Chart | |
| 5. Evidence of serious infectious complications (sepsis, bacteremia, multilobar pneumonia) | Chart | |
| 6. Acute ischemic heart disease (myocardial infarction or unstable angina) | Chart, Interview | |
| 7. History of syncope or hypotension | Chart, Interview | |
| 8. SBP < 90 mm Hg, DBP < 40 mm Hg for longer than 5minutes | Chart | Hypoxic brain injury has very poor prognosis |
| 9. History or evidence of active malignancy | Chart, Interview | |
| 10. History of pre-existing neurologic disorder, such as dementia, mild cognitive impairment, uncontrolled epilepsy, multiple sclerosis, strokes, brain tumors, prior severe TBI, or other disorder that may confound interpretation of MRI or neuropsychological results | Chart, Interview | May confound interpretation of MRI or neuropsychological results |
| 11. History of pre-existing disabling mental illness, such as major depression or schizophrenia | Chart, Interview | Disabling psychiatric conditions confound outcome measures and reduce FU rate |

| | | |
|---|---|---|
| 12. History or evidence of chronic heart failure or chronic renal failure | Chart, Interview | |
| 13. Low likelihood of follow-up (e.g., participant or family indicating low interest, residence in another state or country, unhoused or lack of reliable contacts) | Chart, Interview | Makes FU visits difficult |
| 13. Women who are pregnant or breast-feeding | Chart, Interview | |
| 14. Prisoners or patients in custody | Chart, Interview | |
| 15. Patients on psychiatric hold (e.g. 5150, 5250) | Chart, Interview | |

## 5 SUBJECT RECRUITMENT AND SCREENING

### 5.1 Subject Identification

Study personnel will identify potential subjects in the ED, hospital, and ICU during "peak hours" as appropriate for their study site by review of medical records, trauma logs, and triage notes as well as by conferring with on-duty doctors and nurses to identify potential subjects. Many of the inclusion/exclusion criteria can be evaluated by a review of the potential subject's medical records, such as mechanism of injury, extent of non-head injuries, prior medical history, and prior clinical visits at the center of care. As all eligible patients must receive an acute clinical brain CT due to external force trauma to the head, the ideal place to begin screening is scanning for acute scheduled CT brain studies in the radiology department. When a potential subject is identified and has been screened against the primary set of inclusion/exclusion criteria, they will be approached about the study.

### 5.2 Screening Process

Due to the vulnerability of the subjects and the complexity of the protocol, we envision a three stage screening process. These stages are: 1) review of medical records and test results to determine eligibility, 2) subject completion of a screening evaluation to determine competency to provide informed consent, and 3) subject interview to present and discuss this research participation opportunity. Only after all three of these phases have been completed would the subject be asked to participate and provide formal signed informed consent.

Prior to enrolling a subject, the research personnel will screen the subject for competency to provide informed consent. This is necessary because TBI may result in a period of posttraumatic amnesia (PTA) characterized by confusion, disorientation, and impaired memory for ongoing events. The Galveston Orientation and Amnesia Test (GOAT) will be used as the standard assessment instrument for this screening. A score of ≥75 on the GOAT would indicate that the subject is competent to provide informed consent. If the subject scores <75 on the GOAT, consent must be provided by a Legally Authorized Representative (LAR).

To accomplish the competency evaluation part of the screening process, the research personnel will approach the subject and introduce the study, explaining that the subject may be a candidate but that additional information is required to determine this. It takes approximately 5 minutes for the subject to answer the test questions contained in the GOAT. The research personnel will then score the test as described. If the subject qualifies and wishes to proceed, then the research personnel will move to the third stage of this process. If the subject is not interested in participation in the protocol, the subject will be thanked for their time and any data collected up to this point will be destroyed.

### 5.3 Participation Requirements

An important part of the screening and enrollment process is an interview with the subject, where the research personnel explains the project in detail, presents the consent forms, and responds to all patient questions and concerns. Key points that will be explained during this interview process are:

- Participation in the project is immediate and for all components (clinical, biospecimens, MRI, outcomes), unless contraindicated for MRI.

- Potential subjects will be given time to read the Consent Form(s) and to consult with family members who may be present or by phone. If the subject agrees to participate, then they will sign the appropriate forms. A copy of the form(s) will be given to the subject.
- Upon enrollment, data collection will begin in the hospital. Participation in follow-up activities must be completed within given follow-up assessment windows as specified below (Table 4), but will be scheduled to accommodate the patient:

***Table 4**. Schedule for Follow-Up (FU) Assessment Windows*

| In-person 2 Week Follow-up Assessment Windows | |
|---|---|
| 2 Week FU | MRI: 14 days post-injury ± 4 days |
| | Outcomes + blood collection: ± 3 days of 2-week MRI |

| In-person 6 Week Follow-up Assessment Window | |
|---|---|
| 6 Week FU | Outcomes + blood collection: 42 days post-injury ± 4 days |

| In-person 3 Month Assessment Window | |
|---|---|
| 3 Month FU | MRI: 90 days post-injury ± 14 days |
| | Outcomes + blood collection: ± 3 days of 3 month MRI |

- All efforts should be made to schedule patient return within the specified window for each time point. Patients who are reached and scheduled but fall outside the window for any outcomes testing time point should still have their outcomes assessment completed during the next scheduled follow-up time point. The number of days from date of injury, and the number of days outside of the exact 2-week, 6-week, and 3-month, window will be documented in the QuesGen database.
- Compensation is provided for participation in each study activity. See section 5.4 Subject Compensation for further information.

**5.4 Subject Compensation**

Participants in study activities will receive financial compensation in recognition of the time required by the study. The suggested compensation is in **Table 5** below. While these are the suggested compensation rates for the study, **individual sites have the ability to determine their own reimbursement plans** for participants and Informants as well as a rate per time point within the constraints of their budget and as approved by local IRB.

***Table 5**. Patient Reimbursements*

| Time point | Study Milestone | Subject Reimbursement |
|---|---|---|
| < 6 hours | Blood Draw | $20 |
| 12 hours | Blood Draw | $20 |
| 24 hours | Blood Draw | $20 |
| < 24 hours | Enrollment Outcome Battery | $25 |
| 2-Day | Blood Draw | $20 |
| < 48 hours | MRI | $120 |
| 3-Day | Blood Draw | $20 |
| 5-Day | Blood Draw | $20 |
| 14-Day | MRI | $120 |
| 14-Day | Blood Draw | $20 |
| 14-Day | Outcome Battery | $70 |
| 42-Day | Blood Draw | $20 |
| 42-Day | Outcome Battery | $70 |
| 90-Day | MRI | $120 |
| 90-Day | Blood Draw | $20 |

| | | |
|---|---|---|
| 90-Day | Outcome Battery | $70 |
| Patient travel stipend (Maximum amount per subject) | | $150 ($50/in-person visit) |
| **Maximum amount per subject costs** | | **$775 + up to $150 for travel** |

Compensation will be disbursed at the end of each visit. Subjects must provide a social security number or other form of tax identification to receive these funds.

## 6 INFORMED CONSENT

### 6.1 Study Personnel Obtaining Informed Consent

The individuals responsible for identifying potential subjects, explaining the studies, answering questions, and obtaining informed consent will be study research personnel who are healthcare professionals, including MD, RN, Research Coordinators, and Research Associates (RAs). Qualifications for these positions include clinical experience with TBI patients, patient teaching skills related to home medication administration, excellent interpersonal and problem-solving skills, and knowledge of the clinical research process.

Based on sites' local IRB policies, sites may include language in their informed consent, which will ask patients if they wish to be contacted for future research after the completion of this study.

> Suggested IRB language: *In the future, other studies involving traumatic brain injury may become available. If you agree, then someone from the [site name] Neurosurgery team may contact you in the future about additional research that you may be interested in participating in. You agree to allow someone to contact you about research in the future. _yes _no*

### 6.2 Location and Privacy

Potential subjects will be approached in the ED, hospital wards, or ICU at each IRB-approved enrollment site. All sites have implemented electronic medical records in their hospitals and much of the screening process can be completed via utilizing these resources. Interested subjects are offered the opportunity to participate on-site during their emergency hospital visits or contacted by phone after hospital discharge.

If potential subjects are approached in the ED, all means of ensuring privacy will be undertaken. If the potential subject approaches their time of discharge, then the research personnel will escort the subject and family to a private area to discuss the study and conduct the informed consent process. The approach to potential subjects in the ED will not be made in such a way that it interferes with or delays the diagnosis and treatment process in the ED. Potential subjects will be given as much time as needed to read the informed consent document, discuss it with family members if they choose, and to ask questions of the research personnel.

### 6.3 Electronic Informed Consent (eConsent)

To accommodate any current and future local restrictions on enrollment into research studies during COVID-19, and other similar circumstances, sites should comply with local practices/guidance regarding informed consent procedures. If informed consent can be obtained remotely (i.e., by eConsent) through a secure and locally approved platform (e.g., RedCap, Docusign, etc.), sites should get IRB approval to do so. All eConsent procedures should be documented according to local procedures, and included in the participant's study record in the TRACK-TBI electronic database (i.e., QuesGen).

### 6.4 Competency Screening and Legally Authorized Representatives

TBI often results in a period of posttraumatic amnesia (PTA) characterized by confusion, disorientation, and impaired memory for ongoing events. Thus, TBI patients will be screened for competency using the Galveston Orientation and Amnesia Test (GOAT) to determine whether they are competent to provide informed consent

or whether this must be done by a Legally Authorized Representative (LAR). This competency screening will be performed prior to inviting the subject to participate in the study and while the subject is in the ED or hospital.

The procedure for this competency screening will be:

1. The subject's ED medical record will be reviewed to determine whether the subject has been diagnosed with post-traumatic amnesia or other cognitive deficits.
2. The subject and family, if present, will be approached and informed about the study.
3. If subject and family agree, the GOAT will be administered according to standard procedures.
    a. If the subject scores ≥75 on the GOAT, the subject will be deemed competent to provide informed consent.
    b. If the subject scores <75 on the GOAT, then informed consent must be provided by a LAR.

**6.5 Language and Literacy**

Subjects should be fluent in English to be eligible for the study. Sites with research personnel fluent in Spanish may elect to enroll patients who speak Spanish as their primary language. The informed consent documents are available in both English and Spanish. Patients not fluent in English, or Spanish at certain sites, will be ineligible for the study.

**6.6 Need for Re-consent**

As this is a longitudinal study with multiple assessment time points over the course of 3 months, and knowing that the status of TBI patient cognition may change over this time course, it is likely that subjects may not recall all of the activities or procedures associated with each follow-up visit. To ensure that subjects are still willing to participate, the research personnel will review the Informed Consent document with the subject at the beginning of each follow-up visit.

In the event that a subject was determined incompetent to sign their informed consent document (i.e., GOAT score <75 at time of screening/consent) but later demonstrates competency, then the subject will be asked if they wish to continue participation. If so, then the subject will be asked to sign the Informed Consent Form at that time. If they decline to do so, they will be withdrawn from the protocol.

**6.7 Storage of Consent Documents**

Signed paper consent forms will be stored in a locked file cabinet located in the study office behind locked doors at each site. These documents will be stored for a minimum of 5 years after the conclusion of the study. These documents will be made available, as needed, for review for quality monitoring purposes.

**6.8 Waiver of Consent**

Sites may elect to enroll qualifying patients initially incapable of informed consent who have no legally authorized representative available for LAR consent. This can be done under a "waiver of consent" rule in the emergency setting in order to procure and process all study blood collections. While UCSF will serve as the single IRB (sIRB) for this study (further details about this below), each site who signs on to the study will be able to determine, independently, if they will implement the Waiver of Consent at their local site.
This protocol meets all "Waiver of Consent" criteria under 45 CFR 46.116(f) (3):

(i) The research involves no more than minimal risk to the subjects;

(ii) The research could not practicably be carried out without the requested waiver or alteration. Our research team has extensive experience conducting research in this population; TBI patients very frequently present alone (without a LAR) and with alteration of mental status (ranging from mild disorientation to coma) limiting capacity for self-consent due either to their acute injury or other contraindications.

(iii) The research could not practicably be carried out without using information or biospecimens in an identifiable format. We need to collect identifiable private information (such as patient name, medical record number, and contact information) from patients under waiver so that we can extract data from the EMR, contact a LAR to provide consent as soon as possible, and so that we can complete additional longitudinal follow-up timepoints with the patient/LAR and link this data to previously collected data) in the event that informed consent is obtained;
(iv) The waiver or alteration will not adversely affect the rights and welfare of the subjects. The primary risks of this observational study is loss of privacy and standard risks associated with a blood draw. We have taken steps to ensure the privacy of subjects. In most cases, blood draws will be collected through already placed arterial lines and/or at the same time of clinically indicated blood draws, and thus, there will be minimal to no additional risks associated with these blood draws. Furthermore, in the event that patient or LAR consent cannot be obtained by the time of the 3 month study visit, all data collected on the patient, including blood collected and processed, will be discarded (will not be analyzed, banked, or shared with collaborators);
(v) Whenever appropriate, the subjects or LAR will be provided with additional pertinent information after participation. We will make every effort to obtain patient or LAR consent as soon as possible after enrollment under waiver. A copy of the consent form as well as a study summary document that summarizes the pertinent details about the study will be left at the bedside of a patient enrolled under Waiver of Consent, and study staff will stay in touch with hospital staff to monitor the patient's status while inpatient. If a patient is discharged while still under waiver, then the patient will be discharged with a copy of the consent form, a study summary document that summarizes the pertinent details about the study, and contact information for study staff. Study staff will approach in-person (if the patient remains inpatient/the LAR presents to the bedside) or by phone (if the patient is discharged/a LARis identified who is off-site) to complete the informed consent process as soon as the patient may be able to consent for her/himself and/or a LAR presents her/himself. If self or LAR consent is not obtained by the time of the 3 month study visit, then all data and blood collected under waiver will be destroyed/discarded.

> Suggested IRB language: *If an eligible TBI patient presents to our hospital who is not capable of self-consent, and there is no LAR identified, then a 'Waiver of Consent' will be used to collect baseline clinical data and study blood draws (most critically, the baseline blood draw must be within 6 hours of injury and ideally as soon as possible after injury). Specifically, we will complete study blood draws, blood processing and freezing in our local freezer, and collection of baseline clinical data (including data from the EMR and head CT imaging that was obtained as part of routine clinical care) without consent if the subject does not have the capacity to provide consent and there is no LAR identified. The collected blood will NOT be analyzed in any way (either proteomic or genetic analyses), shared with collaborators, or sent to the National TBI Biorepository (NTBI-BR) at the University of Pittsburgh Medical Center without informed consent from the patient or LAR. Study staff will continue to follow the patient through the hospital course and check daily for capacity to consent or for an available LAR. The length of the study is three months and staff will continue to follow the patient throughout this time period in order to obtain consent from the patient or LAR. In the event that the patient remains incapable of self-consent or a LAR is not identified by the time of the 3 month visit, then all clinical data will be destroyed and all collected blood samples will be discarded. If the patient regains capacity to consent or a LAR is identified before the time of the first follow-up and s/he declines future participation in the study, then the patient/LAR will then be given the opportunity to provide informed consent to permit the use of any already-collected clinical data and/or blood (that was collected under waiver) for research purposes. If the patient/LAR consents to the use of already collected data, then s/he will sign the informed consent form but will then be documented as electively withdrawing from future participation in any additional follow-up data collection.*

### 6.9 Single Institutional Review Board (sIRB) and Human Research Protections Office

As of January 2020, all federally-funded studies are now required to use a Single Institutional Review Board (sIRB). UCSF will serve as the sIRB for this TRACK-TBI Precision Medicine protocol. Participating sites will rely on UCSF as the IRB of Record for this protocol. The UCSF Lead Coordinating Site Study Team is responsible for securing initial IRB approval from UCSF for this MSP (Master Study Protocol) and all other study documents such as the Informed Consent Form, any recruitment materials, and all study Standard Operating Procedures (SOPs) that describe the procedures and operations of the study as they pertain to enrolled subjects. Other sites cannot be added to the protocol until UCSF has initial approval for the study. The UCSF Lead Coordinating Site Study Team will utilize the online SMART IRB system to request, track, and document reliance on UCSF IRB for each Relying Site under the SMART IRB Agreement.

All relying sites' Study Teams will use the Informed Consent Form template(s) provided by the UCSF Lead Coordinating Site Study Team. Site Study Teams will provide any site-specific informed consent form language required for local study team contact details, compensation for injury, study participant reimbursement, etc. The Site Study Team will incorporate these site-specific changes to the Informed Consent with tracked changes, and will provide this form to the UCSF Lead Coordinating Site Study Team for submission to UCSF IRB.

The UCSF Lead Coordinating Site Study Team will be responsible for submitting amendments to the MSP as well as any unanticipated events, protocol deviations, adverse events, and annual continuing reviews, in accordance with UCSF IRB's established procedures and policies. The UCSF Lead Coordinating Site Study Team and participating relying Site Study Teams will follow the procedures as set forth in the UCSF sIRB Standard Operations Procedures (See UCSF sIRB SOP for sites v2.docx saved in Precision Medicine dropbox folder).

After IRB approval is obtained, the approved master study protocol (MSP) and supporting documents will be submitted to the Department of Defense Human Research Protection Office for review. Human subjects research cannot begin at a site until HRPO approval has been obtained.

## 7 SUBJECT PROCEDURES BY CORE

### 7.1 Clinical

The following broad categories of clinical data variable types will be collected from all enrolled patients through medical record and personal interview:

- Baseline demographics e.g., age, gender, race, ethnicity, handedness
- Baseline socioeconomics e.g., education, employment, living situation, types of support
- Baseline medical history by system including substance abuse, prior TBI, and medications
- Mechanism of injury, location, and surrounding circumstances
- Pre-hospital clinical course variables e.g., vital signs, transport times, GCS score
- Brain CT report including presence of skull fracture and intracranial abnormalities
- Emergency department clinical course e.g., vital signs, GCS, fluids, labs, toxicology, complications
- Hospital admission clinical course e.g., complications, surgeries, neuromonitoring
- Hospital daily therapeutic intensity level for ICU patients with neuromonitoring
- Admit and discharge dates and times throughout full clinical course
- Abbreviated Injury Scale (AIS) score and Injury Severity Score (ISS)
- Discharge destination and acute care outcome evaluation

### 7.2 Biospecimens Procedures.

We will obtain 16.0 ml of blood at each of the following time points: within 6 hours of injury, and again at 12h, 24h, 2d, 3d, 5day, 2w, 6w and 3m post-injury (See **Table 6**: Biospecimen Sample Collection Schedule below). Every blood draw will be optional. The TRACK-TBI Precision Medicine procedures around collection,

processing, storage, and shipping of biospecimens collected during the in-person assessment will align with the TRACK-TBI U01 procedures (see TRACK-TBI Precision Medicine Biospecimens SOP on Dropbox at Dropbox\1-TRACK TBI Doc Share\biospecimens core).

- Collection at each time point will consist of one 8.0 ml lavender EDTA tube (plasma and buffy coat) and one 8.0 ml red top tube (serum).
- Whole blood will be processed for serum, plasma, and buffy coat. Serum and plasma will be stored in 500 µl aliquots for future analyses.

***Table 6****. Biospecimen Sample Collection Schedule*

| *Schedule of blood sample Collection (From the time of traumatic brain injury)* | *<6 hr* | *12 hr* | *24 hr* | *Day 2* | *Day 3* | *Day 5* | *Day 14* | *Day 42* | *Day 90* |
|---|---|---|---|---|---|---|---|---|---|
| *Blood volume to be collected at each time point* | 16 mL | 16 mL | 16 mL | 16 mL | 16 mL | 16 mL | 16 mL | 16 mL | 16 mL |
| ***Total blood volume that may be collected in 3 month period=144 ml*** | | | | | | | | | |

- At some sites (subject to IRB approval), cerebrospinal fluid (CSF) will be drawn for patients with ventricular catheters. The CSF collection protocol is detailed in TRACK TBI Precision Medicine Manual Of Procedures (Section 6 of TRACK-PM_MOP_v1.4_20Oct2020). *This is a site-specific protocol item.*
- At some sites (subject to IRB approval), in the case of death, the subject's next of kin will be contacted to request donation of the brain for banking, validation studies of imaging and biomarker findings, and further research. See the TRACK-TBI Brain Donation Protocol for more information (Dropbox\1-TRACK TBI Doc Share\clinical core\Brain Donation Protocol). *This is a site-specific protocol item.*
- Specimens will be kept indefinitely until they are used up or destroyed, and may be used in future research unrelated to this study.

