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ORIGINAL ARTICLE PATHOPHYSIOLOGICAL MECHANISMS

Translational Outcomes Project in Neurotrauma (TOP-NT) Pre-Clinical Consortium Study: A Synopsis

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Abstract

Traumatic brain injury (TBI) has long been a leading cause of death and disability, yet research has failed to successfully translate findings from the pre-clinical, animal setting into the clinic. One factor that contributes significantly to this struggle is the heterogeneity observed in the clinical setting where patients present with injuries of varying types, severities, and comorbidities. Modeling this highly varied population in the laboratory remains challenging. Given feasibility constraints, individual laboratories often focus on single injury types and are limited to an abridged set of outcome measures. Furthermore, laboratories tend to use different injury or outcome methodologies from one another, making it difficult to compare studies and identify which pre-clinical findings may be best suited for clinical translation. The NINDS-funded Translational Outcomes Project in Neurotrauma (TOP-NT) is a multi-site consortium designed to address the reproducibility, rigor, and transparency of pre-clinical development and validation of clinically relevant biomarkers for TBI. The current overview article provides a detailed description of the infrastructure and strategic approach undertaken by the consortium. We outline the TOP-NT strategy to address three goals: (1) selection and cross-center validation of biomarker tools, (2) development and population of a data infrastructure to allow for the sharing and reuse of pre-clinical, animal research following findable, accessible, interoperable, and reusable data guidelines, and (3) demonstration of feasibility, reproducibility, and transparency in conducting a multi-center, pre-clinical research trial for TBI biomarker development. The synthesized scientific analysis and results of the TOP-NT efforts will be the topic of future articles.

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Introduction

The traumatic brain injury (TBI) research field has been plagued by failed clinical trials despite numerous promising therapeutics in pre-clinical animal studies.[1,2] Addressing this crisis in bench-to-bedside translation requires better alignment between the pre-clinical and clinical realms of the field through a more thorough handling of the heterogeneity that exists on both sides.[3] The development of pathophysiological biomarkers has the potential to help. To advance pre-clinical translation for neurotrauma, the Vivian L. Smith Foundation donated funds to National Institutes of Health/National Institute of Neurological Disorders and Stroke (NIH/NINDS) for developing a Translational Outcomes Project in Neurotrauma (TOP-NT) consortium framework. NINDS issued a competitive funding opportunity announcement in the form of a UG3/UH3-phased cooperative agreement funding mechanism with the specific intention of assembling an inter-institutional team to develop, characterize, and validate novel biomarkers that are both clinically relevant and able to be standardized across multiple centers.[4] The UG3/UH3 cooperative agreement supporting TOP-NT is structured as a two-phase funding mechanism that began with site-specific biomarker selection milestones that had to be accomplished by each of the individually funded sites at the end of an initial 2-year period (UG3 phase). Following successful completion of the UG3 milestones, NINDS generated cross-center milestones for second phase (UH3 phase) with 3 additional years of support, charging the individual sites with assessing the reproducibility and rigor of imaging and biofluid biomarker endpoints and standardizing approaches across centers of the consortium. An interesting feature of the funding mechanism is that the five sites selected for funding by NIH study section had no prior collaborations or knowledge of the other projects prior to funding. In this sense, the project represents a social experiment in scientific team building as well as a multi-center scientific project on biomarkers.

To better align bench-to-bedside translation, the TOP-NT researchers approached this challenge by focusing specifically on non-invasive assessment tools that are either currently used or could feasibly be implemented to evaluate patients in the clinic (e.g., neuroimaging and blood-based biomarkers). As clinical practice moves toward implementing biomarkers and magnetic resonance imaging (MRI) into evaluation of patient with TBI, it becomes increasingly important to gain a better understanding of their pathophysiological underpinnings, and thus quantitative histopathology and neurobehavioral assessments are being undertaken to correlate with the non-invasive biometrics.

The primary goal of TOP-NT was to advance biomarkers through distinct phases of initial discovery, internal validation and go/no-go decision-making (phase 1: NIH UG3 award) followed by external cross-validation and multi-laboratory reproducibility testing (phase 2: NIH UH3 award) (Fig. 1). In phase 1 (UG3), candidate biomarkers proposed by the TOP-NT centers were developed in the individual TOP-NT laboratories with pre-negotiated go/no-go criteria. In this initial phase, each individual center employed TBI models that were well established in their laboratory. Biomarkers selected for advancement were then tested in phase 2 across several models of TBI in multiple consortium laboratories. The identification and extensive characterization of TOP-NT biomarkers aims to improve the to improve the rigor, reproducibility, transparency, and set the stage for future translation of TBI biomarkers into clinical implementation. Although the TOP-NT project focuses on the internal and external validation of reproducible, clinically feasible biomarker assessments, the goal of the present article is to present the infrastructure and strategic plan developed for this consortium. The present synopsis may serve as an informative framework for future multi-center efforts that wish to test other important aspects of TBI research such as emerging treatment strategies.

The five independently funded TOP-NT centers were: University of California, Los Angeles (UCLA), Georgetown University/Uniformed Service University (GU/USU), Johns Hopkins University (JHU), University of California, San Francisco (UCSF), and University of Florida which transitioned to Morehouse School of Medicine (UF/MSM) upon investigator relocation. The initiation of the consortium was guided by NINDS programs staff who provided "teaming" milestones which assembled the independently funded laboratories into one team. In order to facilitate a productive "team science" approach, NINDS program staff joined team meetings to provide focused guidance and serve as a facilitator.[5] In addition, the TOP-NT consortium award notices included milestones focused on structural governance with data-sharing agreements, a publication policy, weekly web-conference meetings, an annual all-hands meeting, group-based project deliverables, and go/no-go decision-making regarding which pathophysiological biomarkers should advance to phase 2 (UH3 award phase).

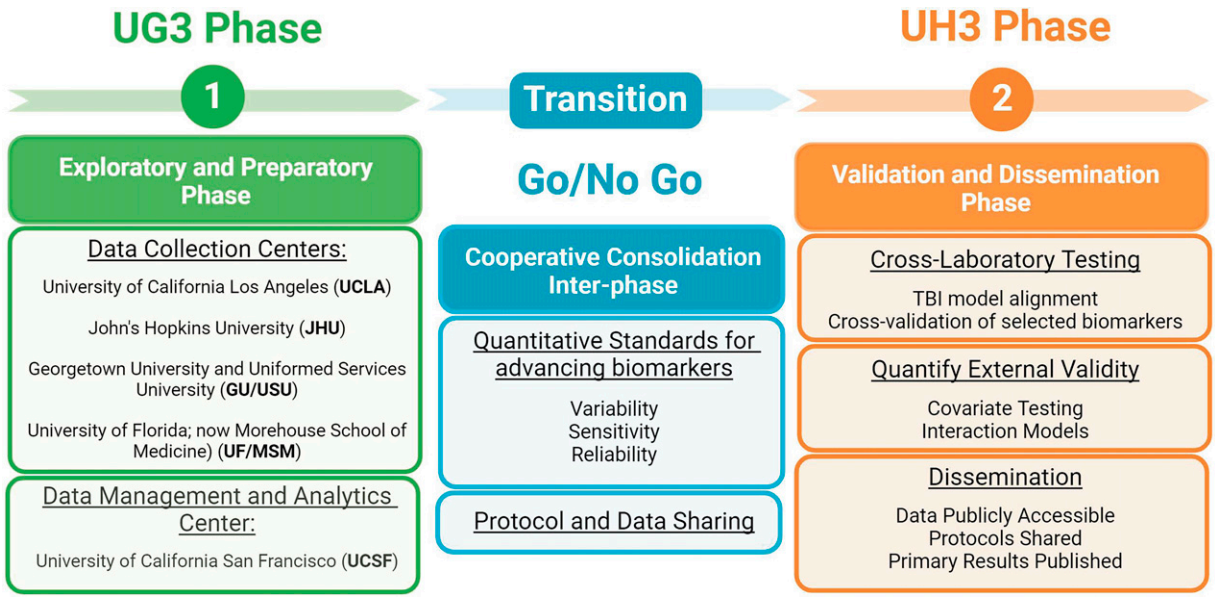

FIG. 1. Overview of the TOP-NT study design. TOP-NT is organized as a phased, multi-center consortium study. The initial, UG3 phase, is characterized by the data collection centers internally validating an extensive list of multimodal biomarkers. Those biomarkers that passed strict go/no-go criteria were advanced. The second, UH3 phase, is characterized by the external validation of biomarkers and dissemination of all research products (e.g., datasets, protocols, and articles). TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma.

A second major goal of TOP-NT involved the generation of harmonized, publicly available datasets for both UG3 and the UH3 phases. Each institution was responsible for carrying out the agreed-upon assessments and formatting the data according to common data elements (CDEs), outlined in the companion article [Wanner et al., *Prospective Harmonization, Common Data Elements and Sharing Strategies for Multicenter Preclinical TBI research: A Translational Outcomes Project in Neuro-Trauma (TOP-NT) Consortium Study*]. UCLA, GU, UF/MSM, and JHU acted as primary data collection sites and UCSF served as the data coordinating center, overseeing dataset harmonization, data sharing, and advanced analytics. Furthermore, TOP-NT is committed to adhering to common data standards with the goal of making datasets findable, accessible, interoperable, and reusable (FAIR).[6] Results of prospective harmonization and detailed descriptions of synthesized findings represent major scientific deliverables of the TOP-NT effort that will be reported in future articles and dataset publications.

Standardizing the collection and reporting of biomarker tools across centers presents an opportunity to compare results across studies with broad inclusion of injury models and for classifying a wide spectrum of TBI. Furthermore, the aggregation and harmonization of datasets from multiple centers can increase the statistical power of studies through the availability of more subjects. Cross-center data pooling presents an opportunity to apply powerful analytical techniques that are typically reserved for much larger datasets (e.g., machine learning). For this reason, the TOP-NT consortium formed a partnership with the Open Data Commons for TBI (ODC-TBI) (odc-tbi.org), an NIH-supported specialist repository specifically designed to support TBI data stewardship and sharing, compliant with the NIH 2023 Data Sharing and Management policy.[7]

At project close, TOP-NT will publish digital object identifiers (DOIs) for each of the datasets created by the collaborating laboratories, generating citable data work products that can be reused under an attribution creative commons license (CC-BY 4.0). This means that datasets are treated like scientific articles: authored, creative works that can be reused in future research, provided that primary data authors are cited for their contribution. Below, we outline the specific and novel features that make TOP-NT a template for next-generation preclinical team science and collaboration.

TOP-NT background and structure

TOP-NT is supported by an NINDS-initiated phased (UG3/UH3) cooperative agreement mechanism (Fig. 1). The initial UG3 phase allowed investigators at the data

collection centers (UCLA, GU/USU, JHU, UF/MSM) to develop and internally validate pathophysiological biomarkers for TBI and associate these biomarkers with progressing symptoms and pathologies (e.g., behavior and quantitative histology). During the UG3 phase, the UCSF team was charged with retrospectively curating data from 11 published articles by their multi-PI team, to create large, multi-lab datasets with imaging and inflammatory biomarkers after TBI.[7] Imaging and biofluid biomarkers included functional MRI (fMRI), diffusion-weighted imaging (DWI), amide proton transfer-weighted imaging (APT), and both blood-based and cerebrospinal fluid (CSF) biomarkers (glial fibrillary acidic protein [GFAP], aldolase C [ALDOC], total tau [Tau], phosphorated-tau [pTau], and neurofilament-light [NF-L]). This represents a combination of established biomarkers already under clinical study as well as newer biomarkers under development for potential advancement into the clinic in the future. The inclusion of both novel and established biomarkers provides side-by-side assessment of developing biomarkers and positive translational controls for cross-lab reproducibility.

In the UG3 phase, the pathophysiological underpinnings of biomarker end-points were established and validated by assessing their correlation to non-invasive measures of functional recovery (e.g., behavioral assessments) as well as their correlation to pathologies at certain time points (e.g., histopathology time-matched and regionally co-registered to imaging modalities). In this phase, each center utilized TBI models and protocols well established by, and already used in their respective labs. The models of TBI included in the UG3 phase were controlled cortical impact (CCI), fluid percussion injury (FPI), accelerated weight drop, and high-frequency head impact. Biomarkers were deemed successful if they survived *a priori* go/no-go criteria established in conjunction with the NIH program directors and were then advanced to the UH3 phase for multi-center cross-validation. During the UH3 phase, the standardized procedures required aligning biomarkers with quantitative histopathology at 1-month post-injury and behavioral assessments observed throughout recovery. As a result, in the UH3 phase each center conducted harmonized assessments and end-points in the models of TBI established in multiple TOP-NT centers (CCI, FPI, closed-head impact model of engineered rotational acceleration [CHIMERA] in rats). Specifically, in the UH3 phase, every center utilized the CCI model, two centers employed the CHIMERA model, and the other two centers employed the FPI model (Fig. 2).