*Analysis.*

A subset of the most promising DAI, MVI, and neuroinflammation blood-based markers identified previously in Phase 1-Base (under a separate IRB protocol) will be analyzed in this cohort. In addition, neuronal injury (UCH-L-1) and astroglial injury (GFAP) biomarkers will also be assayed in these subjects.

**7.3 Neuroimaging**

*Clinical Care Neuroimaging Acquisition*

CT or initial MRI will be obtained as part of clinical care. The subject may be transferred to the study hospital from another hospital at which an initial CT/MRI was already performed. In this case, the site must have the initial image available for collection in order to enroll. All initial and follow-up brain CT scans, and any brain MRI scans that are collected for clinical care will be acquired along with the radiology reports. Images will be read and coded by the Neuroimaging Core radiologist in accordance with the NINDS Neuroimaging TBI-CDEs.

*MRI Procedures*

In this cohort, we will obtain 3T MRIs within 2 week of injury (early), and for a subset of 20% of patients, an additional ultra-early MRI within 24-48 hours of injury. All patients will also receive a follow-up MRI at 3m post-injury. The overall MRI protocol is based on established TRACK-TBI standards for structural imaging, DTI and rs-fMRI that have been harmonized across 3T scanners from all 3 MR vendor platforms. Standard operating procedures for acquisition, QA, QC, and data management of this 3T MRI protocol will align with the TRACK-TBI U01 procedures. Participants will undergo the same MRI procedures as set forth in the TRACK U01 study (Precision Medicine-Neuroimaging SOPfinal-08032020.docx) with a few modifications. In addition to volumetrics, DTI and rs-fMRI, the new MRI protocol will incorporate novel imaging measures of axonal density (using neurite density index (NDI) from NODDI analysis of multishell diffusion MRI), cerebral blood flow (using

ASL perfusion), and neuroinflammation (using free water content (FISO) from NODDI analysis of multishell diffusion MRI), which will all be standardized across sites and MRI vendors prior to participant enrollment.

The proposed MRI protocol includes one additional sequence not represented in the original TRACK-TBI protocol: ASL perfusion imaging of cerebral blood flow (CBF), as well as an additional 64-direction diffusion-weighted shell for the DTI protocol at b=3000 s/mm2 to create the multishell diffusion MRI sequence.

Arterial Spin Labeled Perfusion MRI Protocol: The ASL perfusion protocol is adopted from the new Alzheimer Disease Neuroimaging Initiative 3 (ADNI3) standards for all 3 MR vendors. In brief, the 5-minute sequence consists of 3D pseudo-continuous ASL (PCASL) on 3T GE scanners and 2D pulsed ASL (PASL) on Siemens and Philips scanners, with an additional proton density reference scan using the same ASL readout at a longer TR. These ASL acquisitions for all 3 MR vendor platforms conform to the most recent best practice guidelines for ASL perfusion imaging reported by the International Society for Magnetic Resonance in Medicine.44

The entire proposed MRI protocol can be acquired in 60 minutes. The de-identified neuroimages from each site will be uploaded to the neuroimaging core repository at UCSF.

*MRI Analysis*

We will incorporate axonal density from NODDI analysis of multishell diffusion MRI as an advanced microstructural imaging biomarker of both acute DAI and long-term white matter degeneration. Axonal density, measured as the neurite density index (NDI) of white matter from NODDI, is a metric of the intracellular volume fraction and is not affected by changes in the free water fraction. Therefore, it is a more specific biomarker of axonal loss than any DTI metric such as FA, MD, AD, or RD. In addition, we will incorporate a quantitative imaging biomarker of MVI, specifically, CBF using ASL perfusion MRI.

*MRI Statistical Considerations*

We will calculate Pearson's correlation of each of the baseline early (<6 hours to 1 week) blood-based biomarkers, with the corresponding MRI markers that look promising based on Phase 1 results. There will be no adjustment for multiple comparisons as these comparisons are exploratory given the paucity of information about the time-course of these markers in people with TBI. ROC analysis will be performed to determine the discriminative ability of the blood-based and imaging biomarkers in predicting outcomes. AUC will be calculated and reported along with 95% confidence intervals. Prediction increment will be measured by change in AUCs calculated based on logistic regression models.

Adequacy of sample size: With 50 participants, using correlation power analysis, we have at least 80% power to detect a correlation of .38 or greater.

**7.4 Outcomes**

All clinical outcome assessment (COA) measures will be obtained from the patient, or if cognitively unable, the surrogate (i.e., caregiver). The enrollment outcome assessment battery will be obtained at consent or by phone within 24 hours of injury. It will be administered only to participants who score ≥75 on the Galveston Orientation and Amnesia Test (GOAT). At subsequent follow-up visits, the participants will undergo in-person outcomes testing according to the Flexible Outcome Assessment Battery Decision Workflow (see **Figure 1**) at 2 weeks, 6 weeks, and 3 months from the time of injury. All measures that can be collected by phone will be collected by phone (see Section 8 TRACK-TBI PRECISION MEDICINE ASSESSMENT BATTERY AND ORDER OF ADMINISTRATION tables below), if study staff cannot administer in person.

*Flexible Clinical Outcome Assessment (COA) Battery Framework*

The Flexible Outcome Assessment Battery is designed to assess multiple outcome domains across all phases of recovery in patients at all levels of TBI severity. The battery comprises the original Core CDE measures (TBI-CDE Version 1.0) administered in the TRACK-TBI Pilot study, and additional Basic and Supplemental CDEs (TBI-CDE Version 2.0) that further assess psychological health and cognition. The combined TBI-CDE versions constitute

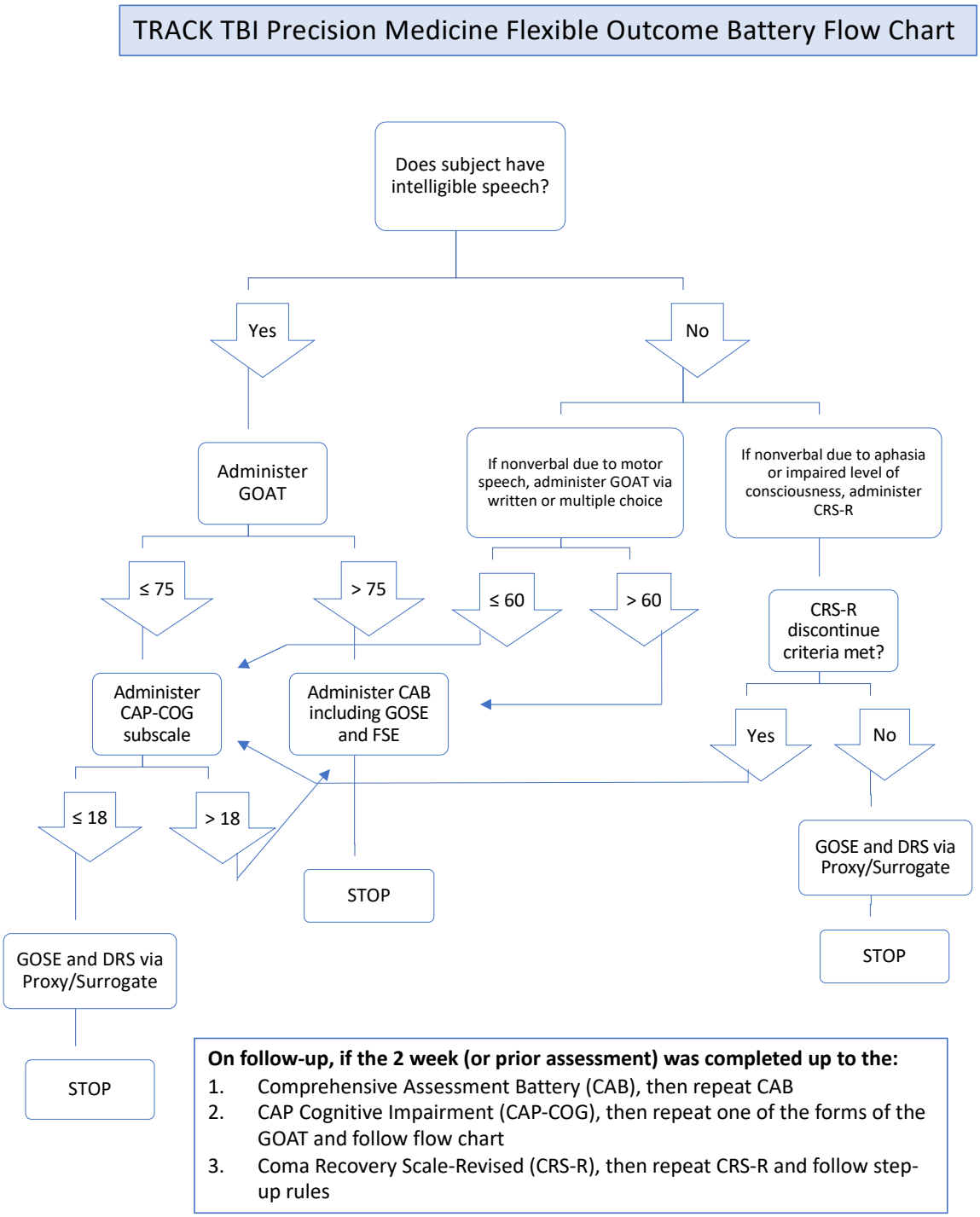
the Comprehensive Assessment Battery (detailed in section 8.2 below). Patients who are too impaired to tolerate the Comprehensive Assessment Battery will undergo assessment on the Abbreviated Assessment Battery (detailed in section 8.3 below), which consists of standardized measures of basic neurobehavioral (e.g., Coma Recovery Scale-Revised [CRSR]) and cognitive (e.g., Confusion Assessment Protocol [CAP]) function. See **Figure 1** for the Flexible Outcomes Assessment Battery Framework Decision Workflow.

*Minimizing in-person outcome assessment procedures and conducting remote outcome assessment*

To accommodate any current and future local restrictions on research study activities during COVID-19, and other similar circumstances, sites should comply with local practices/guidance and reach out to UCSF if they require further guidance on completion of TRACK-TBI-related study activities. The utility and necessity of these procedures will be evaluated over time to see if they need to be modified due to COVID-19, or kept in place after COVID-19 restrictions are lifted.

- **Self-report/Interview measures**: If the PI and study team deem that it is safer to minimize study staff and subject face-to-face contact for study visits that would otherwise be in-person, all self-report and interview outcome measures can and should be completed remotely (e.g., telephone, secure Zoom, or other supported and secure platform) with application of the appropriate test completion code of "1.3 valid administration collected by phone" (even when using Zoom or other, similar platform) with a note added into QuesGen that the visit was conducted by phone due to COVID (or other similar circumstance).
- **Performance Measures**: If it is possible to conduct a shortened in-person visit to administer the cognitive measures that must be collected in-person, sites should do so following all TRACK-TBI procedures and implementing all local safety practices. If it is deemed unsafe to have a shortened in-person visit to collect these cognitive measures, a test completion code of "3.6 Test not attempted due to logistical reasons" should be entered on the electronic and paper CRFs (if paper CRFs are able to be used) with a note added into QuesGen that the visit was conducted by phone due to COVID (or other similar circumstance).
- **In-person procedures that cannot be completed remotely**: MRI and blood collection procedures can only be conducted in-person and should only be attempted once TRACK-TBI leadership has given approval, and any local restrictions on in-person research study activities has been lifted.
- **Paper case report form completion during remote study activities**: The standard for TRACK-TBI studies is to directly enter data onto paper case report forms and then enter the data into the QuesGen electronic database. If remote collection of data is necessary and access to a printer is limited, **direct data entry into QuesGen is acceptable**.

***Figure 1**: Flexible Outcomes Assessment Battery Framework: Decision Workflow*



## 8 TRACK-TBI PRECISION MEDICINE FLEXIBLE OUTCOME ASSESSMENT BATTERY AND ORDER OF ADMINISTRATION

### 8.1 Enrollment Battery (at consent or by phone within 24 hours of injury): ~11 minutes

1. Rivermead Post Concussion Symptoms Questionnaire (RPQ): 3 mins
2. Brief Symptom Inventory 18 (BSI-18): 3 min
3. Standard Assessment of Concussion* (SAC): 5 min

The enrollment outcome assessment battery will be administered only to participants who score ≥75 on the Galveston Orientation and Amnesia Test (GOAT).

*The SAC can only be collected in-person, and cannot be collected by telephone.

### 8.2 Comprehensive Assessment (CA) Battery (2 W, 6 W, 3 M) 91-108 minutes

| Domain | Subdomain | Instrument | Administration Time | Order of Administration In person (~98 min) |
|---|---|---|---|---|
| Screening | Screening | Assessment of Speech Intelligibility* | 2 min | 1, and as needed |
| | | Galveston Orientation and Amnesia Test (GOAT)* | 5 min | 2, and as needed |
| History | Participant Interview | Interview to update occupational status; living situation; medical history (e.g., known neurologic, cognitive, psychiatric conditions)* | 10 min[#] | 3 |
| Daily/Global Function | Global Outcomes | Glasgow Outcome Scale Extended (GOSE)* | 15 min | 5 |
| | | Functional Status Exam (FSE)* | 10 min | 4 |
| Psychological Health/ Neurobehavioral Symptoms | Depression, Anxiety, Somatic | Brief Symptom Inventory-18 (BSI-18)* | 3 min | 13 |
| | TBI-Related Symptoms | Rivermead Post Concussion Symptoms Questionnaire (RPQ)* | 3 min | 11 |
| | Post-traumatic stress | PTSD Checklist for DSM-5 (PCL-5)* | 3 min | 12 |
| | Suicide | Columbia Suicide Severity Rating Scale Screening Version[+] (C-SSRS)* | 5 min | As needed |
| | Life Quality (Brain) | Quality of Life after Brain Injury Overall Scale (QoLIBRI-OS)* | 3 min | 7 |
| Symptom Validity | Symptom Validity | Structured Inventory of Malingered Symptomatology (SIMS)* | 10 min | 6 |
| Cognitive Performance | Episodic Memory | Rey Auditory Verbal Learning Test (RAVLT) | 15 min | 8 (delay after BSI-18) |
| | Executive Function | Trail Making Test A+B | 5 min | 9 |
| | Processing Speed | Wechsler Adult Intelligence Scale – 4th Edition Processing Speed Index (WAIS-IV) PSI | 4 min | 10 |
| Motor | Fine motor (Bradykinesia) | Finger tapping | 5 min | 14 |
| | Gross motor/mobility | Short Physical Performance Battery (SPPB) | 5 min | 15 |

[+]Triggered by /BSI-18

*Measures that should be collected over the phone

**8.3 Abbreviated Assessment (AA) Battery (2 W, 6 W, 3 M) 60-67 minutes**

| Domain | Subdomain | Instrument | Administration Time | Order of Administration In person (~72 min) |
|---|---|---|---|---|
| Screening | Screening | Assessment of Speech Intelligibility* | 2 min | 1, and as needed |
| | | Galveston Orientation and Amnesia Test* | 5 min | 2, and as needed |
| Consciousness and Basic Cognition | Confusion | Confusion Assessment Protocol (CAP) | 15 min | Determined by Flexible Outcome Battery Flow Chart (page 18 of TRACK-TBI Outcomes SOP V 10) |
| | Consciousness | Coma Recovery Scale Revised (CRS-R) | 15-30 min | |
| Daily/Global Function | Global Outcomes | Glasgow Outcome Scale Extended (GOSE)* | 15 min# | 3 |
| | | Disability Rating Scale (DRS)* | 10 min# | 4 |

*Measures that should be collected over the phone

## 9 TRACK-TBI PRECISION MEDICINE ASSESSMENT BATTERY: DESCRIPTION OF MEASURES

### 9.1 Measures to Screen Competency and Selection of Battery

*Speech Intelligibility*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The Speech Intelligibility measure is administered at the beginning of the assessment to determine if the Abbreviated Assessment Battery should be administered and whether to administer the CAP and/or CRS-R. This measure can also be administered at any point during a phone or in-person assessment, if the study coordinator has any concerns about the participant's capacity to consent and complete an assessment.

*Galveston Orientation and Amnesia Test*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The GOAT measure is administered at the beginning of the assessment to determine if the Abbreviated Assessment Battery should be administered and whether to administer the CAP and/or CRS-R. This measure can also be administered at any point during the phone or in-person assessment, if the study coordinator has any concerns about the participant's capacity to consent and complete an assessment.

### 9.2 Interviews

*Participant Interview*

This interview is administered and responses recorded in the same way as described in the TRACK-TBI Outcomes Assessment SOP for the Participant/Surrogate Interview. The TRACK-TBI Precision Medicine participant interview is based on the TRACK-TBI 3M follow-up Participant Interview and adds a neurologic screen for epilepsy as well as questions pertaining to the impact of the COVID-19 pandemic. The Covid-19 survey questions will assess the impact COVID-19 had or is having on the participant and/or someone close to the participant. The Participant Interview should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

### 9.3 Measures of Daily/Global Function

*Functional Status Exam (FSE)*

The FSE[47] measures change in functional status specifically due to traumatic injury. The measure can be administered in relation to changes due to TBI only or both the changes associated with TBI and peripheral injuries. This measure covers 7 areas of functioning: personal care, ambulation, mobility, major activities (i.e.,

work, school), home management, leisure and recreation and social integration. These areas are evaluated using the concept of dependency to operationally define outcome at four levels. The first level signifies no change, the second level signifies difficulty in performing the activity although the person is still independent, the third level signifies dependence on others some of the time, and the fourth level signifies non-performance or inability to perform the activity or total dependence on others. A total score is generated by summing scores from the 7 categories, yielding a range from 0 (return to pre-injury baseline in all areas) to 21 (total dependence on others or can no longer perform any activities across functional areas). Persons who die are assigned a total score of 22. This measure will also be collected from the surrogate. The FSE measure should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.
Additional information regarding the administration of the FSE can be found in the pdf "Functional Status Examination Manual" on Dropbox (Dropbox\1-TRACK TBI Doc Share\TRACK LONG\LONG outcomes training and administration guidance).

*Glasgow Outcome Scale- Extended (GOSE)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. A "TBI" score will be collected for the purposes of TRACK-TBI Precision Medicine. The GOSE should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

*Scoring the GOSE in Relation to the FSE*

There is considerable overlap in the item content of the FSE and GOSE. Because the FSE is administered before the GOSE, the examiner will have extracted information from the subject during administration of the FSE that can be used to score the GOSE. Although it is necessary to independently administer and score *all* the GOSE areas, information obtained during the FSE interview that relates to a specific GOSE area can be directly applied to the GOSE rating. This approach will minimize subject burden and help reduce the completion time of the TRACK-TBI Precision Medicine battery.

*Standard Assessment of Concussion (SAC)*

The Standardized Assessment of Concussion (SAC)[48] is a brief screening instrument designed for the neurocognitive assessment of concussion by a non-neuropsychologist without prior expertise in psychometric testing. The SAC requires approximately 5-10 minutes to administer and includes measures of orientation, immediate memory, concentration, and delayed recall, summing to a total composite score of 30 points, with a higher score indicating better cognitive functioning. The SAC will be obtained only at enrollment and must be collected in-person; the SAC cannot be collected by telephone.

*Disability Rating Scale (DRS)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The DRS measure will be administered during the in-person assessments but can be collected by phone. Only the DRS Caregiver version will be collected in this study.

**9.4 Measures of Psychological Health/Neurobehavioral Symptoms**

*Brief Symptom Inventory 18 (BSI-18)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The BSI-18 measure should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

*Rivermead Post Concussion Symptoms Questionnaire (RPQ)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The RPQ measure should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

*Posttraumatic Stress Disorder Checklist (PCL-5)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The PCL-5 should be administered by telephone but can also be administered in-person if

permitted by local policy and acceptable to the subject or LAR.

*Quality of Life After Brain Injury- Overall Scale (QoLIBRI-OS)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The QoLIBRI-OS should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

*Columbia Suicide Severity Rating Scale (C-SSRS) Screening Version*

The Screening Version of the Columbia will be administered if the participant answers >1 on Q#17 of the BSI-18 (this is the same triggering criteria in TRACK-TBI U01). The Screening Version is a shortened form of the original "Baseline" and "Since Last Visit" forms that assesses suicidal ideation and behavior in the last month, and offers helpful triage categories based on severity. If the participant endorses YES on any question considered "Moderate Risk" (i.e., orange level) or "High Risk" (i.e., red level), examiners should proceed to administer the **TRACK-TBI Suicide Protocol and Safety Plan** found on Dropbox in the "Outcomes Core SOP" folder. The C-SSRS measure will be administered during the in-person assessments but can be collected by phone.