Following the UG3 phase and prior to the initiation of the UH3 phase, TOP-NT Investigators established detailed, stepwise standard operating procedures (SOPs). Each SOP was reviewed by all members to maximize clarity and reproducibility. Consensus SOPs were then shared, adopted, and applied at all sites for the UH3 phase. In parallel, these consensus SOPs led to the development of TOP-NT CDEs that describe the variables articulated in the SOPs. The TOP-NT CDE development process is the topic of a companion article [Wanner et al., *Prospective Harmonization, Common Data Elements and Sharing Strategies for Multicenter Preclinical TBI research: A Translational Outcomes Project in NeuroTrauma (TOP-NT) Consortium Study*].

The thorough application of TOP-NT CDEs enabled the streamlining of the data collection process across centers and allowed for the aggregation of datasets into large, multi-site research products. Application of the consensus SOPs during the UH3 phase supported cross-validation of the primary UG3 findings across models and sites supporting the intended reproducibility, rigor, and transparency goals of TOP-NT. All resulting datasets from TOP-NT have been compiled and are being uploaded alongside detailed data dictionaries to the ODC-TBI (odc-tbi.org). All datasets will be assigned DOIs and be made publicly available.[7]

TOP-NT sites and expertise

The multi-center teaming and organization are shown in Figure 2. At the initiation, proposals from five centers were independently selected for testing and internally validating pathophysiological biomarkers. These proposals included four data collection centers (GU/USU, UCLA, JHU, and UF/MSM) and one center responsible for the centralized aggregation, management, and analysis of both existing and resulting datasets (UCSF). The phased TOP-NT consortium resulting from the NIH's selection of independent proposals and the successful completion of the "team building milestones" took careful advantage of the specific areas of expertise present at each site, while requiring cooperation across individual sites to complete certain milestones. From GU/USU, the biomarker incorporated into TOP-NT was a novel fMRI analysis called Hcorr. Hcorr is a local, regional heterogeneity analysis that uses spontaneous changes in blood-oxygen-level-dependent (BOLD) signal to infer neuronal selectivity, which may have the potential to serve as an indirect measure of synaptic function.[8] From UCLA, the biomarkers incorporated included serum-based, astroglial injury-defined biomarkers comprised of ALDOC and specific protein fragments of GFAP alongside DWI to address edema and microstructural disruption.[9–11] From JHU, APT, a recently developed molecular MRI technique sensitive to tissue pH or concentrations of endogenous proteins and peptides was selected to non-invasively visualize ischemic damage, inflammatory responses, and several other key pathological processes in TBI.[12] Lastly, UF/MSM proposed a multi-model biomarker approach

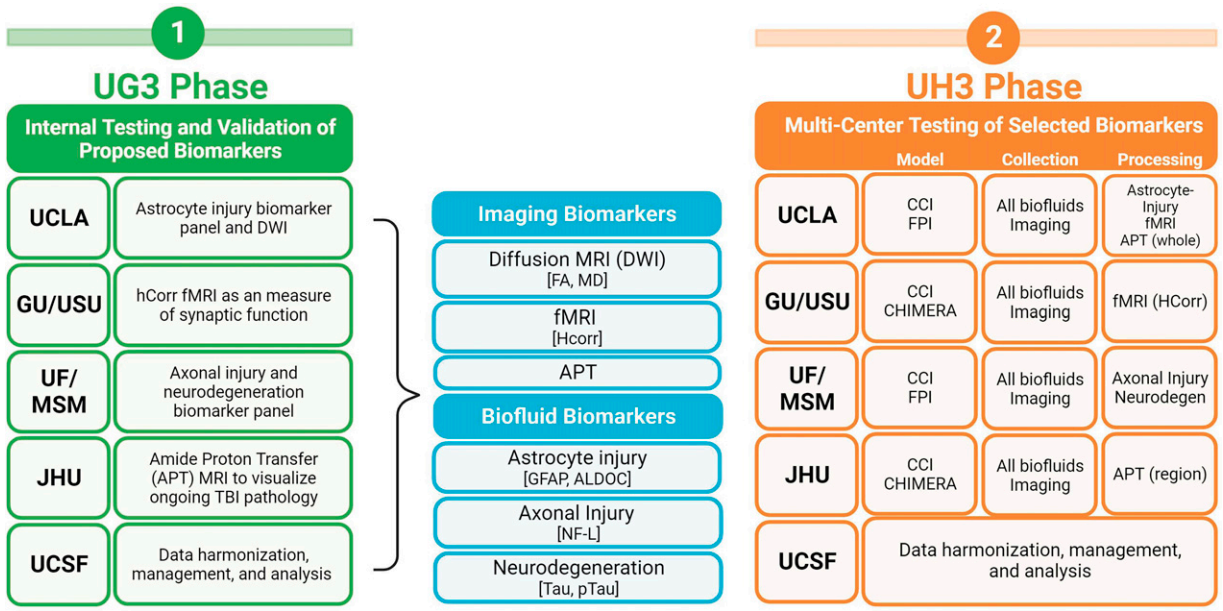

FIG. 2. Specifics of the TOP-NT study design. The UG3 phase was largely dictated by the five centers to take advantage of the local expertise and availability of technologies. Following the transition period where biomarkers were filtered using established go/no-go criteria, at least two centers were assigned to test each variable in the UH3 phase. Standard protocols and training were provided to ensure inter-center consistency. Where needed, samples were prepared and shipped between centers for testing (e.g., serum biomarkers ALDOC and GFAP processed by BRAINBox Solutions *via* UCLA) to collect the most complete dataset feasible. ALDOC, aldolase C; GFAP, glial fibrillary acidic protein; TOP-NT, Translational Outcomes Project in Neurotrauma; UCLA, University of California, Los Angeles.

including a biofluid analyte panel comprised of NF-L, Tau, pTau, and GFAP. These measures were aligned with MRI and applied following multiple models of TBI to capture a more inclusive range of clinically observed TBI pathologies, including axonal injury, contusion/tissue necrosis, loss of synaptic continuity, white matter injury, microvascular injury/brain hemorrhage and neuroinflammation.[13–15]

TOP-NT TBI models

The TOP-NT team tested biomarker candidates in a variety of TBI models, with the goal of providing insight into the pathophysiological features linked to each specific biomarker (Table 1). This multi-model approach is intended to inform future translation of TOP-NT biomarker applications for patient classification, subtyping, and therapeutic stratification for precision medicine.

Table 1. TOP-NT TBI Models: UG3 Initial Scope and UH3 Adaption

UG3-sites	*UG3 animal TBI models used*	*Selection for UG3–UH3 transition*	*UH3-adapted TBI models*	*Type of injury administered*
GU/USU	CCI (mouse), CCI, HFHI (mouse)	Go/no-go criteria	CCI CHIMERA	CCI as a model for focal, contusive, and diffuse injury
JHU	CCI		CCI CHIMERA	
UCLA	CCI		CCI FPI	FPI as model for mild-moderate diffuse injury
UF	CCI, l-FPI, AWD		CCI FPI	CHIMERA as a model of impact-acceleration induced concussion and repeated injury
UCSF	Data Management and analysis		Data integration/harmonization	

Unless otherwise specified, all TBIs were administered in the rat model.

AWD, accelerated weight drop; CCI, cortical impact; CHIMERA, closed-head impact model of engineered rotational acceleration; FPI, fluid percussion injury; HFHI, high-frequency head impact; JHU, Johns Hopkins University; TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma; UCLA, University of California, Los Angeles; UCSF, University of California, San Francisco; UF, University of Florida; USU, Uniformed Service University.

Models established at multiple centers were best suited for cross-center validation testing of the UH3 phase and are described below. Furthermore, the CCI and FPI models are prevalent throughout the TBI pre-clinical literature including detailed reports regarding physiological responses to these injury types. The inclusion of these clinically relevant and common models of TBI was vitally important to test the reproducibility of outcome measures across multiple sites in such a way that is informative for the field as a whole.

CCI injury. To model TBI resulting from a focal impact, the TOP-NT used the CCI rat model. CCI is performed by extending a rigid impactor tip through a parasagittal craniectomy onto the exposed dura resulting in a transient displacement of the underlying dura and neuronal tissue.[16,17] This approach has been well-characterized in the literature as a method of TBI that generates a focal injury to the cortex resulting in contusion and pericontusional areas as well as diffuse fiber damage in both adjacent and remote regions.[16,18,19] The mechanical insult from the impactor is capable of resulting in vast and progressive histopathological, structural, and functional impairments consistent with clinical observations such as cerebrovascular alterations, cell death and dysfunction, ventricular enlargement, and learning and memory deficits. In the TOP-NT study, multiple impact parameters were included to capture a spectrum of pathologies (piston penetration ranged from 1.0 to 2.8 mm; 20 psi; 200 ms dwell time) and model a typical, varied clinical cohort. Differences across parameters and potential implications for injury severity indices will be assessed using imaging and histological assessments able to quantify tissue damage and described in upcoming articles. Equal number of randomly selected male and female adult Sprague Dawley rats were used to determine whether sex is a significant confounding factor in all assessments and predictors. In females, the status of the estrous cycle on injury day added further information on possible influence of female cycling hormone levels on injury and biomarkers.[20,21] Furthermore, the CCI model was used by all data collection centers in the UH3 phase to enable direct cross-validation across sites.

Fluid percussion injury. To model diffuse, open head TBI, TOP-NT used the FPI rat model. FPI is a well-characterized model of TBI that captures clinically relevant deficits and pathologies.[22–24] Similar to the CCI model, FPI also applies a localized, mechanical insult through a parasagittal craniectomy; however, an FPI is administered *via* a pressurized fluid pulse rather than a rigid impactor. This results in a diffuse injury with typically milder or absent focal lesions but expansive and progressing white matter injury.[25] Even in cases where limited histopathology is observed, FPI subjects exhibit manifold neurobehavioral deficits.[23,26] This is consistent with the literature where the majority of TBI cases do not present on conventional (i.e., computed tomography [CT]) imaging assessments, yet patients experience lasting and debilitating symptoms. Similar to CCI, multiple device parameters were included (FPI device: 2.0 atm and 2.5 atm).

Closed-head impact model of engineered rotational acceleration. To model closed-head injuries TOP-NT used the CHIMERA model. CHIMERA is a more recently developed approach than CCI and FPI, developed to capture clinically relevant pathologies such as diffuse axonal injury and neurophysiological impairments.[27,28] CHIMERA involves a closed-head, non-surgical approach that incorporates both linear and angular acceleration in the sagittal plane. This model has been characterized in both mice and rats with previous descriptions of CHIMERA describing pathology consistent with white matter injury in the corpus callosum and optic tract, alongside behavioral impairments including motor deficits and cognitive dysfunction.[27–32] To further adapt the rat CHIMERA model to meet the research goals of TOP-NT, we introduced a 3D-printed interface, placed between the impactor and the scalp to distribute the energy over a larger surface area and avoid skull fracture (as used in the mouse CHIMERA).[31] This interface allowed us to induce impacts at approximately 10 m/s generating 5 J of kinetic energy. Mirroring the inclusion of multiple parameters in the other two injury models, one group received a single insult, and a second group received four insults distributed over 2 days (two insults at 1-h interval each day). Assessing the implications of different model parameters with respect to injury severity will be the topic of forthcoming articles.