### 9.5 Measure of Symptom Validity

*Structured Inventory of Malingered Symptomatology (SIMS)*

The Structured Inventory of Malingered Symptomatology™ (SIMS™)[49] is a 75-item, true-or-false screening instrument that assesses both malingered psychopathology and neuropsychological symptoms. It is a multi-axial, self-administered measure developed to serve as a screening tool for the detection of feigned or exaggerated psychiatric disturbance and cognitive dysfunction among adults ages 18 years and older across a variety of clinical and forensic settings. The SIMS consists of 75 items that yield a summary score reflective of a general feigning presentation (Total score), as well as five nonoverlapping scales that reflect theoretical and statistical considerations of the more commonly feigned or exaggerated disorders: (a) Psychosis, (b) Neurologic Impairment, (c) Amnestic Disorders, (d) Low Intelligence, and (e) Affective Disorders. The SIMS is intended to serve multiple functions as (a) an initial screening tool for individuals who may not otherwise be referred for specific evaluation of potential feigning within a forensic or medico-legal context or setting; (b) an initial screening tool for individuals suspected of feigning to determine the need for more extensive evaluation; and (c) convergent data in a comprehensive evaluation for potential feigning. The SIMS' brief, easily administered self-report format and fifth-grade reading level reduce clinician burden and allow for completion by a wide range of individuals at varying educational/cognitive levels. The SIMS measure should be administered by telephone but can also be administered in-person if permitted by local policy and acceptable to the subject or LAR.

### 9.6 Measures of Cognitive Performance

*Rey Auditory Verbal Learning Test (RAVLT)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP, and can only be collected at an in-person assessment. The Delayed Recall trial will be administered after the BSI-18.

*Trail Making Test*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP, and can only be collected at an in-person assessment.

*Wechsler Adult Intelligence Scale – 4th Edition Processing Speed Index (WAIS-IV) PSI*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP, and can only be collected at an in-person assessment.

### 9.7 Measures of Motor Function

*Finger tapping*

The Finger Tapping Test (FTT) measure is one of the most widely used measures of motor functioning While there are several FTTs available, the most popular and well-known administration of this test is as part of the

Halstead–Reitan Neuropsychological Test Battery (HRNB)[50]. The purpose of this test is to measure the tapping speed of the index finger of each hand. A finger tapping counter device ("key") is provided and should be used for this test. Examinees are instructed to place their hand on the board, allowing only the index finger to move. The base of the hand (not the palm), the thumb and the tips of the other fingers should rest on the board (the hand will be slightly cupped). They then raise and lower the index finger of their dominant hand for five consecutive trials, each lasting 10 seconds, enough to cause the counter on the device to record each tap (or oscillation). This procedure is then repeated for the non-dominant hand, with the requirement that the individual completes five trials within 5-point range. **For purposes of this study, two 10-second trials will be given for each hand during the in-person assessment.** Total number of finger taps for each trial on each hand should be recorded.

**Instructions for administrators.** Tell the Participant:

**Now we are going to do a test to see how fast you can tap. We will use this little key here** (show the key to the subject) **and I want you to tap just as fast as you can, using the forefinger** (point to the subject's index finger) **of your right hand** (or left, if the subject is left-handed). **When you do it, be sure to use a finger movement: do not move your whole hand or your arm. When you tap this key, you will have to remember to let the key come all the way up and click each time, or else the number on the dial won't change.**

(Demonstrate to the subject how the key operates and how it should be allowed to "click." Also, demonstrate actual tapping, for a five or six second period, going as fast as possible).

**Now you move the board to a comfortable position for your hand and try it for practice.** After a brief practice period, say: **remember to tap as rapidly as you possibly can.** Be sure that the subject knows what to do and is properly challenged to tap as fast as possible.

Then say: **alright. Ready! Go!**

Begin timing with a stop watch when the participant's finger touches the key. At the end of 10 seconds, say: **STOP!**

Note the number of taps on the dial when saying "STOP" as some participants may continue tapping.

The subject may rest his or her hand after any trial.

After completing the test consisting of two 10-second trials with the preferred hand, finger tapping speed for the index finger of the non-preferred hand is determined with two 10-second trials. Do not alternate between right and left-hand trials.

SCORING: The total number of finger taps for each trial on each hand should be recorded. So, a total of 4 raw scores will be recorded (2 for each hand).

**Issues in administration**

1. The base of the hand (not the palm), and other fingers should rest gently on the tapping board. The hand will have a slightly cupped look allowing the participant to reach the key easily.
2. At times, the participant's middle finger or thumb will also move as they are tapping. Cue the participant to keep their other fingers still. If it is still a problem, the examiner may hold down the middle finger. Usually placing your finger lightly on their fingernail is enough to prevent extra movement.
3. Some participants will want to make a fist. This can be allowed but for many participants using this method will cause excess movement in the hand. The examiner may want to encourage them to spread their hand out as described above.
4. If the participant is tapping but not bringing the key up far enough to record the tap, the examiner can remind them to do so. "Remember to bring the key all the way back up."

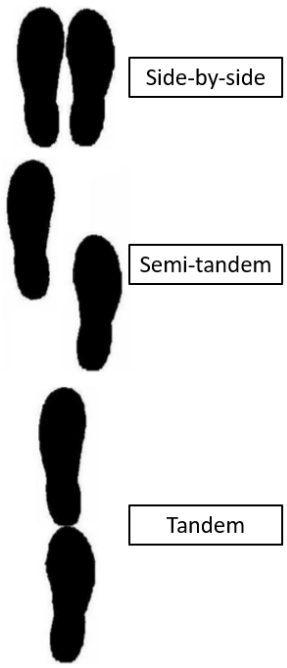
*When demonstrating speed for a significantly impaired participant, it is not necessary to go as fast as you can. Instead, demonstrate a moderated speed.

*Short physical performance battery (SPPB)*

The SPPB is a performance-based, three-part assessment that measures functional status and predicts future functional decline. The SPPB assesses gait speed, balance, and lower extremity strength (gross motor ability). The SPPB takes approximately 5-10 minutes to administer and will be assessed during the In-person Assessment. An online training video with a detailed explanation and administration instructions can be found here: *https://www.youtube.com/watch?v=N_rJOGhQqZ4*. This measure will require a Timer and measuring tape to administer, and can only be administered in person.

You will be testing the patient in three areas: Balance, Gait speed, and Lower Extremity strength. Each section is scored out of 4 points, so the highest total score for the SPPB is 12 points. Use the scoring sheet to calculate the total points.

**Balance Test** (3 different positions - The patient must be able to stand on their own without an assistive device, though you can help the patient get up if needed. If the patient cannot hold a posture for 10 seconds, skip the remaining balance postures and move to the next section of the test.)




**"I would like you to try to maintain your balance in different positions. I will describe and show each position to you, then I would like you to try to do it. If you cannot do a particular position or feel it would be unsafe, tell me and we will move onto the next activity. I do not want you to try any exercise you feel might not be safe. Do you have any questions before we begin?"**

**"Now I will show you the first position"**. (Demonstrate stance. Don't let the patient start yet)

**"You will stand with your feet together, side-by-side, for ten seconds. You may use your arms, bend your knees, or move your body to maintain your balance, but try not to move your feet. Try to hold this position until I tell you to stop."**

(Have patient assume position. Assess safety: be ready to stabilize patient if needed. Get ready with timer)

**"Ready?" … "Begin"** (Start Timer and tell patient to stop after 10 seconds.)

Demonstrate and give instructions for the semi-tandem and tandem foot positions. Stop after 10 seconds for each position. Assess the safety of patient for each stance. If the patient cannot hold a position for 10 seconds, score the section and move to Gait Speed Test.

**Gait Speed Test** (Make sure you have a 4-meter course measured out in advance and a timer that goes to the hundredths mark. If the patient uses a cane or other walking aid and feels they need it to walk a short distance, they can use it)

**"Now I'm going to observe how you normally walk. Here is our walking course. I want you to walk to the other end of the course at your usual speed, as if you were walking down the street to go to the grocery store. Walk all of the way PAST the end of the tape before you stop. Do you feel this would be safe?"**

(If the patient appears unstable, tell them that you will walk next to them.)

(Demonstrate the walk for the patient. Have the patient stand with both feet touching the starting line. Prepare the timer.)

**"Ready?" … "Begin"** (Start timer when the patient's foot crosses the line. Walk next to the patient for safety. Stop timer when BOTH of the patient's feet cross the line.)

If they score less than 4 points, repeat the walking test a second time and record the fastest time.

**Chair Stand Test** (Before testing the patient, you will make sure it is safe by having the patient complete one untimed chair stand.)

**"The last test measures the strength in your legs. Do you think it would be safe to try to stand up from the chair without using your arms?"** (If no, stop and record score as zero for this section.)

**"Fold your arms across your chest and sit so that your feet are flat on the floor. Now stand up keeping your arms folded across your chest."** (If patient cannot rise without using their arms, this is the end of their test. Record the results on the scoring sheet. If they are able to rise with their arms folded, continue with the chair stand test.)

**"Do you think it would be safe for you to try to stand up from a chair five times without using your arms?" "Please stand up straight as QUICKLY as you can five times, without stopping in between. After standing up each time, sit down and then stand up again. Keep your arms folded across your chest. I'll be timing you with a stopwatch. Let me demonstrate.** (Demonstrate)

**Do you have any questions? Remember to do this as QUICKLY as you can five times. Ready? ... Stand."** (Begin timing when the patient starts to rise. Count out loud as the patient stands each time, up to 5 times. Stop if the patient becomes tired or short of breath during repeated chair stands. Stop the stopwatch when the patient has straightened up completely for the fifth time. Also stop if the patient uses their arms, has not completed 5 rises by 1 minute, and at your discretion if you are concerned for patient safety.)

### 9.8 Measures of Consciousness and Basic Cognition

*Confusion Assessment Protocol (CAP)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP. The CAP measure can only be collected during an in-person assessment.

*Coma Recovery Scale Revised (CRS-R)*

This measure is administered and scored in the same way as described in the TRACK-TBI Outcomes Assessment SOP, and can only be collected during an in-person assessment.

## 10 Protocol for Sharing Outcome Data with Participants

Release of outcomes testing results is a site-by-site issue to be addressed in accordance with local IRB and Risk Management policies. Upon request, sites that agree to provide results to subjects can do so after completion of the Precision Medicine battery using the following guidance:

- Information will be released only after a written request has been made by the subject or the guardian.
- The study PI should ensure that the results are communicated only by a licensed psychologist (neuropsychologist) who is familiar with the TRACK Precision Medicine outcome assessment battery, and has been authorized by the site PI to serve in this capacity. This consultation can be completed in person or over the telephone.
- If a licensed psychologist is not available, the information should be released in the form of raw data with the name of the measure and the score without any interpretation.
- A disclaimer statement must be included in the released records (i.e., "These data are not meant to replace diagnostic testing/evaluation that would be ordered by a personal physician. We cannot interpret the data and provide recommendations as the data we collect is meant for research purposes only.")
- Test record sheets *should not be released under any circumstances* (risk of copyright violation and test invalidation), and any outcome data provided will be stripped of the Study ID.

**11 Examiner Training and Certification Procedures**

All examiners are required to complete CITI and HIPAA training in accord with local IRB requirements. In addition, they will be required to demonstrate competency in administration and scoring of all the measures included in the Precision Medicine outcome assessment battery. Training seminars will be conducted via webinar and will be supplemented with printed materials. Training materials and CRFs for all assessment measures can be found on Dropbox. Competency in administration and scoring of the Precision Medicine battery will be established through review of videotaped simulated assessment sessions prepared by the examiner. Videotapes will be reviewed and certified by members of the Outcomes core. Examiners who have been previously certified on the TRACK-TBI battery will only be required to prepare video simulations for new measures that have been added to the Precision Medicine battery.

After recording simulated test administration, simulations and scanned copies of the paper CRFs should be uploaded to Dropbox electronically by requesting an invitation link from Dr. Sabrina Taylor (Sabrina.Taylor@ucsf.edu). **Do not post any videos containing test material to publicly accessible websites such as YouTube.**

**12 SUBJECT RISKS AND BENEFITS**

**12.1 Foreseeable Risks By Core**

The potential risks to the subject are minimal across all domains of data collection. The subject's signed consent form may become part of their medical record, but no research data collected as part of this study will become part of the subject's medical record.

**Clinical.** The TBI event will already be part of the subject's medical record, so involvement in this study will not have any effect on obtaining care or coverage under insurance. The risks involve some degree of loss of privacy. This will be minimized as much as possible. All data will be confidential and stored in locked areas to which only authorized study personnel have access. Records will be coded with a Study ID as soon as the patient is enrolled so that names and other identifying information will not be linked to personal or sensitive data, in compliance with federal regulations of the Health Insurance Portability and Accountability Act (HIPAA). The Study ID is automatically generated by the QuesGen System as soon as the patient is entered into the database. In addition, subjects and their families will be informed that participation is completely voluntary, that they may decline response to any questions, and that they may withdraw from the study at any time, all without jeopardizing medical treatment to which they are otherwise entitled. Subjects and their families will not be required to answer any interview or assessment questions that they find distressing or sensitive in nature.

**Biospecimens.** The blood sample will be drawn from an arterial or central venous catheter placed as a part of standard care for those patients consented while in the ICU. Those patients consented on the ward or in the emergency room will need to undergo phlebotomy, but every attempt will be made to collect the blood sample at the same time a clinical sample is drawn to reduce needle sticks. The subject may experience the discomfort associated with a needle stick and may suffer bruising at the site of the needle stick. No more than two venipuncture attempts will take place.

**Genetic Research.** There is a possibility that, if the results of a research study involving genetic material were to become generally known, this information could affect one's ability to be insured, employed, future decisions regarding children, or family relationships. As noted, all data will be de-identified and linked by Study ID. Data will be stored in locked areas to which only authorized study personnel have access.

The Genetic Information Nondiscrimination Act of 2008 (GINA) prevents employers and health insurers from discriminating based on genetic information. Below GINA language will be included in the informed consent form for this study:

*A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:*

- *Health insurance companies and group health plans may not request your genetic information that we get from this research.*
- *Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.*
- *Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.*

*Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.*

**Neuroimaging.** Participants will undergo noninvasive brain imaging using FDA-approved 3 Tesla MR scanners within 24-48hr, at 2 weeks and at 3 months post-injury. No exogenous contrast agents and no sedation will be used. The MRI procedures are noninvasive and painless. The MRI does require the subject to lie still with the head and part of the body confined in a tunnel-like device for a considerable length of time (total scan time of approximately 60 minutes). The subject will have frequent communications with the MRI technologist and study staff, and will be able to stop the MRI scan at any time. Contraindications for the MRI examination include those who have cardiac pacemakers, neural pacemakers, surgical clips in the brain or blood vessels, surgically implanted steel plates, screws or pins, cochlear implants, intrauterine devices, or non-MRI compatible metal objects in their body, especially in the eye. Subjects will be required to remove all ferromagnetic items (e.g., keys, phones, credit cards, belts, loose change, and others) before entering the MRI examination room. Claustrophobia may also preclude successful MR imaging. Careful screening will prevent such individuals from participating in this study, as well as preventing the introduction of any ferromagnetic objects into the scanner room. Dental fillings do not present a problem.

The FDA has set recommendations for exposure in MRI studies and the proposed 3T examinations satisfy those criteria. The guidelines from the Bureau of Radiological Health of the FDA will be followed in regard to specific absorption rate (SAR) of radiofrequency energy and time varying magnetic fields (dB/dt). Precautions will be maintained so that SAR will be less than 8 watts per kilogram in any 1 gram of tissue. This is the estimated power required to raise the temperature 1 degree centigrade. The maximum dB/dt will be set at 20T/sec for > 120usec or 200T/sec for < 12usec. These levels are well below peripheral nerve stimulation threshold in humans, both children and adults. In rare cases, subjects may still experience some peripheral nerve stimulation during portions of the MRI procedure. These experiences are transient and harmless. MRI participants will be instructed prior to examination to refrain from skin-on-skin contact of their extremities (e.g., clasping hands or legs) to further reduce this risk. The MRI will produce loud noises during image acquisition. The decibel intensity of these noises is not considered harmful per FDA regulations, and is below FDA guidelines of 140 dB peak referenced to 20 micropascals. Subject will be provided with earplugs and noise-cancelling headphones/earpads to minimize discomfort.

Subjects will always be in communication with the MRI technologist and will be given a squeeze ball that triggers an alarm. If the subject indicates at any point that they have a desire to stop the procedure, the exam will be terminated immediately and without any penalties to the subject in any way.

If any unexpected findings are identified that may be clinically significant, the participant will be counseled by the Site PI and recommended to seek medical care from their primary care physician.

**Outcomes.** Some of the questionnaires and interviews used in this research ask about personal and potentially sensitive information. This will be explained to subjects both orally and in the consent document. Further, only trained study personnel who are sensitive to these issues will administer such interviews and questionnaires. If a subject endorses any of the questions regarding suicide, it will be documented in the research record and the study staff will follow the **TRACK-TBI Suicide Protocol and Safety Plan**.

**12.2 Protections Against Subject Risks**

**Recruitment and Informed Consent.** All study sites are experienced with recruiting TBI subjects. All sites will obtain IRB approval through a central UCSF IRB to enroll patients into TRACK-TBI Precision Medicine. Research staff will locate eligible patients in the hospital (emergency department, hospital wards, intensive care unit), explain the research study, review the consent form, ask the subject or LAR if s/he voluntarily agrees to participate, and obtain consent. Prospective subjects will be given as much time as needed to consider study participation. If the subject is not capable of self-consent, all efforts will be made to locate a LAR to sign in person or via fax.

**Clinical.** The potential risk to subjects is minimal. We will take all necessary steps to reduce risk for all study participants. We will inform subjects of the potentially sensitive nature of some of the research questions and create an atmosphere of security and trust prior to collecting data. We carefully explain the steps taken to assure the confidentiality of all participant data. Subjects are always given permission to not answer questions with which they feel uncomfortable. In our experience with TRACK-TBI Pilot and TRACK-TBI U01, with the establishment of rapport by a sensitive, experienced research team, the majority of subjects welcome the opportunity to participate in research. Following initial consent, subjects will be reminded before every procedure that participation is completely voluntary, that they may decline to respond to any questions, and that they may withdraw from the study at any time without jeopardizing medical treatment to which they are otherwise entitled.

To protect confidentiality, no paper copies of study forms will include subjects' names, but instead will contain a Study ID as the identification key to match subjects over the repeated measures. The subject names will be entered into the QuesGen web-based eCRFs in order for study personnel to contact patients and conduct follow-up visits. However, only designated study site personnel will be able to view the subject name fields in QuesGen. All data communication between the QuesGen browser and secure servers is through an encrypted secure socket layer connection. Servers are located in a Statement on Auditing Standards-70 compliant data center behind a dedicated firewall. QuesGen has procedures in place for full compliance with HIPAA security standards for protection of PHI. User password accounts are assigned according to user types and access roles which allow or restrict the viewing of any PHI fields. An algorithm is applied to each data element to determine if it should be considered PHI. The default determinations can be overridden if incorrectly classified as PHI. Administrative users can set up accounts for users to only view the data or set filters that limit viewing of records according to their study site. Every data modification is tracked and all views and deletions are logged so that data tampering is not possible. Study sites that are not covered entities by their institution will be required to establish Business Associate Agreements with QuesGen Systems, Inc.

Study data will be entered into eCRFs using designated laptop and desktop study computers with secure, encrypted connections to the eCRF data. No PHI will be stored on the hard drives of any study computers. Study computers will have encrypted drives conforming to IT standards at each respective site. Study computers will be password-protected and securely maintained with virus protection software installed to automatically update and scan the drives. Only research personnel responsible for data entry or review will have password access for study computers. Paper copies of surveys will be filed by their Study ID in locked file cabinets behind locked doors at each study site. A list linking the Study ID and names will be kept in a separate locked file cabinet behind locked doors at each study site. Once the final outcome assessments are completed and checking for data quality monitoring purposes is completed, PHI for the subject will no longer be

accessible to previously authorized personnel except the study site PI. At project conclusion, PHI data will be stored in a password protected PDF file and given to the site PI for long-term protected storage. The QuesGen Data Manager will then remove the PHI data from all server hard drives and all backup devices. QuesGen will not retain any copies of the PHI data long-term. These identifiers will then be accessible only by the study site PI and would be used at a later time only if it becomes necessary to contact the subject for additional studies or for regulatory purposes.