Neuroimaging biomarkers

TOP-NT assessed the generalizability of the structural/functional markers in different MRI facilities, with different field strengths, and using different TBI models (Table 2). Neuroimaging biomarkers were selected by the TOP-NT Executive Committee based on the UG3 go/no-go criteria and cross-validated in three models (CCI, CHIMERA, and FPI) in four labs (GU/USU, JHU, UCLA, and UF/MSM) during the UH3 phase. Three neuroimaging sequences (fMRI, APT, and DWI) and four biomarkers (Hcorr, APT, fractional anisotropy [FA], and mean diffusivity [MD]) were selected representing a broad range of known TBI pathophysiologies. Specifically, Hcorr serves as a biomarker for synaptic damage/dysfunction (host site: GU/USU), APT imaging serves as a biomarker for protein density associated with neuroinflammation (host site: JHU), and FA and MD quantify microstructural damage (scalar maps derived

Table 2. TOP-NT Neuroimaging-Based Biomarkers: Initial Scope in UG3 and Selection for UH3 Phase

UG3	*Selection for UG3 to UH3 transition*	*UH3 adapted biomarkers*	*TBI pathological mechanism*
Biomarker candidates including T2, T2*, T1, DWI (MD, AD, RD, FA), CBF, MTC, APT, resting state fMRI, and Hcorr	Go/no-go criteria	FA	Axonal injury—white matter and gray matter injury
		MD	Swelling and vasogenic edema Diffuse white and gray matter injury
		Hcorr	Synaptic connectivity
		APT	Neuroinflammation, astrogliosis, microgliosis

AD, axial diffusivity; APT, amide proton transfer-weighted imaging; CBF, cerebral blood flow; DWI, diffusion-weighted imaging; FA, fractional anisotropy; fMRI, functional magnetic resonance imaging; MD, mean diffusivity; MTC, magnetization transfer contrast; RD, radial diffusivity; TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma.

from fitting data to the diffusion tensor), including FA to capture diffuse axonal injury and MD to reflect edema (host site: UCLA).

MRI scanners, software, and coils were used at each site in the UH3 phase including GU/USU (Bruker 7T, ParaVision 6.0.1, 86-mm quadrature coil transmit/4-channel phased array coil receive); JHU (Bruker 11.7T, ParaVision 6.0.1, 72-mm birdcage volume coil transmit/4-channel phased array coil receive); UF (Bruker 11.1T, ParaVision 6.0.1, in house quadrature surface coil/transceive mode); and UCLA (Bruker 7T, ParaVision 5.1.0, 72-mm birdcage volume transmit coil/quadrature, receive-only surface coil). Different MRI scanner hardware and software (e.g., different field strengths, different software versions, and different coils) may largely affect the cross-lab validation. Based on the sequences established by the host sites, the neuroimaging working group has worked closely to harmonize all related MRI sequences at the beginning of the UH3 phase. Our goal is to use the same imaging parameters and analyses pipelines that are optimized and acceptable for all labs (see Wanner et al., in press)[75]. However, due to different field strengths and scanner hardware limitations, reasonable adjustments were made for some parameters, such as the acquisition average number and echo time. To assess cross-site MRI variability, the sites tested an MRI phantom but these efforts were not successful due to the early state of technological development of the phantom. We therefore adopted an approach of scanning rats that were obtained from the same vendor/litter and used site-specific anesthetic sham rats to control for site–site variability.

fMRI and Hcorr. fMRI scans were applied to measure the selected Hcorr, or local regional heterogeneity, biomarker which can effectively estimate synaptic dysfunction across brain regions through an fMRI scan. Synaptic changes have been extensively reported after experimental TBI, with synapse loss occurring in multiple different animal models. Thus, the loss of synapses appears to be a common event, regardless of injury type and injury severity. We have selected an assessment battery consisting of validated techniques (including Hcorr from non-invasive fMRI scans) to measure synapse integrity, pathobiology, and function.

Amide proton transfer. APT is a relatively new, protein-based molecular MRI technique. Early APT or APT-weighted MRI studies have shown promise in detecting tumors, stroke, and other diseases.[12,33–35] This application in TOP-NT aimed to demonstrate the feasibility, potential, and reproducibility of APT-MRI signals as functional markers for TBI. Currently, there are no standard APT-MRI products available in Bruker MRI systems. To run APT-MRI experiments, TOP-NT designed, tested, and standardized novel pulse sequences.

Diffusion-weighted imaging. DWI tensor data were used to derive maps of FA and MD. These two scalars were determined by the prior G phase studies to be most promising for determining group assignments and predicting outcomes. Data were processed using the same pre-processing script shared among the sites in order to harmonize methods (see companion article, Wanner et al., in press)[75]. Data were co-registered to a common brain (i.e., mean deformation template created from the data at each site) followed by a computerized strict threshold-determined voxel-based change from average sham resulting in volumes of "injury burden" for each injured animal.

TOP-NT biofluid biomarkers

The TOP-NT team developed and performed a cross-validation assessment of selected blood-based biomarkers after TBI (Table 3).

Astrocytic injury markers. GFAP is an intermediate filament found in astrocytes with strong expression patterns reported in the hippocampus, cerebellum, and white matter.[36] Additionally, GFAP is highly induced in the hippocampus and cortex following TBI.[37] ALDOC is a brain-specific isoform of a glycolysis enzyme and is among the most abundant proteins found in the brain. ALDOC is highly expressed in healthy gray matter and white matter astrocytes.[38,39] Both GFAP and ALDOC

Table 3. TOP-NT Blood-Based Biomarkers: Initial Scope in UG3 and Selection for UH3 Phase

UG3 phase	*Selection for UG3 to UH3 transition*	*UH3 adapted biomarkers*	*TBI pathological mechanism*
Biomarker candidates for neuronal injury, astrocyte injury, axonal injury, white matter injury, neuroinflammation, vascular injury	Go/no-go criteria	GFAP	Astrocyte demise, white matter injury
		ALDOC	Astrocyte membrane wounding, gray and white matter injury
		NF-L	Axonal degeneration
		Tau	Neuronal cell injury
		Tau/pTau	Neurodegeneration

ALDOC, aldolase C; GFAP, glial fibrillary acidic protein; NF-L, neurofilament-light; pTau, phosphorated-tau; Tau, total tau; TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma.

are highly enriched markers of astrocytes whose expression is upregulated by the transcription factor STAT3; thus, implicating their role as targets of reactive astrogliosis following injury.[39,40] Additionally, GFAP and ALDOC are elevated in biofluids of patients with TBI with distinct profiles related to release kinetics and stability.[9,37,41] Released GFAP proteolytic fragments are associated with mortality and astrocyte demise, whereas ALDOC is released from membrane-wounded astrocytes and has greater biofluid stability.[9,42] GFAP, together with neuronal ubiquitin C-terminal hydrolase-L1, protein elevations in the blood are associated with intracranial lesions on head CT scans supporting their Food and Drug Administration approval for identifying CT-positive patients with more severe TBI and ruling out CT-negative patients with mild TBI.[43] On the contrary, ALDOC elevation has been reported in serum samples of both patients with mild and severe TBI.[9] Together, these two astroglial biomarkers capture white and gray matter injuries with distinct kinetics and sensitivities making them suitable for determining TBI types and underlying pathophysiology in pre-clinical studies.