**Biospecimens.** For the blood draw, no more than two venipuncture attempts will be performed and specimens will be coded when the draw is complete.

**Neuroimaging.** As noted, the total scan time is approximately 60-75 minutes during which time the subject will have continuous communication with the experimenter. Some patients find the loudness of the oscillating gradients during image acquisition to be discomforting, but the acoustical noise level is below FDA guidelines of 140 dB peak referenced to 20 micropascals. In addition, all patients are provided with earplugs to reduce the noise. There is no evidence for long-term negative effects of MRI procedures on the body. The 3T scanner is an FDA-approved system. For patients' safety, only MRI-compatible monitoring equipment will be used in the scanner suite for any patients requiring that for the ultra-early MRI.

**Outcomes.** An experienced outcomes team designated by the Outcomes Core will train all outcomes personnel for TRACK-TBI. Only trained study personnel sensitive to the inherent issues across cognitive, mental health, psychological, and quality of life domains will administer the outcome instruments for the study. Subjects will be re-introduced to the study at each outcomes time point for understanding and approval to continue participation. Subjects are informed that they are free to not answer any question that may be uncomfortable for them. If a subject endorses any of the questions regarding suicide, it will be documented in the research record and the research personnel will notify the Site PI and activate local suicidality protocols.

### 12.3 Certificate of Confidentiality

As an additional level of safeguard for study participants, we will obtain a Certificate of Confidentiality from the NIH. Having this Certificate means that investigators and study personnel cannot be forced to disclose research information that might identify the subject in any federal, state, or local criminal, civil, administrative, legislative, or other proceedings. This is important because TBI patients are often involved in high-risk behaviors that result in their injuries.

### 12.4 Potential Benefits of Proposed Research

There is no direct benefit to study participants. The results will be directly relevant to society in general and to future patients who suffer TBI. Tokens of thanks for study participants are especially important in longitudinal studies, where the burden on the respondent, even if small, tends to multiply over time. Each site will establish a reimbursement schedule for each of the time-consuming components of the study, including travel, MRI, telephone and in-person outcomes testing.

TRACK-TBI subjects will undergo extensive neuropsychological testing and brain imaging. These procedures are not part of the standard of care for mild TBI.

- All subjects will have access to the results of their research MRI results within 2 weeks of the MRI collection. If requested, subjects will receive a CD of their conventional MR imaging data and a viewer application tool. The study ID will be stripped from the MRI scan.

**For details regarding the release of outcomes testing results to the participants refer to the section '10 Protocol for Sharing Outcome Data with Participants'.**

## 13 SUBJECT COMPLIANCE AND RETENTION

We will monitor subject compliance with the observational portion of the protocol, and research personnel will maintain scheduled contact with the subjects and their family members to ensure on-going compliance through the 3-month study duration. Upon consent and enrollment, participants will be asked to provide multiple forms of contact information including phone, address, and email. Upon their permission, participants will be asked to provide one or more alternate contacts. The Site will maintain a schedule of contacts to maximize the chance of successful communication and scheduling for follow-up time points as soon as their window of return opens for that time point. This will involve training Research Coordinators to monitor their own site progress, which will be supplemented by automated reminders of upcoming windows for follow-up generated by QuesGen Systems and emailed to each site coordinator weekly. Every effort will be made to schedule return visits at the subject's convenience, and multiple procedures for that time point will be scheduled on the same day. When subjects return for their follow-up, they will be met by research personnel who will escort them to the various testing locations. Subjects will have full opportunity to ask any questions before, during, and at the end of the follow-up appointment. Site Coordinators will be encouraged to have relevant resource packets and materials for TBI assembled to provide to study patients.

In the event that a participant misses a follow-up time point, every effort should be made to schedule subsequent visits for outcome assessment, neuroimaging, and blood draws.

- If an in-person assessment cannot be scheduled despite multiple attempts, outcome assessments at that time point should be collected by phone.

## 14 DATA MANAGEMENT AND COMPLIANCE

### 14.1 Clinical database

Upon study enrollment, the subject will be entered onto the QuesGen System, which will automatically generate a Study ID. This Study ID is not generated from personally identifiable information (PII). Study IDs will be coded in the format of [Site Code (2 digits)] – [Patient Number (4 digits)], e.g., XX-XXXX, with Site Codes for the 5 participating study sites. MRI scans will have an additional label at the end to distinguish the time point and whether the scan is a patient or phantom. The additional labels are as follows: (XX-XXXX_Ultra-early for MRI within 24-48 hours, _2WK for 2-week MRI, _3MO for 3-month MRI and _PHA for phantom). Clinical data will be entered into eCRFs via a web-based portal to the secure, fully HIPAA-compliant QuesGen clinical database.

**Site Codes for participating sites are as follows:**

| Site Name | Site Code |
|---|---|
| UCSF | 03 |
| Univ. of Pittsburgh | 07 |
| Univ. Pennsylvania | 12 |
| MCW | 14 |
| Univ. of Utah | 15 |

**Automated data integrity monitoring.** All clinical data will be entered into electronic Case Report Forms (eCRFs) and managed by the QuesGen data management platform. As data is entered into each form, the system will run data validation checks that include conditionally required data, validation across fields, and validation requirements based on subject type. If any validation check fails, the user is alerted immediately that the data does not meet QA criteria and the issue can be addressed and corrected at that point. If a data element fails a validation check, yet the value entered is correct, the user can enter an exception to the problem and provide a notation as to why the out-of-range data is actually correct.

- **Date/time value checks:** all dates and times entered into the database are checked to ensure that events recorded are accurate and in sequence.
- **Range value checks:** all numeric, non-date fields have range values specified to minimize data entry errors.
- **Selection lists:** all categorical data fields have predetermined drop-down lists, check boxes, or resettable radio buttons instead of free text to ensure accuracy.
- **Logic checks:** data fields from different sections of the eCRF will be compared to pass logical integrity
- **Required fields:** the eCRF will be programmed to require input into fields when appropriate to minimize missing information.
- **Score calculation** will be performed and programmed into eCRFs for tests and measures with numerical score summations or norming to avoid mathematical errors by the examiner. All automated scoring computations will be fully documented and validated by QuesGen and the Clinical Core, and must pass User Acceptance Testing.
- **Electronic data audits** will be automated in the QuesGen database through a series of pre-determined queries against the study database at regular intervals. These queries will be designed for the Clinical Core to monitor data quality and completeness and identify protocol variations/deviations/violations.
- **Data audits against source documents**, where available, will be conducted prior to the final "lock" of each subject's data set. Errors found will be corrected at this time.

All investigators and designated study personnel will have unique and confidential password access to the QuesGen database. All access to the database and to study data will be logged in an audit trail and monitored. Any indication of inappropriate access will be reported immediately to the Clinical Core.

The QuesGen system will also provide checks for form completion based on the subject type. Validation rules will establish when forms for a particular subject should be entered, and any missing forms can be tracked by the Study Site and Clinical Core immediate follow-up. Once subject forms are marked complete, a dataset for sharing can be created. The QuesGen platform stores the exact dataset that is shared for future reference.

Due dates for eCRF completion windows are set by the Clinical Core. The Subject and Presentation eCRFs need to be initiated as soon as possible following enrollment in order to assign subject IDs for the biospecimen vials. In general, every effort should be made to complete eCRFs within 2 business days of enrollments, inpatient stays, follow-up milestones and discharges. It is understood that some forms and fields within forms may not yet have complete information available to report (e.g., Hospital Admission/Discharge, AIS/ISS, Surgeries, Concomitant Medications, etc.). The QuesGen System will automatically generate reminders to complete eCRFs for enrolled patients. Monthly reports of enrollment, timeliness of eCRF completion and error correction will be monitored and adjudicated by the Clinical Core.

**Integration with analytics platforms.** All de-identified electronic study data in the TRACK-TBI database will be maintained in secure storage by QuesGen Systems for the duration of subject enrollment and follow-up and for a period afterwards for data analysis and preparation of publications. We estimate that the analysis and publication period will last for several years after the conclusion of subject enrollment.

Together with QuesGen Systems, the Clinical Core will ensure that data standards are established for the data model e.g., conformity of field formats, field codes and names to ensure consistency across all datasets. After the initial approval of the data model and eCRFs, any proposed changes to the database will be reviewed by QuesGen and the Clinical Core for impact upon the existing data in the repository. Approved changes will be fully documented with dataset updates to maintain data quality and accuracy.

**14.2 Biospecimens**

*Biospecimens collection*. Study sites will collect, process, and ship blood and CSF (if collected) biospecimens according to the NINDS TBI-CDE Biospecimens Protocol, to a central biorepository at University of Pittsburgh. Each site will batch and ship biospecimens to the central repository on a quarterly basis. Formalized QC/QA policies for collection, processing and storage were developed and validated for TRACK-TBI Pilot. Refer to the TRACK TBI Precision Medicine Biospecimens Protocol for detailed information regarding management of collection supplies (disposables and reagents), identification (using Study ID), labeling conventions, collection and processing methods, storage and retrieval, shipping and receiving, training, and security. Together these pre-analytic QC/QA policies minimize circumstances that could adversely affect scientific results, ensure the safety of research personnel, and aid in the efficient operation of the TRACK-TBI Biospecimen Core. The Biospecimen Core will review the efficiency of existing processes and procedures on a quarterly basis.

*Whole brain collection*. When possible, sites will approach next of kin for donation of subject brains. We expect to collect brains from individuals who die in both the acute and sub-acute time periods. The brains will be processed locally and shipped to the TRACK-TBI Brain Bank at the University of Washington in Seattle, WA (Dr. Dirk Keene, Director). This will facilitate neuropathologic studies of subjects who have been characterized with MRI, proteomic, and genetic studies, providing further opportunity to validate imaging and biomarker results.

*Biospecimen Repository*. The NTBI-BR at the University of Pittsburgh will manage receiving biospecimen shipments from all participating sites, inventory of those shipments, storage of all study biospecimens, and shipment of study biospecimens to relevant analytic partners, where genomic and proteomic analyses will be used to discover new TBI biomarkers.

**14.3 Neuroimaging**

*Standardization of MR across sites*. All study MR systems will initially be characterized with the Magphan® Quantitative Imaging Phantom (Phantom Laboratory, Salem, NY) designed to measure signal-to-noise ratio, object size scaling, and spatial distortion. The Magphan® Phantom has been extensively used for high-resolution structural brain imaging in numerous trials, including ADNI, as follows:

1. Serial imaging of the phantom will track scanner performance over the study enrollment period using an online MR Distortion and Image Quality service (ImageOwl, Salem, NY) which identifies scanner errors or defects and corrects for scaling errors and gradient non-linearity.
2. Standardization for diffusion MR imaging using an ice water phantom adopted by ACRIN for multi-site diffusion imaging trials.
3. Performance of fMRI will be assessed using the Biomedical Informatics Research Network (BIRN) phantom, with serial data analyzed to assess signal mean and standard deviations, temporal fluctuations, and drift.

Standardized MR protocols will maximize consistency among study sites and across vendor platforms. Within vendors the protocols will be identical and shared via manufacturer-generated tools (e.g. edx files, examcards). Across vendors, protocols will match spatial coverage, voxel dimensions, and primary contrast parameters (e.g. echo time, repetition time). A TRACK-TBI board-certified neuroradiologist will evaluate the test-retest scans from all sites to assure diagnostic image quality and for pathoanatomic analysis of structural MRI sequences.

*Neuroimaging Repository*. All study neuroimages (CT and MRI) will be de-identified at each study site before uploading to the TRACK-TBI neuroimaging core repository (Flywheel) as DICOM files, utilizing the Neuroimaging Core's de-identification and transport protocol. Image data will then be passed into one of a series of modality-specific semi-automated quality assessment pipelines and evaluated by the Neuroimaging Core for protocol conformance and quality. QA results will be provided to acquisition sites within 48 hours and the scan repeated if it does not meet QA criteria. Images passing QA will be sent into a modality-specific image analysis pipeline and the resulting processed images and measures will be returned to the neuroimaging

repository. The existing image processing provenance collection method will ensure that derived images and data are properly annotated and preserved for future research.

*Integration and analysis*. The Neuroimaging Core will direct the coding of all TRACK-TBI neuroimages. Final versions of the curated TBI-CDE compliant clinical and outcomes data, neuroimaging, and molecular measures will be integrated within Flywheel. Analytic systems within Flywheel will support real-time inspection of data, hypothesis testing, data subsetting, and data exploration across studies.

## 15 ADVERSE EVENTS

Events may be categorized as Adverse Events (AEs) if the distress felt by the subject requires termination of testing or procedure (e.g., outcomes testing, MRI). Anticipated AEs in TRACK-TBI include:

- Excessive discomfort, pain, or bruising during venipuncture
- Claustrophobia or severe anxiety in the MRI
- Anxiety during outcomes battery administration due to sensitivity of material discussed
- Anxiety due to fear of legal discovery associated with high-risk/illegal behaviors during interview

**15.1 Reporting Procedures.** AEs will be documented in the AE section of the QuesGen database. Each Site PI will be informed on a weekly basis regarding the number and nature of the AEs at their site. The Clinical Core will review the number and nature of AEs at each site on a monthly basis. Given that each site must reach the 80% completion of follow-up milestones, if AEs exceed 10% of site enrollment the Clinical Core will contact the site to discuss potential methods for reduction of AE incidence.

**15.2 Other Serious Events.** Any other serious events that do not meet the above criteria will be reported to the Clinical Core within five working days. These AEs will be recorded for individual subjects during the 3-month study period. In addition to submission as required per IRB regulations, AE data will be analyzed quarterly and reported in the quarterly reports submitted to the Executive Committee.

## 16 DATA SHARING

TRACK-TBI Precision Medicine internal and external data sharing procedures will align with the TRACK-TBI data sharing procedures (see Appendix 2: TRACK-TBI Data Use Agreement/Human Materials Transfer Agreement of TRACK-TBI Research Collaboration Policy_06-19-2020_Final_Current.pdf saved in Precision Medicine drop box folder).
UCSF lead team has registered the study on Clinicaltrials.gov under identifier NCT # NCT04602806. The results information from this study will be submitted to ClinicalTrials.gov.

## 17 CLINICAL PROTOCOL MAINTENANCE

### 17.1 Protocol Modifications

See TRACK TBI U01 protocol-appendix 7 for the procedure for revisions to the Precision Medicine Protocol (Dropbox\1-TRACK TBI  Doc Share\clinical core\Clinical Protocol\Clinical Protocol Appendices).

### 17.2 Protocol Deviations

Protocol compliance and study performance will be monitoring by the Clinical Core using the study reports and dashboards provided by QuesGen Systems. Any protocol deviations should be reported and described in full under the research subject's study record in QuesGen, "Subject → Protocol Deviations" tab.

Protocol deviations may include:

Clinical

- Subject enrolled with unclear time of injury (for assessment of enrollment <24 hours)
- Baseline interview information missed on enrollment
- Other:

Biospecimens

- Blood collected for the baseline sample outside of <6 hour window
- Blood processing times deviated from protocol
- Blood collection was missed at any time point until day 5 only if the patient was still hospitalized
- Blood collected outside of the 2-week, 6-week, or 3-month window without prior approval for exception by the Executive Committee approval.
- Blood collection attempt unsuccessful
- Blood draw missed (i.e., follow up completed by phone, examiner error, subject refused)
- Other:

Neuroimaging

- MRI collected outside of the pre-specified time windows
- Certain MRI sequences were not completed or required separate visits to complete
- MRI missed within 24-48 hour, at 2 weeks, and 3 months
- Other:

Outcomes

- MRI and Outcomes not completed within 3 days of each other
- Certain outcome measures incomplete
- Certain outcomes measures missed
- Other:

In most instances and especially concerning enrollment, MRI, or outcomes administration dates, protocol deviations must be reported to the Executive Committee for approval before data collection can resume for the subject at the respective time point of deviation. Due to the time sensitivity of blood draws and processing, deviations can proceed at the local level, but must be reported to the Clinical Core within 2 business days. Under circumstances in which the permissible window for outcome assessment cannot be met, with agreement from the subject, data collectors can request permission from the Executive Committee to complete the scheduled follow-up out-of-window. A protocol deviation will need to be reported within the QuesGen database, even if permission is obtained in advance.

In the event that a consistent pattern of poor performance (e.g., not enrolling allotted amount of patients per month, not achieving at or above 80% follow-up rate across all time points) or inadequate compliance (e.g., insufficient blood draw amount, CT or MR imaging, CRF completion without errors, full outcomes battery completion, or any time point completed outside the approved window) is detected, the responsible site investigator will be notified and required to present a plan for improvement and a time line for accomplishing this to the Clinical Core. Failure to meet objectives specified in this plan may result in termination of the project or assignment of the project to another investigator.

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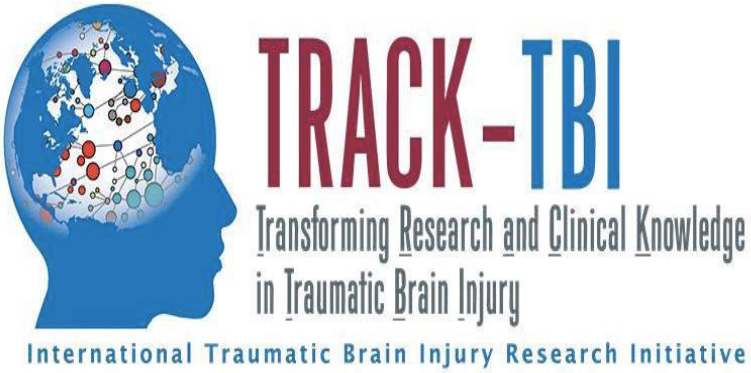
**19 ACRONYMS**

| | |
|---|---|
| AEs | Adverse Events |
| ASL | Arterial Spin Labeled |
| AUC | Are Under the Curve |
| BSI-18 | Brief Symptom Inventory-18 |
| CBF | Cerebral Blood Flow |
| CDEs | Common Data Elements |
| CRF | Case Report Forms |
| CSF | Cerebrospinal Fluid |
| DAI | Diffuse Axonal Injury |
| DoD | U.S. Department of Defense |
| DTI | Diffusion Tensor Imaging |
| eCRF | Electronic Case Report Forms |
| FDA | U.S. Food and Drug Administration |
| FISO | Free Water Volume Fraction |
| Fn | Fibronectin |
| GFAP | Glial Fibrillary Acidic Protein |
| GOSE | Glasgow Outcome Scale - Extended |
| ICU | Intensive Care Unit |
| LAR | Legally Authorized Representative |
| MD | Elevated Mean Diffusivity |
| MRI | Magnetic Resonance Imaging |
| mod-sTBI | Moderate To Severe TBI |
| mTBI | Mild Traumatic Brain Injury |
| MVI | Microvascular Injury |
| NIH | U.S. National Institutes Of Health |
| NODDI | Neurite Orientation Dispersion And Density Imaging |
| PASL | Pulsed ASL |
| PCASL | Pseudocontinuous ASL |
| PCL-5 | Post Traumatic Stress Disorder Checklist-5 |
| RAVLT | Rey Auditory Verbal Learning Test |
| RPQ-18 | Rivermead Post Concussion Symptoms Questionnaire-18 |
| rs-fMRI | Resting State Functional MRI |
| SWI | Susceptibility Weighted Imaging |
| TBI | Traumatic Brain Injury |
| TMT | Trail Making Test |
| TRACK-TBI | Transforming Research And Clinical Knowledge In Traumatic Brain Injury Study |
| UCH-L1 | Ubiquitin C-Terminal Hydrolase-L1 |
| WAIS-IV | Wechsler Adult Intelligence Scale-Fourth Edition |
| SOP | Standard Operating Procedure |
| DRS | Disability Rating Scale |
| FSE | Functional Status Exam |
| SPPB | Short Physical Performance Battery |

Appendix 2




# TRACK-TBI Add-On study Precision Medicine

# Biospecimens Procedures

# 10 October 2020

- **You are receiving this MOP because your site has agreed to participate in this Precision Medicine study as an add-on to the TRACK-TBI protocol. The 5 participating sites include:**

  **03 UCSF**

  **07 Pittsburgh**

  **12 Pennsylvania**

  **14 MCW**

  **15 Utah**

- **DO NOT BEGIN USING THIS PROTOCOL UNTIL YOUR INSTITUTION HAS RECEIVED THE NECESSARY IRB APPROVAL**
- **The <u>first blood draw</u> must be within 6 hours of injury.**
- **A waiver of consent may be used to collect and process blood draws within the first 24 hours of injury (waiver language will vary by site).**
- **DO NOT SHIP SPECIMENS COLLECTED UNDER WAIVER UNLESS CONSENT IS SIGNED WITHIN WAIVER PERIOD.**
- **Buffy coat samples may be collected under waiver, but only ship specimens for subjects who have consented to possible future genetic testing.**
- **Biospecimen Sample Collection Schedule**

| *Schedule of blood sample Collection (from time of traumatic brain injury)* | ***<6 hr*** | ***12 hr*** | ***24 hr*** | ***Day 2*** | ***Day 3*** | ***Day 5*** | ***Day 14*** | ***Day 42*** | ***Day 90*** |
|---|---|---|---|---|---|---|---|---|---|
| *Blood collection* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

- **The NTBI-BR will be serving as the biorepository for TRACK-TBI projects, including the Precision Medicine study.**

## Precision Medicine Biospecimen Collection Summary (Baseline and Follow-up Visits)

| Sample Type | Tube Type | Number of Blood Tubes and Cryovials Supplied in Kit | Processing/ Aliquoting | Cryovials to NTBI-BR |
|---|---|---|---|---|
| **Whole Blood for Isolation of Plasma and Buffy Coat (for DNA extraction)** | **8 ml Purple Top EDTA Tube** | 1 | **N/A** | **N/A** |
| | **Plasma:** 1.5 ml cryovials with purple caps | 8 cryovials + 8 purple screw tops | 0.5 ml plasma aliquots per 1.5 ml cryovial | 7-8 |
| | **Buffy Coat:** 1.5 ml cryovial with clear cap | 1 cryovial + 1 clear cap | 0.5 ml buffy coat aliquot per 1.5 ml cryovial | 1* (baseline visit only) |
| **Whole blood for isolation of serum** | **8 ml Red Top SCA Tube** | 1 | **N/A** | **N/A** |
| | **SERUM:** 1.5 ml cryovials with red caps | 8 cryovials + 8 red screw tops | 0.5 ml serum aliquots per 1.5 ml cryovial | 7-8 |
| **Blood TOTAL** | 16 ml | 17 cryovials with color-coded caps 1 lavender top- 8 ml 1 red top- 8 ml | | 17 cryovials from baseline visit; 16 cryovials from follow-up visits |

## Table of Contents

# 1. Abbreviations

| | |
|---|---|
| EDTA | Ethylene Diamine Tetra-acetic Acid |
| IATA | International Air Transport Association |
| NTBI-BR | National TBI Biospecimens Repository |
| PPE | Personal Protective Equipment |
| RCF | Relative Centrifugal Force |
| RPM | Revolutions Per Minute |
| TBI | Traumatic Brain Injury |

# 2. Purpose

The purpose of this Manual is to provide all study personnel (Principal Investigators, study coordinators, phlebotomists, nursing and laboratory staff) at the enrolling sites with instructions for the collection of whole blood, fractionation of blood from vacutainer tubes, aliquoting, labeling, freezer storage and shipping frozen samples to the NTBI-BR located in Pittsburgh. The following samples will be collected:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)

# 3. Biorepository Information

## 3.1 NTBI-BR Contacts

NTBI-BR Director: Ava Puccio, RN, PhD
Neurotrauma Clinical Trials Center
University of Pittsburgh
Department of Neurological Surgery
200 Lothrop Street, Suite B-400
Pittsburgh, PA 15213
Office: 412-648-9246
Mobile Phone: 412-298-7033
Email: puccioam@upmc.edu

NTBI-BR Manager: Miri Rabinowitz, PhD
Office: 412-648-2031
Mobile Phone: 412-491-6199
Email: rabinowitzmk@upmc.edu

NTBI-BR Supervisor: Mike Mancinelli
Office: 412-648-2389
Mobile Phone: 484-885-8211
Email: mancinellimd@upmc.edu

## 3.2 Hours of Operation

The NTBI-BR operates from 9 AM to 4 PM EST, Monday through Friday.

Frozen samples must be shipped Monday – Wednesday only.

Frequency of shipments will depend on enrollment rate at each study site. Shipping volume may vary by site enrollment. Shipments should contain no less than four (4) cryoboxes, and no more than sixteen (16) cryoboxes. For common scenarios with packing and shipment, refer to Section 8 of this MOP.

Frozen samples should be shipped at least quarterly or as determined by the Study Monitor. Please ensure adequate storage at -80°C prior to shipment. Contact the NTBI-BR Manager prior to arranging any shipments.

Check weather reports to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples.

## 3.3 Holiday Schedules

Please be sure to verify with your courier's schedule prior to shipping close to a holiday.

**Holiday Observations* – United States**

| Date | Holiday |
|---|---|
| New Year's Day | January 1 |
| Martin Luther King Day | 3rd Monday in January |
| President's Day | 3rd Monday in February |
| Memorial Day | 4th Monday in May |
| Independence Day | July 4 |
| Labor Day | First Monday in September |
| Columbus Day | 2nd Monday in October |
| Veteran's Day | 2nd Monday in November |
| Thanksgiving | 4th Thursday of November |
| Day after Thanksgiving | 4th Friday of November |
| Christmas Day | December 25 |

***Additionally, each year the University of Pittsburgh is officially closed from December 24 through January 2. Do not schedule any shipments during this time**.

# 4. Supplies and Equipment

## 4.1 Supplies and Equipment Provided by the Enrolling Sites:

The following are necessary for the collection of whole blood and are to be **supplied by the local institution**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol prep pad
- Gauze pad
- Bandage
- Butterfly needle
- Microcentrifuge tube rack
- Cryogenic Gloves
- Sharps bin and lid
- Biohazard disposal

Each site must have access to the following equipment:

- Centrifuge capable of ≥ 1500 rcf (1500 x g)
- -80°C Freezer

## 4.2 Specimen Collection Kit Contents

The NTBI-BR will provide research specimen collection kits. The kits will include the Vacutainer tubes needed for whole blood collection, and cryovials and color-coded caps for plasma/serum/buffy coat aliquots. Labels pre-printed with study information specific to the type of sample being drawn will also be provided, as will 81-slot cryoboxes for freezer storage.

Collection kits contain the following supplies to collect samples from a given subject-timepoint. **Do not replace or supplement any of the kit components with your own supplies unless you have received approval from Biorepository Laboratory Manager to do so.**

**Kit Supplies**

| Quantity | Baseline Kit Components |
|---|---|
| 17 | Polypropylene microcryovial tubes |
| 8 | Purple caps (for plasma) |
| 8 | Red caps (for serum) |
| 1 | Clear cap (for buffy coat/DNA) * |
| 1 | 8 ml EDTA (purple top) blood collection tube |
| 1 | 8 ml Serum determination tube (red top) |
| 3 | Disposable graduated transfer pipettes |

* The baseline visit includes aliquoting the buffy coat in cryovials using the clear caps; the buffy coat is NOT collected in visits after the baseline visit unless the sample was compromised, and a replacement can be obtained from the next obtained plasma sample.

## 4.3 Initial Supply of Study Materials to Study Sites

Each site will initially be supplied with 30 blood draw kits, 6 cryoboxes, and 15 sheets of pre-printed labels.

## 4.4 Resupply to Study Sites

Each site will be responsible for maintaining and requesting adequate inventory after the initial supply has been sent. Regularly check your supplies (including Vacutainer expiration dates*) and order additional kits and sheets of pre-printed labels before you run out so you are prepared for both scheduled and unanticipated visits.

Email the Biorepository Manager to request a resupply of kits. Allow **14 days** for kit orders to be processed and delivered.

*Take note that Vacutainer expiration dates on the tubes are in the format: **YYYY-MM-DD**

# 5. Blood Collection and Processing Procedures

*****Important Note*****

**In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the procedures detailed in the following pages. Please read the following instructions before collecting any specimens. Have all study supplies, forms and equipment out and prepared prior to drawing blood. Draw blood in the order of the most essential* for this research study, i.e. first collect the purple top tube for plasma, then the red top tube for serum.**

***This blood draw order differs from standard clinical practice.**

## 5.1 Sample Collection and Quality

Care must be taken during collection of study samples to prevent hemolysis and/or contamination.

**The following techniques shall be used to prevent possible backflow and hemolysis:**

- Place donor's arm in a downward position.
- Avoid drawing blood from a hematoma
- If drawing from an existing IV using a syringe, avoid drawing the plunger back too forcefully.
- Avoid using very small needles.
- Make sure the venipuncture site is dry.
- Avoid a probing, traumatic venipuncture
- Hold tube in a vertical position, below the donor's arm during blood collection.
- Avoid prolonged tourniquet application or fist clenching
- Release tourniquet when final tube is nearly filled.
- Make sure tube additives do not touch stopper or the end of the needle during venipuncture.
- **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.**
- Vacutainer tubes are designed to draw the correct volume of blood into the tube to mix with any additive.

When obtaining blood through venipuncture, **no more than two attempts** may be performed.

## 5.2 Labeling Samples

- Each kit is supplied with preprinted labels for the cryovials (18 for the baseline blood draw and 16 for all subsequent blood draws. Buffy Coat is collected only once for each subject).

- Each cryovial will be labeled with a unique alphanumeric string. For example: **PM-07-1010-D-06P** Is the 6th plasma aliquot (06P), from the Day 2 blood draw (D) on subject 1010 from the Pittsburgh site (07).
    - The naming convention is as follows:

| Study | Site | Subject ID | Blood Draw (from time of injury) | Specimen Type and Vial |
|---|---|---|---|---|
| PM | 03 (UCSF)<br>07 (Pitt)<br>12 (Penn)<br>14(MCW)<br>15(Utah) | 1001 to 1076 | A thru I<br>**A:** <6 hr (baseline)<br>**B:** 12 hr<br>**C:** 24 hr<br>**D:** Day 2<br>**E:** Day 3<br>**F:** Day 5<br>**G:** Day 14<br>**H:** Day 42<br>**I:** Day 90 | Plasma: 01P to 08P<br>Serum: 09S to 16S<br>Buffy Coat: 17D*<br>CSF: 01C to 05C |

*D for DNA. Since DNA will only be collected at the baseline blood draw, only samples with an "A" designation will have labels 17D.

**Note:** There are 8 aliquot cryovials for plasma, 8 aliquot cryovials for serum, and 1 aliquot cryovials for buffy coat. The provided cryovial caps are color-coded. Use the **Purple caps** for plasma; **Red caps** for serum; and **Clear caps** for buffy coat.

In order to ensure the pre-printed label adheres properly to the cryovial, follow these instructions:

- Place labels on **ALL** aliquot cryovials **BEFORE** sample processing/freezing. This should help to ensure the label properly adheres to the cryovials before exposure to moisture or different temperatures.

- Place each label **vertically** on the cryovials.

- Take a moment to ensure the label is **completely adhered** to each cryovial. It may help to roll the cryovial between your fingers after applying the label.

- Be sure to place only **plasma** in cryovials labeled with the suffix **01P to 08P**
  - Depending on the amount of blood drawn you may fill less than 8 cryovials:
    - Use the lowest numbered labels, i.e. 01P, 02P and 03P rather than 03P, 04P and 05P if only 3 cryovials used.
  - Do not fill more than 8 cryovials; all specimens must have a pre-printed label.
  - Cap with the **PURPLE** caps.

- Be sure to place only **buffy coat** in cryovials labeled with the suffix **17D**
  - Collect the entire white buffy coat layer and as little pellet as possible.
  - Cap with the **CLEAR** caps.

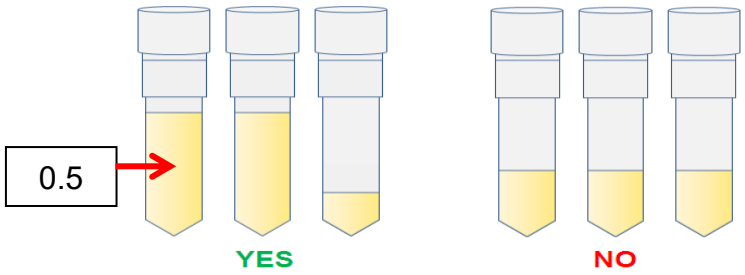
- Be sure to place only **serum** in cryovials labeled with the suffix **09S to 16S**
  - As before, you may fill less than 8 cryovials with serum - use the lowest numbered labels first.
  - Do not fill more than 8 cryovials; all specimens must have a pre-printed label.
  - Cap with the **RED** caps.

- In summary, only place **plasma** in "**P**" labeled cryovials, **buffy coat** in "**D**" labeled cryovials and **serum** in "**S**" labeled cryovials

### 5.3 Filling Aliquot Tubes (Plasma, Serum, and CSF)

Each kit is provided with 3 disposable transfer pipettes – one each for plasma, buffy coat, and serum - to be used to transfer the fractionated sample to its appropriately labeled cryovial. Cryovials should be filled with 0.5 milliliter of the respective biologic material. Over-filled cryovials may burst once placed in the freezer, resulting in a loss of that sample. You do not have to fill all cryovials provided; you should attempt to fill as many cryovials as possible with 0.5 ml of sample.

**Example**: if 2.7 ml of sample is obtained, you should fill 5 cryovials each with 0.5 ml, and the 6th cryovial with the remaining 0.2 ml of sample.




*****Important Note*****

**Plasma, serum and buffy coat specimens must be placed in the -80° C freezer within 2 hours of whole blood collection.**

## 5.4 Plasma and Buffy Coat Collections

**Whole Blood Collection for Isolation of Plasma and Buffy Coat: 8 ml EDTA Purple Top Vacutainer Tube (for processing of plasma and buffy coat aliquots).**

1. **Store EDTA Purple Top Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

2. Using a blood collection set and a holder, collect blood into the **8 ml EDTA-Purple tube** using your institution's recommended procedure for collecting whole blood by venipuncture or an indwelling catheter.

   **The following techniques shall be used to prevent possible backflow:**
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet when final tube is nearly filled.
   d. Make sure tube additives do not touch stopper or the end of the needle during venipuncture.

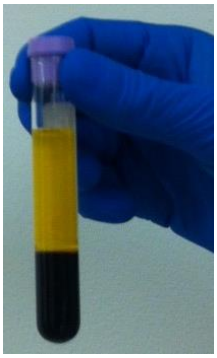
3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 8 ml of blood into the tube.

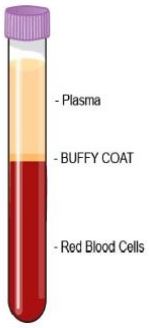
4. **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

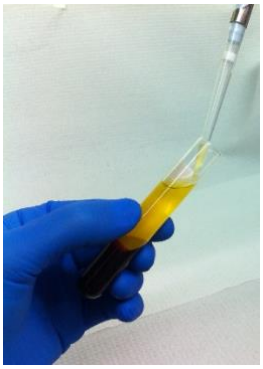
5. Within 60 minutes of whole blood collection centrifuge balanced tubes for 20 minutes at 1500 RCF (x g).
   - **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM in your particular rotor).**
   - Refrigeration prior to or during centrifugation is not recommended.

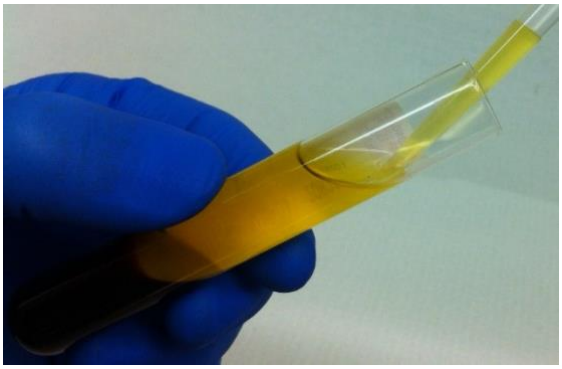
6. Place:
   - Pre-printed "**PLASMA**" labels (01P – 08P) on the 1.5 ml cryovial tubes (8)
   - Pre-printed "**BUFFY COAT**" labels (17D) on 1.5 ml cryovial tubes (1).

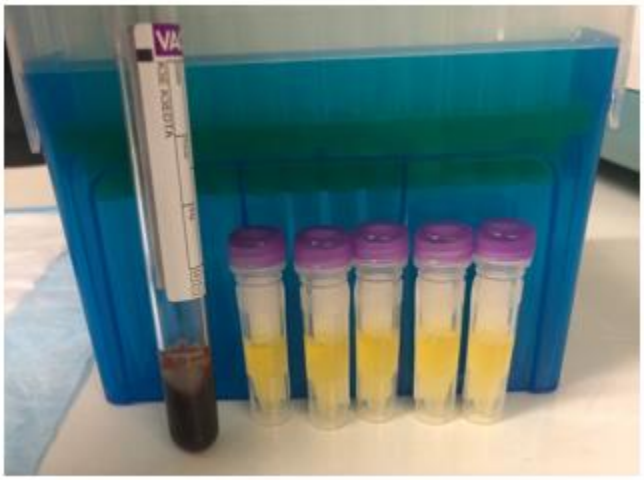
7. Remove the plasma, being careful not to agitate the buffy coat layer or the packed red blood cells at the bottom of the vacutainer tube.
   a. Tilt the tube and place the pipette tip along the lower side of the tube wall without touching the buffy coat layer or the pellet below so that plasma is not contaminated by these materials (see below).
   b. Using a disposable graduated transfer pipette, transfer plasma into the pre-labeled cryovials.
      i. The EDTA vacutainer tube should yield, on average, 4 ml of blood plasma. Aliquot 0.5 ml per cryovial (total vials = 7-8 with 0.5 ml each).
   c. Be sure to place only **plasma** in cryovials labeled with the suffix **01P to 08P.**
   d. Place a PURPLE cap on each cryovial filled with plasma.







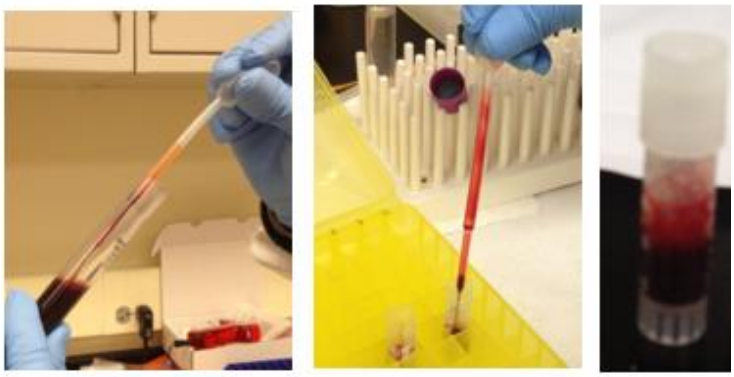


8. Place the labeled cryovials in the 81 grid cryovial box and freeze samples immediately following aliquoting by transferring to **-80°C Freezer**. Store all samples at **-80°C until shipped** to the Biorepository on dry ice.