Neurofilament-light. Neurofilaments (NFs) are neuron-specific, class IV intermediate filament proteins (10 nm diameter). NFs are major components of the axonal cytoskeleton, determine axon caliber, and support organelle trafficking and synaptic activity.[44] NFs are abundant in axons, dendrites, and perikarya.[45] The major neuronal intermediate filaments in the CNS are those assembled from the NF triplet proteins: the NF-L (68–70 kDa), NF-medium (150 kDa), and NF-heavy (200 kDa). As a TBI biomarker, NF-L is the most studied among neurofilaments. Shahim and colleagues reported CSF and serum NF-L levels correlate with post-concussive symptoms after sports-related concussion/mild TBI and relate to diffusion tensor imaging measures, including atrophy rates and FA.[46] In a rat model of mild TBI, NF-L was found to be substantially elevated at all acute (2 h, 1, and 3 days) and sub-acute time points (day 7–14) after a single mild TBI, with a peak at 1 day.[47] In a penetrating ballistic brain injury model in rats, Li et al. also found that serum NF-L was elevated on both days 1 and 2 post-injury.[48]

Tau and pTau. Tau protein is a microtubule-associated protein that functions as a major structural element in the axonal cytoskeleton.[49] Tau protein is prone to different post-translational modifications (PTMs) where phosphorylation at multiple epitopes is among the most assessed PTM in the context of neurodegeneration. Tau and pTau proteins are deposited in the brain in numerous neurodegenerative conditions including Alzheimer's disease, frontal temporal dementia, and TBI-linked chronic traumatic encephalopathy. These conditions are, therefore, termed tauopathies.[49–51] Using an ultra-high sensitive assay (rolling cycle amplification-surrounded optic fiber), Rubenstein and colleagues reported that both Tau and pTau (Thr-231) are elevated acutely (day 1) in patients with TBI (Glasgow Coma Score 3–15), and pTau (Thr-231) remains elevated in chronic TBI (average >1 year post-injury).[52] In two mouse models of repeated close-head injury, both serum Tau and pTau were elevated acutely and sub-acutely (day 1 to day 30 following injury) with pTau showing a higher fold increase.[53] Furthermore, Rubenstein et al. found both plasma Tau and pTau elevations on day 1 with Tau levels plateauing early but remaining elevated and plasma levels of pTau continuing to rise up to 12 months post-injury.[54]

TOP-NT neurobehavioral end-points

The TOP-NT team used several neurobehavioral end-points to help assess and validate the neuroimaging and biofluid biomarkers developed by the constituent laboratories (Table 4).

Neuroscore. The neurobehavioral status of the rats was assessed using the neuroscore, a composite score that includes tasks evaluating motor function, alertness, and physiological behavior.[55] Lower neuroscore values indicate more severe deficits. Animals were scored on a scale of 0 (severely impaired) to 4 (normal) for each of the following seven indices: (a) left and right (two indices) forelimb flexion during a tail suspension task, (b) left and right (two indices) hindlimb flexion when the forelimbs remained on a hard surface and the hindlimbs were lifted up and back by the tail, (c) ability to resist a lateral pulsion toward the left and right (two indices),

Table 4. TOP-NT Neurobehavioral End-Points: Initial Scope in UG3 and Selection for UH3 Phase

UG3 neurobehavioral end-points	*Selection for UG3–UH3 transition*	*UH3 neurobehavioral end-points*
Neuroscore, rotarod, water maze, elevated plus maze, Y-maze	Consensus discussion	Neuroscore Rotarod Y-maze Barnes maze

TOP-NT, Translational Outcomes Project in Neurotrauma.

and (d) performance on an angled board. The composite neuroscore (ranging from 0 to 28) was generated by summing the scores from each of the seven tests.[55,56]

Rotarod. The rotarod test is a widely used method for assessing the motor coordination of rats and mice.[57] It involves placing animals on a circular rod that rotates at a constant or increasing speed. The test evaluates maximal motor performance as animals attempt to maintain balance on the rotating rod rather than falling onto a platform below. Automation of this test allows for simultaneous evaluation of multiple animals on the same rod, facilitated by vertical barriers that separate them. Motor performance on the rotarod can be influenced by various factors, including motor coordination, learning ability, and cardiopulmonary endurance.

Barnes maze. The Barnes maze was used to assess spatial learning and memory impairments. The apparatus for rats is a light gray circular platform (122 cm in diameter) with 20 circular holes (10 cm in diameter) evenly spaced around the periphery, with a platform located 90 cm above the floor. The platform is illuminated with a bright light. An escape box that provides a dark cover preferred by rats is located under 1 of the 20 holes, and false bottom trays are placed below the other holes to prevent the rat from falling. The escape box and all the false bottoms are made of the same material as the platform. A white curtain with visual cues is placed around the apparatus. The task consists of one trial per day for 5 consecutive days. For each trial, the rat is placed in the middle and can freely explore the platform to enter the escape box. If the rat does not enter the escape box within 90–240 sec, it will be gently pulled into the escape box and be allowed to stay for 60 sec before being returned to home cage. The time for exploration to enter the escape box and the path traveled are tracked with a video camera and analyzed with Any-maze software.

Y-maze spontaneous alternation. This variant of the Y-maze task was used to assess short-term, spatial working memory impairments.[58,59] The apparatus is comprised of three identical arms (50 cm long, 10 cm wide, and 20 cm high, at an angle of 120° with respect to the other arms). Each individual rat is placed in the center of the Y-maze field and allowed to explore the maze freely for 8 min. The experimenter then leaves the room, and the movement of the rat is video recorded. A valid entry requires all four paws to be inside the arm. A spontaneous alternation is counted when the rat enters three different arms consecutively. Percentage of spontaneous alternation is calculated = [(number of alternations)/(total number of arm entries-2)] × 100.