*****Important Note*****

**BUFFY COAT MUST NOT BE COLLECTED IF THE SUBJECT HAS NOT CONSENTED TO POSSIBLE GENETIC TESTING**

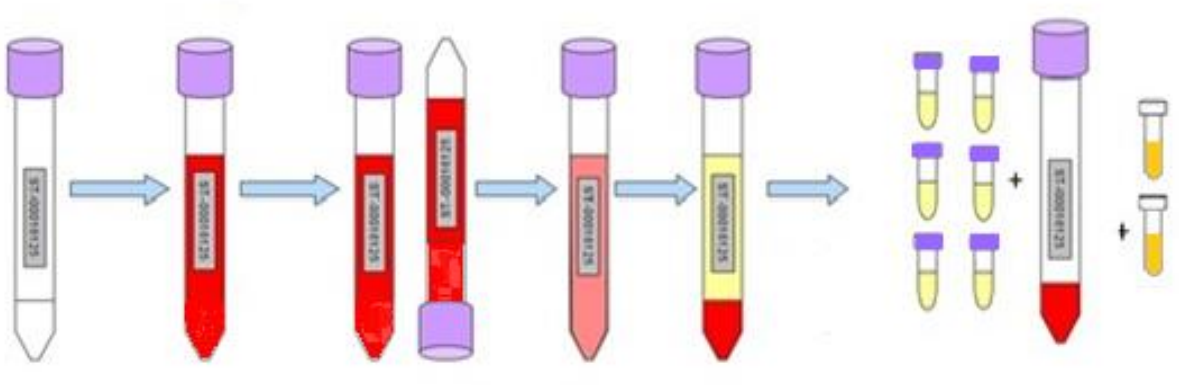
9. In the BASELINE visit ONLY, **after** plasma has been removed from the EDTA, purple top tube, aliquot the buffy coat layer into labeled cryovials with a disposable graduated pipette. Collect all of the white buffy coat layer, with as little incidental collection of pelleted red blood cells as possible.
    a. Each aliquot should be 0.5 ml of buffy coat per cryovial tube in one cryovial tube (as supplied in each specimen kit).
    b. Be sure to place only **buffy coat** in cryovials labeled with the suffix **17D**.
    c. Place a CLEAR cap on each cryovial filled with buffy coat.



10. Place labeled buffy coat cryovials in 81 grid cryovial box and place the box in **-80°C Freezer.**

11. Dispose of the purple EDTA tube (vacutainer) with cell pellet into a biohazard container.

12. Fill in TRACK Precision Medicine Sample Collection Form (Appendix C) during processing with:
    a. Sample Collection Date and Time
    b. Date and Time Plasma Spin Begins
    c. Number of Plasma Aliquots Collected
    d. Number of Buffy Coat Aliquots Collected
    e. Date and Time of Plasma and Buffy Coat in Freezer



| 1: Store tubes at *room* temperature | 2: Collect blood into the purple top tube, allowing blood to flow 10 seconds and ensuring blood has stopped flowing. | 3: Immediately after blood draw, invert tube gently 8-10 times to mix sample. | 4: Within 60 minutes of whole blood collection Centrifuge sample for 20 min at 1500 x g. | 5: Label cryovial tubes with preprinted "plasma" and "buffy coat" labels. Using graduated pipette, aliquot 0.5 ml plasma into each "plasma" cryovial and 0.5 ml buffy coat into "buffy coat" cryovials. Cap plasma cryovials with purple caps; and buffy coat cryovials with clear caps. Store at -80°C until shipment. |
|---|---|---|---|---|

## 5.5 Serum Collection

**Whole Blood Collection for Isolation of Serum: 8 ml Red Top Tube (for processing of serum aliquots).**

1. **Store 8 ml red top tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.**

2. Using a blood collection set and a holder, collect blood into an 8 **ml red top tube** using your institution's recommended procedure for collecting whole blood by venipuncture or an indwelling catheter.

   **The following techniques shall be used to prevent possible backflow:**
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet when final tube is nearly filled.
   d. Make sure tube additives do not touch stopper or the end of the needle during venipuncture.

3. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 8 ml of blood into the tube.

4. **Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.**

5. **Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes.**

6. After 30 minutes of clotting and within 60 minutes of whole blood collection, centrifuge the balanced vacutainer tube for 15 minutes at 1500 rcf (x g). **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation. (See worksheet in Appendix A to calculate RPM in your particular rotor)**

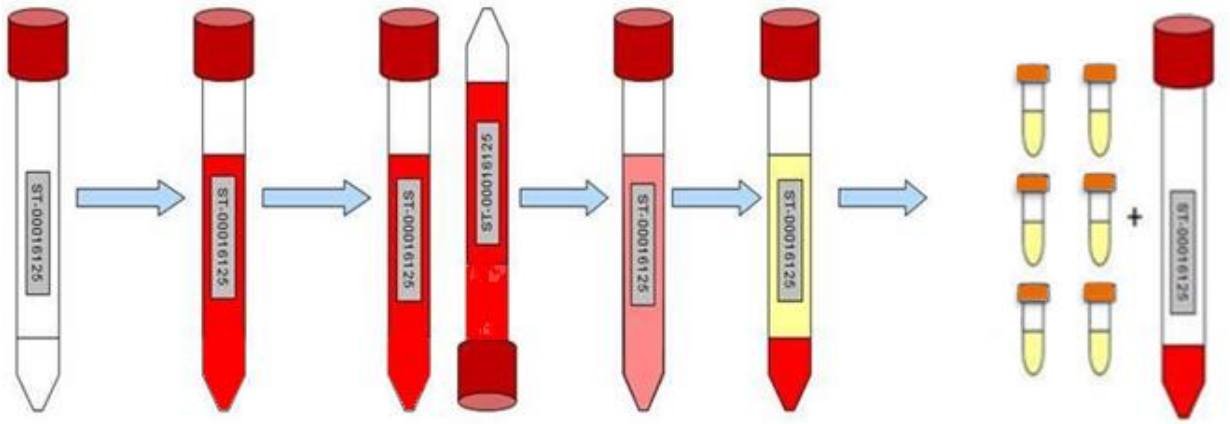
7. Place pre-printed "**SERUM**" (09S-16S) labels on the 1.5 ml cryovial tubes.

8. Remove the serum, being careful not to agitate the clot at the bottom of the vacutainer tube.
    a. Tilt the tube and place the pipette tip along the lower side of the tube wall without touching the pellet.
    b. Using a disposable graduated transfer pipette, aliquot serum into the pre-labeled cryovials.
        i. The red top tube should yield, on average, 4 ml of blood serum. Aliquot 0.5 ml per cryovial (total vials = 7-8 with 0.5 ml each).
    c. Be sure to place only **serum** in cryovials labeled with the suffix **09S to 16S** (as before, you may fill less than 8 cryovials with serum- use the lowest numbered labels first).
    d. Place an RED cap on each cryovial filled with serum.

9. Place cryovials in 81 grid cryovial box and freeze samples immediately in **-80°C Freezer**.

10. Dispose of red top serum tube (vacutainer) with clotted blood in the bottom of the tube into a biohazard container.

11. Fill in the TRACK Precision Medicine Sample Collection form (Appendix C) during processing with.
    a. Date and Time Serum Spin Begins
    b. Number of Serum Aliquots Collected
    c. Date and Time of Serum in Freezer




| | | | | |
|---|---|---|---|---|
| 1: Store tubes at room temp | 2: Collect blood into red top tube, allowing blood to flow for 10 sec and ensuring blood has stopped flowing. | 3: Immediately after blood draw, invert tube gently 8-10 times to mix sample. | 4: Allow blood to clot for 30 - 60 mins. Then centrifuge tubes at 1500 x g for 15 minutes. | 5: Label cryovials with PM "serum" labels. Use graduated pipette to aliquot 0.5 ml of serum into each cryovial. Cap serum cryovials with red caps. Store at -80°C until shipment. |

# 6. CSF Collection and Processing Procedures

## 6.1 General Guidelines

The decision to place an External Ventricular Drainage (EVD) is a local clinical decision and is not affected by a patient's participation in TRACK PM. Similarly, indications and procedures for CSF drainage (continuous vs. intermittent drainage) is a local clinical decision and not prescribed in the TRACK PM protocol.

CSF collected for research purposes is fluid that would otherwise be discarded.

- Procedures for inserting the EVD and for collecting fluid from the system are also governed by local Neuro ICU protocols.

- Published guidelines from the American Association of Neuroscience Nurses are available (Am Assc Neurosci Nurses (2011) Care for the patient undergoing intracranial pressure monitoring/external ventricular drainage or lumbar drainage. Glenview (IL) 37 p. [164 Refs]). Link to PDF

- A video demonstrating CSF collection is available here: https://vimeo.com/user120054989/CSFfromEVD
  **DISCLOSURE**: This tutorial is to assist trained personnel in CSF collection from an EVD.
  - Each site may differ in procedure. Check your local Neuro ICU protocol.
  - Also, this video shows betadine for cleaning the port in a sterile fashion; at some institutions, this may have been changed to chlorhexidine.

- The collection of CSF from the EVD system is performed by trained Neuro ICU nurses or physicians; however, trained research personnel may be granted permission at your institution (check local hospital protocols).

- At most centers, collection of 0.5 – 1 mL of CSF is routinely done daily, to monitor for infection. CSF for research purposes will in most cases be collected at the same time as the daily routine accession of the system.
  - If insufficient CSF is produced, priority will be given for fluid required for patient care.

- An effort should be made to collect the first CSF available at the time of insertion of the EVD. Up to 5 mL should be collected.

## 6.2 CSF Collection

**Steps for Whole CSF Collection and Processing**

1. CSF is collected daily from the buretrol. If a bag change occurs in the morning, allow at least 2 hours before collection from buretrol.

2. **Fresh fluid** is collected as follows:
   a. Fluid is collected **using sterile technique** directly from the buretrol.
   b. Up to 5 mL is collected (although in most cases it will be less) and transferred to single polypropylene conical centrifuge tube.

c. Fluid is allowed to drain into buretrol by gravity (**never aspirated**).

3. Cell contamination of ventricular CSF is a significant confound. To minimize, CSF is centrifuged.

4. Transport fluid within 30 minutes of collection to the laboratory and centrifuge at 1500 RCF (x g) for 15 minutes. This can be done at the same time as blood processing.

5. Place pre-printed **"CSF"** (01C-05C) labels on the 1.5 ml cryovial tubes.

6. Aliquot supernatant into 1.5 ml polypropylene cryovials using a micropipette with disposable tip.
   a. Up to 5 aliquots are prepared, each containing to 0.5 mL. If more fluid is collected, increase volume of aliquots up to a maximum of 1.0 mL.
   b. Examples:
      i. If 5 mL are collected, distribute into 5x 1 mL aliquots
      ii. If 2.0 mL are collected, distribute into 4 x 0.5 mL aliquots.
      iii. If 1.2 mL are collected, distribute into 2 x 0.6 mL mL aliquots.
   c. Place a CLEAR cap on each cryovial filled with CSF.

7. Place cryovials in 81-grid cryovial box and freeze samples immediately in -80$^{o}$C freezer.

8. The following are noted on the applicable CRF:
   a. Appearance of fluid (clear, cloudy, bloody)
   b. Date and Time of collection
   c. Time of centrifugation and freezing

9. This CSF collection protocol can run for a maximum of five (5) days:
   a. Collect up to three (3) CSF samples on Day 1 (A, B, and C visits).
   b. Collect one (1) CSF sample on Day 2 (D), Day 3 (E) and Day 5 (F).
   c. If possible, collect the TRACK PM blood sample at the same time as collecting the CSF sample. This will provide a paired blood sample for some of the CSF samples.

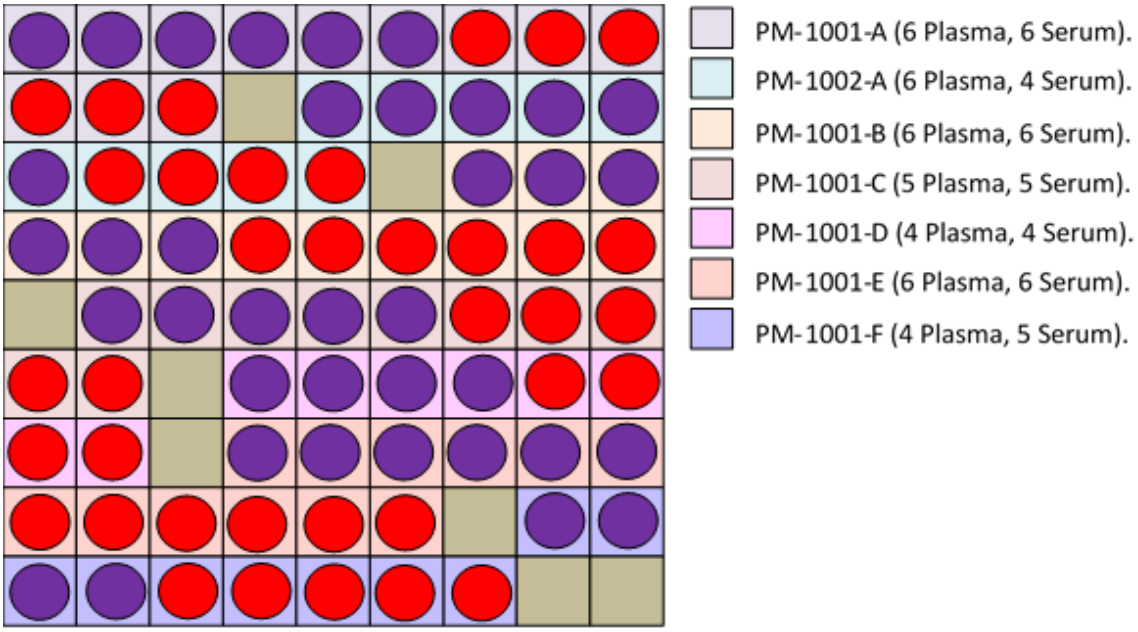
Samples will be shipped to the NTBI-BR at the University of Pittsburgh using same procedures as done for blood samples.

# 7. Storage of Specimens

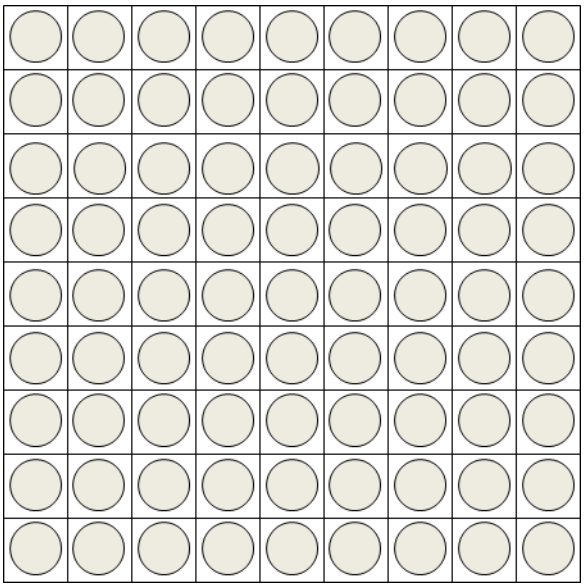
## 7.1 Storage of Serum, Plasma, Buffy Coat, and CSF Cryovials in Freezer

The supplied cryoboxes should be filled in chronological order of collection to minimize misplacement of samples within the box due to reshuffling of cryovials to keep subject samples next to one another.

- Plasma and serum from all visits may be placed in the box
- All aliquots from a single subject/timepoint are kept together
- Leave an empty space in the box between subject/timepoint

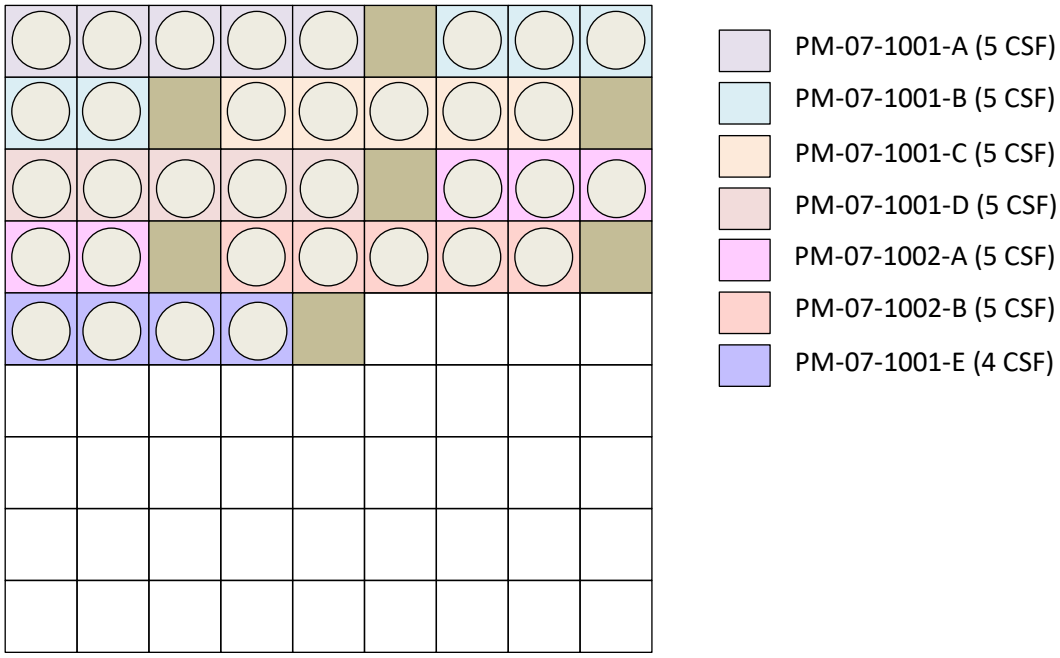



Buffy Coat should be placed in a separate cryobox. It is not necessary to leave spaces between subject/timepoint as done in the plasma/serum box above.



CSF should be placed in a separate cryobox from plasma/serum and buffy coat. CSF should be stored within the cryobox chronologically (as in the plasma/serum cryobox above).

- Plasma and serum from all visits may be placed in the box
- All aliquots from a single subject/timepoint are kept together
- Leave an empty space in the box between subject/timepoint
- Multiple subjects may be placed in the same box. No extra spaces need to be placed between different subjects (i.e., only a single space between subject/timepoint regardless of subject).




## 7.2 Storage of PAXgene Tubes in Freezer

PAXgene tubes must be stored upright until the specimen is fully frozen. It is best to keep PAXgenes in a freezer-safe rack in the -20°C freezer until transfer to -80°C freezer for long-term storage prior to shipping.

- PAXgenes must be kept in -20°C freezer for a minimum of 24 hours but may be stored at the temperature indefinitely.
- It may be most efficient to keep rack at -20°C until full, then transfer entire rack to -80°C freezer.
- PAXgene tubes need not be stored in any specific order, but it is imperative that PAXgenes are shipped concomitant with their respective plasma, serum, and CSF.

# 8. Packaging and Shipping Instructions

## 8.1 Biospecimens to be sent to the NTBI-BR Laboratory:

The following samples will be collected on each subject:

- Serum
- Plasma
- Buffy Coat (for DNA extraction)

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academics or industry through the NTBI-BR. A copy of the consent form for each subject should be kept on file by the investigator.

ALL study personnel responsible for shipping should be certified in biospecimen shipping and can obtain training and certification through the CITI training site (Course titled "Shipping and Transport of Regulated Biological Materials" at https://www.citiprogram.org/). Check your local institution's Environmental Health & Safety if additional training in the Shipment of Hazardous Material is needed.

## 8.2 Biorepository Shipping Instructions

*****Important Note*****

**For frozen shipments, include no more than FIVE Pathopouch A3 envelopes per GDI-80 shipping container in order to have room for enough dry ice to keep samples frozen up to 36 hours.**

**The labeled, processed, aliquoted and frozen cryovials of plasma, buffy coat, serum will be shipped to the Biorepository as outlined below.**

**Baseline and Follow-up Shipments to the Biorepository include the following:**

- **Frozen 0.5 ml aliquots of plasma (FROZEN SHIPMENT)**
- **Frozen 0.5 ml aliquots of buffy coat (for DNA, BASELINE visit only, FROZEN SHIPMENT)**
- **Frozen 0.5 ml aliquots of serum (FROZEN SHIPMENT)**

Specimens being shipped to the Biorepository should be considered as Clinical/Diagnostic specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

| **IMPORTANT!** |
| --- |
| **FROZEN SAMPLES MUST BE SHIPPED ON MONDAY - WEDNESDAY ONLY!** |

### 8.3 World Courier Instructions

World Courier will arrange delivery of packaging and dry ice to your site. **Packaging and shipping labels should be ordered three days in advance of shipment.** These will be delivered directly to your site prior to the shipping day. Dry ice will be delivered at the time of pick up. Please note that World Courier drivers cannot assist with packing your shipments.

**To arrange for the packaging and pick-up of samples, please contact:**

**World Courier Tel: (800) 221-6600**

Provide the World Courier Representative with the following information:

1. Study Account Number: # XXXXX
2. Time that pick-up is required **(Ship only on Monday-Wednesday!)**
3. Specify the type of samples being sent:
   Blood, serum, plasma. Biological substance category B, Frozen at -80°C.
   on dry ice, to Pittsburgh.
4. State that you will need ALL shipping materials delivered to your site.
5. Specify that dry ice is required at time of shipping

## All shipments require the following items:

| Shipping materials | Quantity | Description |
|---|---|---|
| **House WayBill** | **1** | Comes pre-printed with Shipper and Consignee information |
| **Box labels** | | World Courier provides these (both UN 3373 and Dry Ice labels.) |
| **Dry ice (10-kilo bag)** | **1-2 (see below)** | World Courier will bring when picking up your shipment. Specify that you need pellets rather than blocks of dry ice. |
| **Intelsius DGP Pathopouch 95 (A3 size)** | **Varies (see below)** | The Pathopouch A3 is a large Secondary envelope.<br>One Pathopouch A3 can maximally hold 4 81-grid cryoboxes, stacked 2 high and 2 deep. One Pathopouch A3 can maximally hold 24 PAXgenes in 6 dividers. |
| **Absorbent material vial dividers** | **Varies (see below)** | **World Courier provides these. Be sure to ask for them.**<br>Include 1 absorbent material pad in the Secondary envelope with the cryoboxes. Additionally, one divider may be used to hold 6 PAXgenes. |

## Use the following guide to determine which size of shipping container is necessary:

| Shipping Box | Cryoboxes | PAXgene Dividers | Description |
|---|---|---|---|
| **GDI-80** | **Up to 16** | **Up to 8** | A GDI-80 insulated box can hold 6 Pathopouch A3 packages. This should be large enough for 16 cryoboxes (4 boxes in each of 4 Pathopouches) + 48 PAXGene tubes (24 tubes in each of 2 Pathopouches) + dry ice (2 bags). |
| **GDI-45** | **Up to 12** | **Up to 8** | A GDI-45 insulated box can hold 3 Pathopouch A3 packages. This should be large enough for 12 cryoboxes (4 boxes in each of 3 Pathopouches) + 48 PAXGene tubes (24 tubes in each of 2 Pathopouches) + dry ice (order 2 bags, may need more than 1 to fill |
| **GDI-30** | **Up to 4** | **Up to 4** | A GDI-30 insulated box can hold 2 Pathopouch A3 packages. This should be large enough for 12 cryoboxes (4 boxes in 1 Pathopouch) + 24 PAXGene tubes (24 tubes in 1 Pathopouch) + dry ice (1 bag). |
| **GDI-15** | **1** | **1** | A GDI-15 insulated box can hold 1 Pathopouch A3 package. This should be large enough for only a single cryobox + 6 PAXGene tubes (all in 1 Pathopouch) + dry ice (1 bag, about half of the bag should fit). |

## World Courier model GDI-80 insulated shipper


The GDI-80 insulated shipping box is large enough for 5 filled A3 Pathopouches

5 A3 Pathopouches should fit 20 cryoboxes, along with adequate dry ice.

Outer Dimensions: 23 x 21 x 25 inches (57.0 x 52.0 x 63.5 cm)
Inner Dimensions: 17.4 x 15.2 x 19 inches (44.4 x 38.8 x 48.5 cm)

## Intelsius DGP Pathopouch 95, Size A3


Each A3 Pathopouch can fit up to four 5 x 5 x 2 inch cryoboxes, stacked 2 x 2.

Each A3 Pathopouch can fit up to four 6-place absorbent vial dividers, stacked 2 x 2.

Outer Dimensions: 16.5 x 11.5 inches (41.9 x 29.2 cm)
Inner Dimensions: 14.1 x 10.3 inches (35.9 x 26.1 cm)

The International Air Transport Association packing instructions for shipping Biological materials, IATA 650, can be found at https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

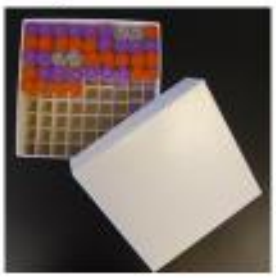
**Triple packaging consists of a primary receptacle, secondary packaging and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.**

| **IMPORTANT!** |
| --- |
| **IT IS ESSENTIAL TO KEEP YOUR SAMPLES FROZEN AT ALL TIMES DURING THE PACKING PROCESS** |

**Shipment Packing Instructions for Frozen Shipments (Plasma/Serum Cryovials)**

Watertight Secondary (DGP Pathopouch 95)

Cryovial box



Watertight Primary (cryovial)

Stack boxes 2 x 2 inside Pathopouch. Ensure that boxes pushed into pouch such that the adhesive-lined edges can meet.

Place an absorbent sheet above AND below boxes.

## *** Sealing Pathopouches ***

- It is imperative that the Pathopouches are fully sealed. Do not remove the protective tape covering the adhesive until the pouch has been filled.
- Once pouch is filled, remove tape and fold at opening. Line up black lines on both sides of the opening.
- When packing cryoboxes, ensure that corners are adequately sealed.

## Shipment Packing Instructions for filling the shipping container with sealed secondary envelopes and dry-ice

Place a layer of dry ice on the bottom of the Styrofoam-lined shipping carton.

Place the sealed Pathopouches **UPRIGHT** in the Styrofoam-lined shipping carton. You should be able to fit 6 within a GDI-80 box (not pictured).

FILL the remaining space in the shipping carton with dry ice, ensuring dry ice surrounds the envelopes and reaches the TOP of the carton

**The following video is a guide to filling your GDI-80 shipping box:**
https://vimeo.com/211217233
**You may ignore the portions pertaining to the cardboard stabilizer (we will be shipping too large a volume to make use of these) and temperature monitor.**

**IMPORTANT!**

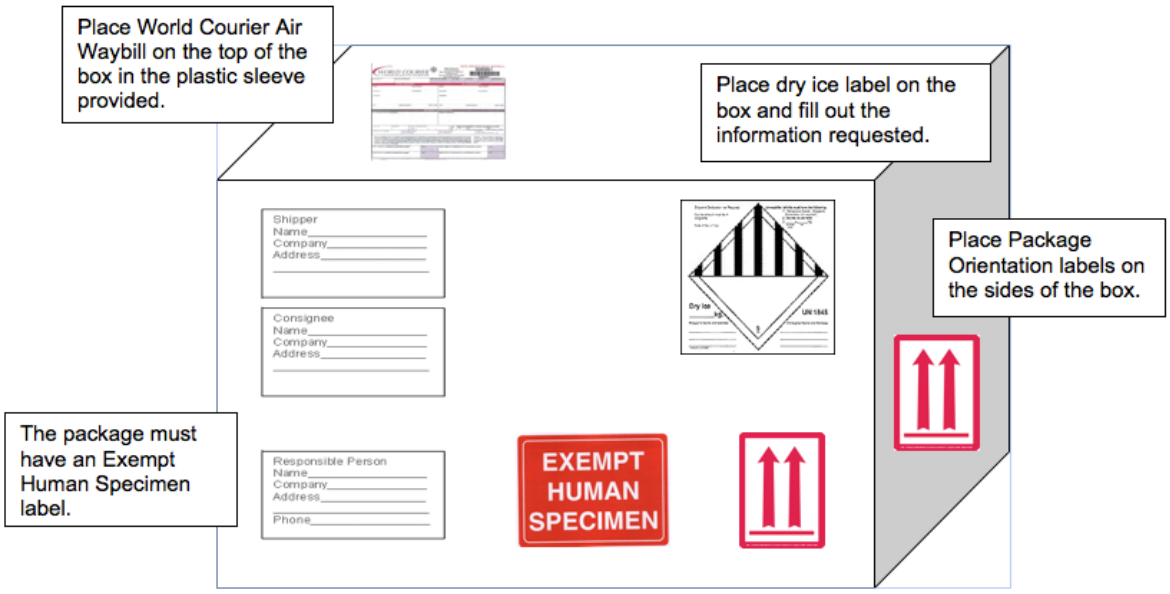
**AN ITEMIZED LIST OF CONTENTS MUST BE ENCLOSED BETWEEN THE SECONDARY PACKAGING AND THE OUTER PACKAGING.**

## *** Packing and Labeling Guidelines ***

IATA 650 guidelines: https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_PI650_EN.pdf

- The primary receptacle (frozen cryovials) must be leakproof and must not contain more than 1L total.
- The secondary packaging (DGP Pathopouch 95) must be leakproof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovial box containing the frozen cryovials) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging (i.e. between the Styrofoam container and cardboard box).
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

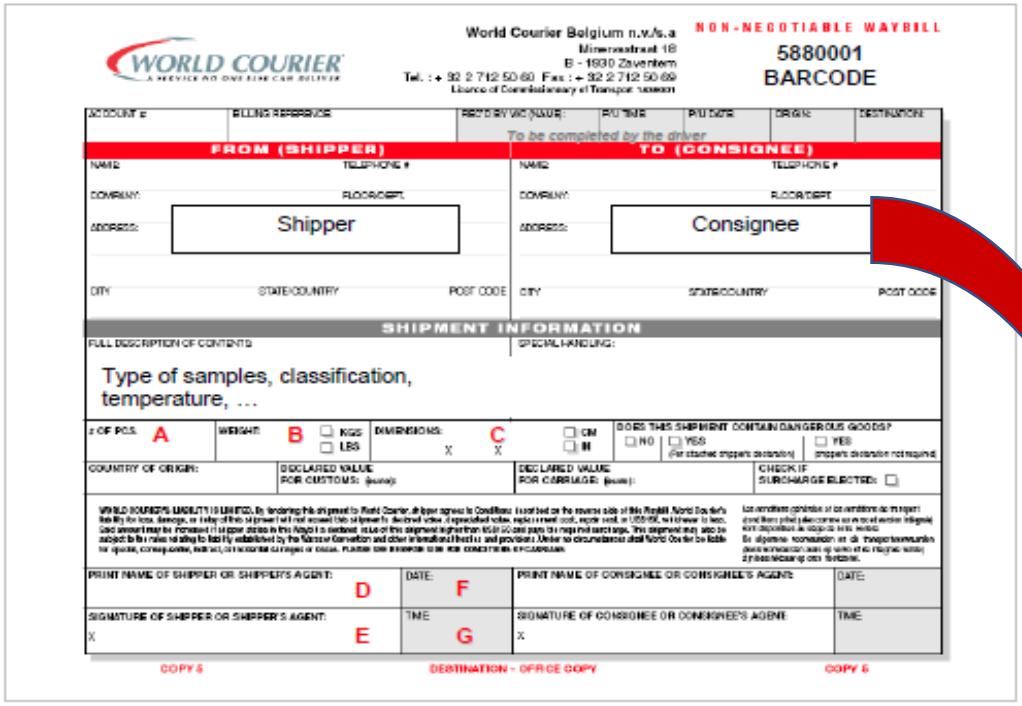
**Labeling & Marking Instructions**



# Required documents

## House Waybill (HWB)

- Please affix a waybill (or HWB) to the exterior of each shipment tendered to World Courier.
- World Courier will provide these forms with shipper and consignee information pre-printed for your convenience at the time of pick-up.
- This form is an internal tracking form used to identify your shipment from pick-up to delivery. When inquiring about your shipment, please reference the waybill number in the right hand corner.




**Please complete the following information :**

- A = number of packages
- B = total weight (kg)
- C = dimensions of the box (thermal box)
- D = full name of the shipper (in capitals)
- E = signature of the shipper
- F = collection date
- G = collection time

**Ensure that the Consignee address is:**

University of Pittsburgh, Neurotrauma Clinical Trials
ATTN: Miri Rabinowitz
3550 Terrace Street
Scaife Hall, Room S-916
PITTSBURGH, PA 15261

1. Contact World Courier to confirm service is available and schedule package supplies to be delivered and schedule the container to be picked up.

2. Notify the Biorepository of your intent to send a shipment by sending an email to the following addresses:

   - rabinowitzmk@upmc.edu
   - mancinellimd@upmc.edu

3. When the shipment is sent send an email to the address above and include the **Excel electronic manifest**

   a. See Appendix E for format of electronic manifest spreadsheet

   b. The Excel electronic manifest is uploaded into the database and should match the specimens being sent. This helps in accessioning the specimens into the database at the Biorepository.

4. If you have any questions or concerns, contact Miri Rabinowitz, Biorepository manager.

   a. Email: rabinowitzmk@upmc.edu
   b. Phone number: (412)-648-2031

**SHIP ALL FROZEN SAMPLES MONDAY-WEDNESDAY ONLY!**
**BE AWARE OF HOLIDAYS!!**
**BE AWARE OF INCIPIENT INCLEMENT WEATHER THAT MAY DELAY SHIPMENT/DELIVERY OF SAMPLES**

# 9. Sample Quality Checks and Feedback to Projects

In addition to tracking and reconciliation of samples, the condition and number of samples received are tracked by the Biorepository for each sample type. (See Shipment Tracking form – Appendix D) Investigators and clinical coordinators for each project are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process.

# 10. Data Queries and Reconciliation

The Precision Medicine Sample Collection Form (Appendix C) must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

QuesGen will be collaborating with the NTBI-BR to reconcile information captured in the QuesGen database compared to samples received and logged at the NTBI-BR. Information that appears incorrect in the QuesGen database will be queried through the standard system.

Data queries or discrepancies with samples shipped versus received at the BR may result from:

- Missing samples at the NTBI-BR
- Incorrect samples collected and shipped to the NTBI-BR
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information logged at the BR compared to information entered into the QuesGen database

Protocol compliance and study performance will be monitored by the Clinical Core using the study reports and dashboards provided by QuesGen Systems. Any protocol deviations should be reported and described in full under the research subject's study record in QuesGen, "Subject → Protocol Deviations" tab. Protocol deviations related to biospecimens may include:

- Blood collected for the baseline sample outside of <6 hour window
- Blood processing times deviated from protocol
- Blood collection was missed at any time point until day 5 only if the patient was still hospitalized
- Blood collected outside of the 2-week, 6-week, or 3-month window without prior approval for exception by the Executive Committee approval.
- Blood collection attempt unsuccessful
- Blood draw missed (i.e., follow up completed by phone, examiner error, subject refused)
- Other: (Any deviation not detailed above)

# 11. Appendices

**Appendix A:** **Rate of Centrifugation Worksheet**

**Appendix B:** **Lab Cheat Sheet**

**Appendix C:** **Blood Sample Collection Form**

**Appendix D:** **CSF Sample Collection Form**

**Appendix E:** **Shipment Tracking form**

**Appendix F:** **Biorepository Electronic Manifest Form**

## 11.1 Appendix A: Rate of Centrifugation Worksheet

Rate of Centrifugation Worksheet

**Rate of Centrifugation Worksheet**

Please complete and email this form to the NTBI-BR Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Include the completed copy of this form with your study files.

**Please call (412) 648-2031 with any questions**

**Submitter Information**
Name:

Site Number:

Submitter Email:

**Centrifuge Information** (Please answer the following questions about your centrifuge)

**Centrifuge Type:** ☐ Fixed Angle Rotor ☐ Swing Bucket Rotor

**Radius of Rotation (mm):**
Determine centrifuge's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

**Comments**

**These values are calculated using the formula below:**

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

**RCF** = relative centrifugal force (x g)
**RPM** = rotational speed (revolutions per minute)
**r** = centrifugal radius in mm = distance from the center of the turning axis to the bottom of the centrifuge.

**Email this form to:**
**NTBI-BR Manager**
**rabinowitzmk@upmc.edu**

**It is vital to this study that all samples be processed correctly.**

Rate of Centrifugation Worksheet
Created Date: 10 September 2020
Last Updated: 10 September 2020

Page 1 of 1

SAMPLE

## 11.2 Appendix B: TRACK-Precision Medicine Specimen Processing Quick Reference

### TRACK-Precision Medicine
### Specimen Processing Quick Reference

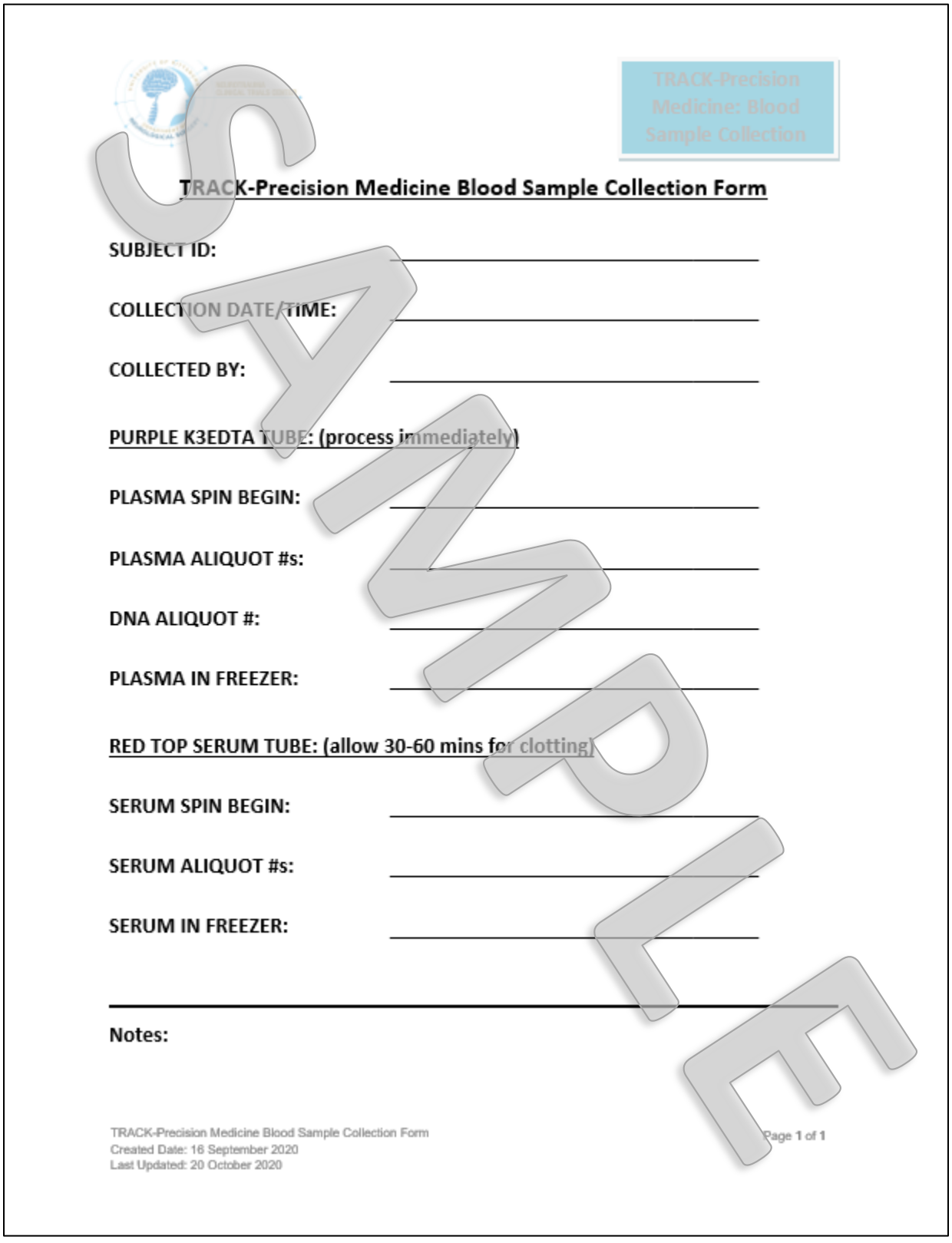
**1 EDTA Purple Top Tube**

- Spin for 20 Mins. @ 1500 RCF Immediately
- Aliquot Plasma into prelabeled 1.5 mL cryovials: 01-08P with purple caps
- Aliquot in portions of at least 500 uL (0.5 mL)
- DAY 1 ONLY: Aliquot Buffy Coat into prelabeled 1.5 mL cryovial: 09D with clear cap
- Store in -80°C

**1 Red Top Tube**

- Allow to clot 30-60 mins.
- Spin for 15 Mins. @ 1500 RCF
- Aliquot Serum into prelabeled 1.5 mL cryovials: 10S-17S
- Aliquot in portions of at least 500 uL (0.5 mL)
- Store in -80°C