Elevated zero maze. The elevated zero maze is used to assess anxiety-related behavior in rodents.[60] The apparatus consists of an annular platform where two diametrically opposite quadrants of the maze are enclosed by walls and adjacent to quadrants open to room light. Rats are randomly placed at the boundary between open and closed-walled quadrants, facing the closed zone, and permitted to explore the maze for 10 min. During the test, the animal's behavior is recorded by a video camera mounted above the maze and analyzed using a video tracking system for the position of the rat to determine the time spent in the open zones of the mazes, and the distance traveled during the test. Preference for being in the closed quadrants indicates a higher level of anxiety-like behavior.

TOP-NT histopathology end-points

The TOP-NT consortium incorporated histopathology as an unbiased quantitative approach to validate biomarker pathophysiology in both the UG3 and UH3 phases (Table 5). The features quantified during the UG3 phase included acute cortical astrocyte compromise and cell death, neuronal fiber damage, acute proteinopathy (injury day and 1 day post-injury; UCLA), and early inflammatory cell infiltration as well as microgliosis (3 days post-injury; JHU). Importantly, this approach provided regional metrics for the same markers as those measured in serum (GFAP, ALDOC, NF-L, and Tau). While the aforementioned proteins provided information on neuronal and astroglial injury, ionized calcium-binding adaptor-1, bisbenzimide, and Luxol fast blue were added to the histopathology assessments to capture microgliosis, cell density, and myelin changes at 1-month post-injury. Total amount and intra-area (within remaining tissue) changes were quantified in the ipsilateral cortex, cingulum, corpus callosum, and the dorsal hippocampus, with the latter allowing direct correlation to Hcorr data. Furthermore, true-to-position profiles enabled correlation

Table 5. TOP-NT Histopathology End-points: initial Scope in UG3 and Selection for UH3 Phase

UG3 histopathological end-points	*Selection for UG3–UH3 transition*	*UH3 histopathological end-points (profiles of areas, amounts, intensities)*	*TBI pathological mechanism*
Biomarker candidates for acute and chronic cytological injury: astrocyte injury, neuronal injury, (Image J driven process damage assays; astrocyte densities); neuroinflammation, scarring; vascular injury (IgG extravasation)	Consensus Discussion	GFAP	Astrogliosis
		ALDOC	Chronic astrocyte depletion and astrogliosis
		NF-L	Chronic neuronal loss and axonal degeneration
		Iba-1	Microgliosis
		Tau	Neuronal injury and tauopathy
		Luxol fast blue staining	Demyelination and white matter atrophy
		Bisbenzimide (Hochst) automated nuclear count	Inflammatory proliferation and atrophic/chronic cell loss

ALDOC, aldolase C; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adaptor-1; IgG, Immunoglobulin G; NF-L, neurofilament-light; Tau, total tau; TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma.

with co-registered MRI slice data, while summated data were used for correlation with serum biomarker levels (see companion article Wanner et al., in press).

TOP-NT data management, curation, and sharing

The TOP-NT consortium created a large number of datasets as scientific work products that will be disseminated to the research community to drive future research (Fig. 3). Preparing the datasets for public dissemination involved intensive collaboration between data scientists at UCSF and the data collection sites (see Fig. 2). To support this effort, the team partnered with an NIH-supported data repository, the ODC-TBI.[7] TOP-NT labs work closely with ODC-TBI data science staff members to adopt international data citation standards, conform to data formatting standards, and help with data wrangling to make datasets FAIR.[6]

Data management and sharing plan: ODC-TBI.

As previously noted, TOP-NT uses ODC-TBI (odc-tbi.org) as the data management and sharing platform. ODC-TBI supports storage of tabular data in comma separated value (.csv) format in a secure, cloud-based platform designed to support large-scale TBI collaborations, along with study metadata, data dictionaries, and ancillary files such as portable document format files that are associated data files. Large-volume imaging data (e.g., MRI files) are uploaded to the Federal Interagency TBI Research (FITBIR) Informatics system, and direct links to these resources will be made available within the ODC-TBI to ensure provenance and semi-federated access. In addition to privately sharing data with lab members or selected collaborators, ODC-TBI also enables researchers to publish their datasets with persistent and citable DOIs.[7] This approach conforms to the NIH data sharing policy and, with the inclusion of a detailed data dictionary, adheres to FAIR data standards.[61] In order to access datasets in the ODC-TBI, users must first register for a free account. Once logged in, users can access datasets, corresponding data dictionaries, and provenance pages specific to each dataset through the ODC-TBI portal.

FAIR data standards for TOP-NT.

FAIR data stewardship was a requirement of the original TOP-NT funding announcement.[4,6] A *findable* dataset enables any person wishing to work directly with the dataset the ability to locate all necessary files for viewing and understanding that content of the dataset (data dictionaries, SOPs, metadata, etc.). In the case of TOP-NT, the datasets are made public through the ODC-TBI platform and indexed in a manner similar to scientific articles. Public datasets include full author lines and DOIs that are persistent identifiers, web-discoverable, and citable entities. Thus, any article that uses a TOP-NT dataset, whether it is our group or an external researcher, must cite the issued dataset DOI alongside the publication. Web searches for dataset DOIs resolve to the ODC-TBI landing page for that dataset, where the associated files and their descriptions can be located.[62–64] Once located, the *accessible* nature of FAIR data stewardship ensures that files are made available in a clear and widely accessible manner. That is, the files must be downloadable in a format that is free and familiar to most users. The TOP-NT data are released under a Creative Commons Attribution license (CC-BY 4.0), which allows users to freely access and reuse data, provided that they cite the original data authors. In the case of TOP-NT and ODC-TBI, tabular data are stored in flat.csv files that can be easily opened in Excel and other common spreadsheet software and analyzed *via* common analytical programming languages such as R or Python. In the case of imaging files that do not follow the same tabular format, datasets will be stored as a .tar format in FITBIR, and direct links are provided on the ODC-TBI pages relevant to those datasets. Accessibility requirements for the TOP-NT datasets follow those set by the ODC-TBI infrastructure. Users must register for an account with ODC-TBI using their institutional email. Once the email has been validated,