## 11.3 Appendix C: Blood Sample Collection Form



TRACK-Precision Medicine: Blood Sample Collection

**TRACK-Precision Medicine Blood Sample Collection Form**

**SUBJECT ID:** _____

**COLLECTION DATE/TIME:** _____

**COLLECTED BY:** _____

**PURPLE K3EDTA TUBE: (process immediately)**

**PLASMA SPIN BEGIN:** _____

**PLASMA ALIQUOT #s:** _____

**DNA ALIQUOT #:** _____

**PLASMA IN FREEZER:** _____

**RED TOP SERUM TUBE: (allow 30-60 mins for clotting)**

**SERUM SPIN BEGIN:** _____

**SERUM ALIQUOT #s:** _____

**SERUM IN FREEZER:** _____

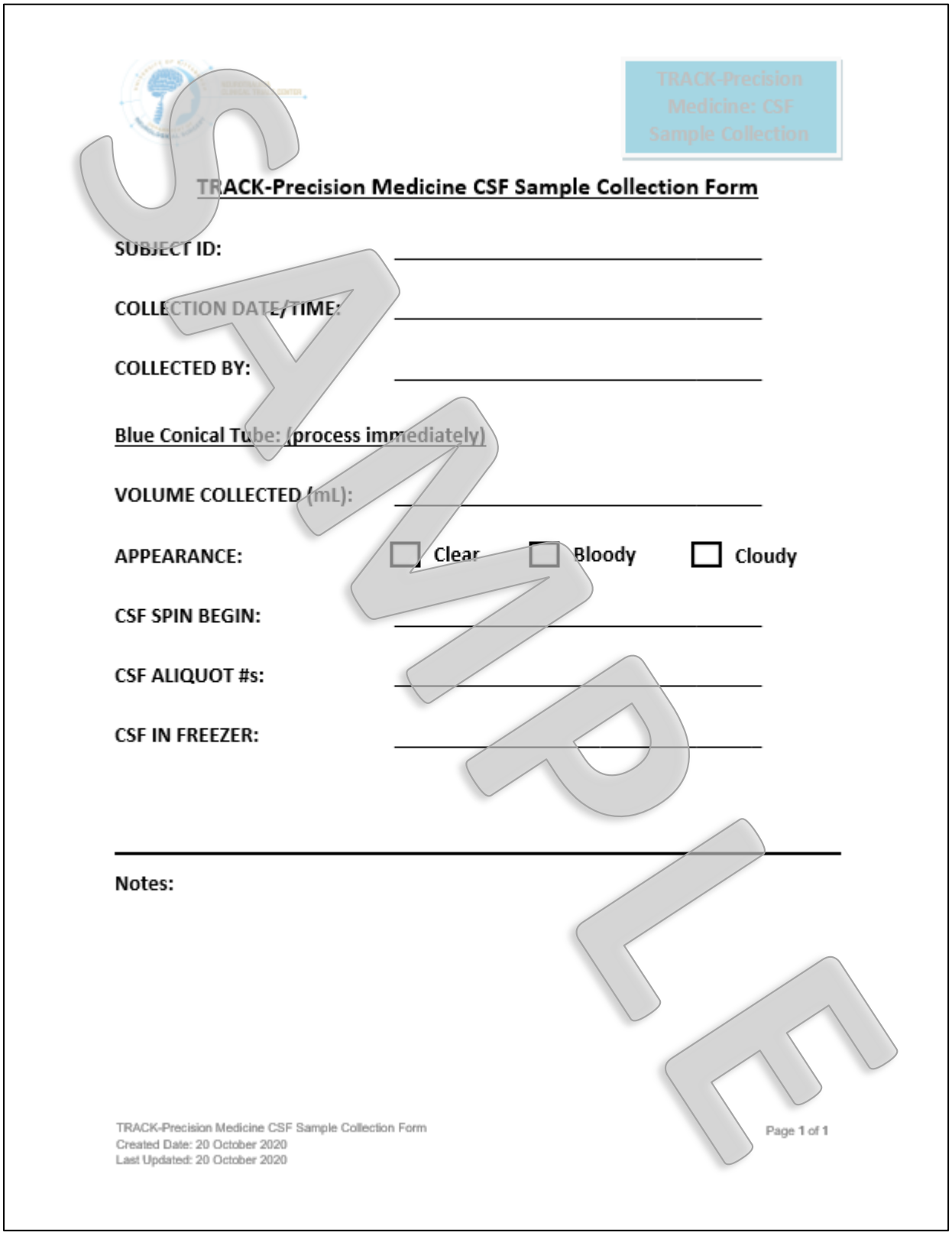
**Notes:**

TRACK-Precision Medicine Blood Sample Collection Form
Created Date: 16 September 2020
Last Updated: 20 October 2020

Page 1 of 1

SAMPLE

## 11.4 Appendix D: CSF Sample Collection Form



TRACK-Precision Medicine: CSF Sample Collection

**TRACK-Precision Medicine CSF Sample Collection Form**

**SUBJECT ID:** _____

**COLLECTION DATE/TIME:** _____

**COLLECTED BY:** _____

**Blue Conical Tube: (process immediately)**

**VOLUME COLLECTED (mL):** _____

**APPEARANCE:** ☐ Clear ☐ Bloody ☐ Cloudy

**CSF SPIN BEGIN:** _____

**CSF ALIQUOT #s:** _____

**CSF IN FREEZER:** _____

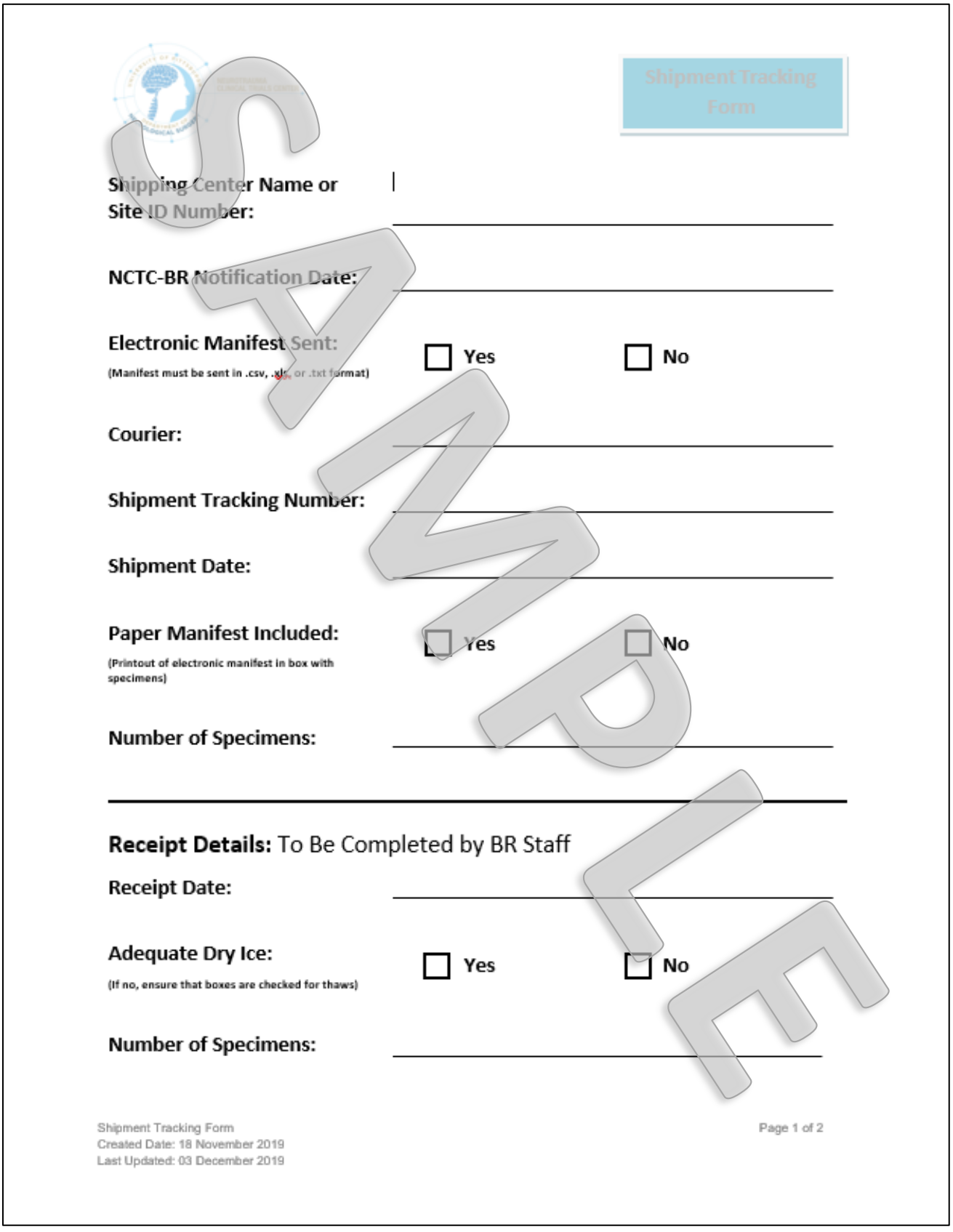
**Notes:**

SAMPLE

TRACK-Precision Medicine CSF Sample Collection Form
Created Date: 20 October 2020
Last Updated: 20 October 2020

Page 1 of 1

## 11.5 Appendix E: Shipment Tracking Form



Shipment Tracking Form

**Shipping Center Name or Site ID Number:** _____

**NCTC-BR Notification Date:** _____

**Electronic Manifest Sent:** ☐ Yes ☐ No
(Manifest must be sent in .csv, .xls, or .txt format)

**Courier:** _____

**Shipment Tracking Number:** _____

**Shipment Date:** _____

**Paper Manifest Included:** ☐ Yes ☐ No
(Printout of electronic manifest in box with specimens)

**Number of Specimens:** _____

---

**Receipt Details:** To Be Completed by BR Staff

**Receipt Date:** _____

**Adequate Dry Ice:** ☐ Yes ☐ No
(If no, ensure that boxes are checked for thaws)

**Number of Specimens:** _____

Shipment Tracking Form

**Instructions:**

**Shipping Center:** Name and Site ID of collection site shipping samples to BR

**NCTC-BR Notification Date:** Date of first notification to BR staff (Mike Mancinelli or Miri Rabinowitz). **BR MUST BE NOTIFIED PRIOR TO SHIPMENT.**

**Electronic Manifest Sent:** In initial notification email, an electronic manifest of all specimens included in shipment must be attached. Manifest must be in .csv, .xls, or .txt format.

**Courier:** The name of courier used for shipping. This should be World Courier except in extremely rare cases.

**Shipment Tracking Number:** Air Waybill number that will be used for shipment.

**Shipment Date:** Anticipated date of shipment.

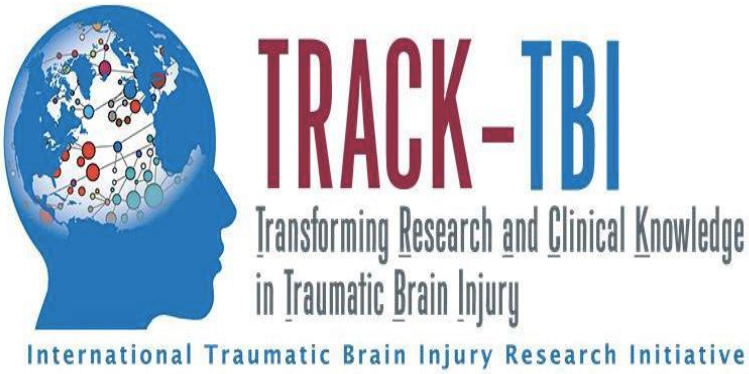
**Paper Manifest Included:** A printout of the electronic manifest should be included in the shipping box.

**Number of Specimens:** Total number of specimens included in shipment.

## 11.6 Appendix F: Biorepository Electronic Manifest Spreadsheet

| Box Label | Sample ID (Subject) | Visit | Material Type | Material Modifier | Date/Time Drawn | Date/Time Processed | Date/Time Frozen | Date/Time Shipped | Site |
|---|---|---|---|---|---|---|---|---|---|
| PM-03 Box 1 | PM-03-1001-A-01P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-02P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-03P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-04P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-05P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-06P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:20 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-07P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-08P | <6 Hours | Plasma | EDTA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-09S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-10S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-11S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-13S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-14S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-15S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-A-16S | <6 Hours | Serum | SCA | 1/3/2020 9:00 | 1/3/2020 9:42 | 1/3/2020 10:06 | 8/1/2020 11:00 | UCSF |
| PM-03 BC Box 1 | PM-03-1001-A-17D | <6 Hours | Buffy Coat | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-01P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-02P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-03P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-04P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-05P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-06P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-07P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-08P | 12 Hours | Plasma | EDTA | 7/8/2020 15:00 | 7/8/2020 15:05 | 7/8/2020 15:30 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-09S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-10S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-11S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-13S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-14S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-15S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |
| PM-03 Box 1 | PM-03-1001-B-16S | 12 Hours | Serum | SCA | 7/8/2020 15:00 | 7/8/2020 15:32 | 7/8/2020 15:51 | 8/1/2020 11:00 | UCSF |

Appendix 3




# Appendix 12: TRACK-TBI Add-On study Precision Medicine Manual of Procedures

## June 30, 2020

**You are receiving this protocol because your site has agreed to participate in this Precision Medicine study as an add- on to the TRACK-TBI protocol. The 5 participating sites include:**

**03 UCSF**
**07 Pittsburgh**
**12 Pennsylvania**
**14 MCW**
**15 Utah**

**SPECIFIC AIM 2**

**To validate early and ultra-early blood-based and novel imaging biomarkers of DAI, MVI, and neuroinflammation that may serve as predictive and pharmacodynamic biomarkers in a new cohort of moderate-severe subjects.**

In the TRACK-TBI U01study, MRIs were first obtained at 2-weeks after injury, and the initial blood sample was obtained within 24 hours after injury. Since it is likely that many effective TBI therapies will have to be started within hours or days post-injury, to obtain the necessary precision to design future clinical trials, we must have more granular information about the early evolution of the imaging and molecular biomarkers identified in Phase 1. In Aim 2 we will use the TRACK-TBI clinical recruitment procedures (see Appendix 9- TRACKTBI U01 Clinical Protocol V18-January 18 2019) to recruit a new cohort of subjects (n=50) with moderate to severe TBI defined according to VA/DoD criteria[1] through four of the existing TRACK-TBI Network sites. In this cohort, we will validate blood-based biomarkers at early (<24h) and ultra-early time periods (~6h), and neuroimaging biomarkers at early (2w) and ultra-early (within 24 to 48h) time periods.

**I) Specific Aim 2.A.**

**Validate early and ultra-early predictive blood biomarkers as well as pharmacodynamic molecular biomarkers in the acute phase after injury.**

**II) Specific Aim 2.B.**

**Validate early and ultra-early novel imaging biomarkers in the acute phase after injury.**

In this cohort we will obtain MRIs within 2 week of injury (early), and for a subset of 20% of patients, an additional ultra-early MRI within 24-48 hours of injury. All patients will also receive a follow-up MRI at 3m postinjury. In addition to volumetrics, DTI and rs-fMRI, the new MRI protocol will incorporate novel imaging measures of axonal density, cerebral blood flow, and neuroinflammation, which will all be standardized across sites and MRI vendors prior to participant enrollment.

***Hypothesis 2.B-1****: MR imaging biomarkers of DAI at ultra-early (within 24-48h) and early (2w) time points will correlate with elevated plasma biomarkers of acute DAI and predict progressive atrophy (on 3D T1 structural MRI), white matter degeneration and loss of functional connectivity in the period from 1 week to 3 months post-injury.*

***Hypothesis 2.B-2****: MR imaging biomarkers of MVI at ultra-early (within 24-48h) and early (2w) time points will correlate with elevated plasma biomarkers of acute MVI and predict progressive atrophy (using volumetric analysis of the serial 3D T1-weighted MRI scans) from 1 week to 3 months post-injury.*

***Hypothesis 2.B-3****: We further hypothesize that acutely elevated plasma biomarkers of MVI will correlate with reduced CBF and brain parenchymal microhemorrhages at <24 hours and at 1 week post-injury, both reflecting vascular endothelial dysfunction, and predict global and regional brain volume loss from 1 week to 3-months post-injury on volumetric analysis of 3D T1-weighted imaging.*

***Hypothesis 2.B-4****: MR imaging biomarkers of neuroinflammation at ultra-early (within 24-48h) and early (2w)time points will correlate with elevated plasma neuroinflammatory biomarkers and predict progressive atrophy from 1 week to 3 months post-injury.*

**Research Approach to Aims 2.A and 2.B.**

***Neuroimaging biomarkers****:* The overall MRI protocol is based on established TRACK-TBI standards for

structural imaging, DTI and rs-fMRI that have been harmonized across 3T scanners from all 3 MR vendor platforms. Standard operating procedures for acquisition, QA, QC, and data management of this 3T MRI protocol are available from the TRACK-TBI Imaging Core and have been adopted by other multicenter TBI imaging studies. The proposed MRI protocol includes one additional sequence not represented in the TRACKTBI protocol described in Aim 1: ASL perfusion imaging of CBF, as well as an additional 64-direction diffusion-weighted shell for the DTI protocol at b=3000 s/mm2 to create the multishell diffusion MRI sequence

**The entire proposed MRI protocol can be acquired in 60 minutes.** Standardization of structural MRI, diffusion MRI and rs-fMRI across sites will be performed using the same procedures already established for the TRACK-TBI study.

Arterial Spin Labeled Perfusion MRI Protocol: The ASL perfusion protocol is adopted from the new Alzheimer Disease Neuroimaging Initiative 3 (ADNI3) standards for all 3 MR vendors. In brief, the 5-minute sequence consists of 3D pseudocontinuous ASL (PCASL) on 3T GE scanners and 2D pulsed ASL (PASL) on Siemens and Philips scanners, with an additional proton density reference scan using the same ASL readout at a longer TR. These ASL acquisitions for all 3 MR vendor platforms conform to the most recent best practice guidelines for ASL perfusion imaging reported by the International Society for Magnetic Resonance in Medicine.[2]

MR Imaging Analysis: Analytic methods for volumetrics (to detect progressive atrophy over serial scans), DTI, and rs-fMRI will be the same as described for Phase 1-Aim 1A of TRACK-TBI Precision Medicine . Presence and number of microhemorrhages on high-resolution 3D T2* susceptibility imaging will follow the methods described for TRACK-TBI Precision Medicine Phase 1-Aim 1.B. Free water content (FISO) measurements from NODDI of multishell diffusion MR imaging will also follow the methods of Phase1-Aim 1.C.

ASL Perfusion MRI Analysis: CBF maps will be calculated from the difference between the labeled and control ASL perfusion images using the method of Buxton et al. (1998) for 3D PCASL imaging[3] and the method of Wong et al. (1998) for 2D PASL imaging.[4] For correction of proton density (PD) effects and radiofrequency coil in homogeneities, a scaling factor from the separately acquired PD scan will be employed to yield absolute CBF quantitation in units of mL/min/100 mL.[2]

**The TRACK-TBI Precision Medicine MRI procedures**

The TRACK-TBI Precision Medicine MRI procedures such as standardization of MR across sites, integration and analysis will align with the TRACK-TBI U01 procedures; however, the de-identified neuroimages from each site will be uploaded to the neuroimaging core repository at UCSF. TRACK-TBI Precision Medicine participants will undergo the same MRI procedures as set forth in the TRACK U01 study, Exhibit 5 with the exception of one additional ASL perfusion sequence not represented in the TRACKTBI protocol (See the 'Research Approach' above).

**MRI TIME POINTS**

CT or initial MRI will be obtained as part of clinical care. 3T MRI will be obtained within 24-48h, at 2 weeks and 3 months from CA+MRI subjects only. All initial and follow-up brain CT scans, and any brain MRI scans that are done for clinical care and their reports will be collected. Images will be read and coded by the Neuroimaging Core radiologist in accordance to the Neuroimaging TBI-CDEs.

The CA + MRI Cohort will receive a 3T MRI according to this protocol at the following time points:

## MRI Scans Schedule

| *Schedule of MRI scan (from time of traumatic brain injury)* | ***24 hr*** | ***Day 2*** | ***Day 14*** | ***Day 90*** |
|---|---|---|---|---|
| MRI scan | (X) | (X) | X | X |

(X)—Option either day 1 or day 2

- **The first ultra early scan- within 24-48 hour from time of injury**

- **2-Week/14 days (+/- 3 days) post-injury**

Subsequent cognitive testing must be completed within 3 days of this MRI, but not to exceed 8-18 days post-injury (for example, if MRI was done on Day 15, outcomes still need to be completed by Day 18).

- **3-Month/90 days (+/- 7 days) post-injury**

Subsequent cognitive testing must be completed within 7 days of this MRI, but must also fall between 83-97 days post-injury.

## References

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