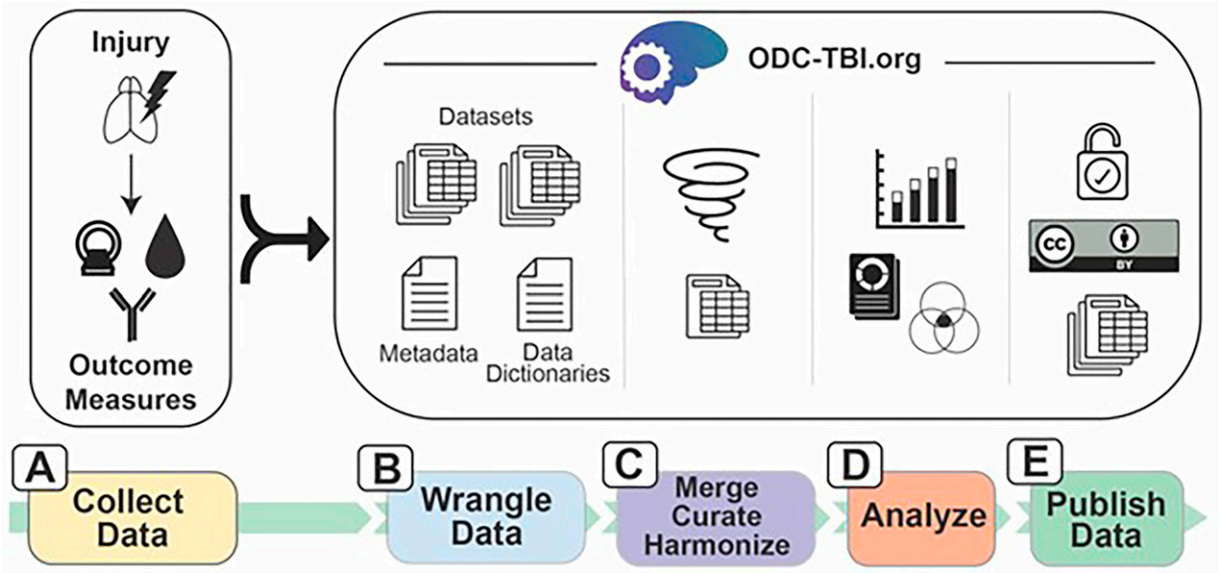

FIG. 3. Data management workflow. **(A)** Collected experimental data from the TOP-NT UG3 and UH3 phases were digitized and uploaded to the ODC-TBI (odc-tbi.org), an NIH-funded biomedical data repository for TBI data management and sharing. **(B)** Individual TOP-NT sites worked with ODC-TBI data scientists to wrangle data into machine-readable formats, providing structured metadata and data dictionaries that support FAIR principles and international data citation standards.[66] **(C)** Multi-center TOP-NT data were merged and cross-curated to generate large data pools. **(D)** Large sample sizes achieved through data pooling enabled reaching statistical power for hypothesis testing and provided rich feature sets for advanced machine learning approaches. **(E)** The individual datasets as well as pooled derivative data represent citable primary scientific work products that are publishable units much like articles. Each TOP-NT dataset received its own persistent digital object identifier with complete indexing information. TOP-NT datasets are made publicly available under a Creative Commons Attribution (CC-BY 4.0) license upon publication of associated peer-reviewed articles or grant end (whichever comes first). This makes TOP-NT data FAIR and open and conforms to federal data sharing policies including the 2023 NIH data management and sharing policy. FAIR, Findable, Accessible, Interoperable, and Reusable; ODC-TBI, Open Data Commons for TBI; TBI, traumatic brain injury; TOP-NT, Translational Outcomes Project in Neurotrauma.

the user can view and download publicly available datasets that have been issued citable DOIs. As TOP-NT progresses through the publication process, all datasets will be made available in this manner.

To ensure *interoperability* of multi-center datasets, TOP-NT adopted and further developed the federal pre-clinical CDEs for TBI. Development of the TOP-NT CDEs involved an intensive effort, detailed in a companion article (Wanner et al., in press). The TOP-NT CDEs were aligned to pre-clinical NINDS CDEs and version controlled to enable incorporation with future interagency efforts to maintain CDEs for the field. In cases where TOP-NT data elements cover areas not yet addressed by prior NINDS CDEs, TOP-NT-specific data elements were derived from SOPs and implemented across centers to ensure interoperability.

Lastly, FAIR data standards emphasize the importance of dataset *reusability* for secondary analyses and cross-validation. In the case of scientific research, this standard assists with addressing the rigor and reproducibility of data reporting. To ensure that any user who wishes to analyze a TOP-NT dataset has all the information necessary, the ODC-TBI published datasets include rich metadata and provenance information ODC-TBI.[7] These features provide a high-level overview of the dataset contents, and any published peer-reviewed articles that report findings derived from the dataset. As of February 2024, TOP-NT laboratories have uploaded 44 datasets to the ODC-TBI multi-laboratory controlled-access space for TOP-NT Investigators, representing $N = 3444$ rats and mice. The first of the UG3 datasets, which are based on UCSF UG3 phase retrospective curation of data from 11 prior articles ($N = 1250$ animals) (see "TOP-NT background and structure" section), have been published through publicly available DOIs.[62–64] UH3 datasets from

UCLA, GU/USU, UF/MSM, and JHU will be finalized for public release in the coming months.

Summary and Conclusion

As of 2024, the majority of pre-clinical TBI literature consists of siloed studies by individual laboratories rather than coordinated multi-center efforts. Most laboratories focus on a single animal model of TBI and a small number of target end-points. This results in a disjointed literature of heterogeneous pre-clinical studies, each modeling a specific patient subpopulation. The development of biomarkers that help define injury subtypes has great potential to accelerate patient stratification and precision medicine. Toward this goal, the TOP-NT project performed multi-center collection and standardization of pre-clinical data on pathophysiological biomarkers and end-points for multiple TBI models. In doing so, we now have the opportunity to compare the reproducibility, rigor, and transparency of biomarker results across centers, studies, and TBI types. Aggregation and harmonization of multi-center pre-clinical data enables pooling of subjects, increasing statistical power of studies and opening opportunities for advanced analytics such as machine learning that are typically reserved for much larger datasets.

TOP-NT is not the first large-scale, multi-center study in pre-clinical neurotrauma. The spinal cord injury (SCI) field executed the Multicenter Animal Spinal Cord Injury Study (MASCIS) in the mid-1990s with the goal of testing drugs in standardized injury models with standardized outcomes.[65] The major outputs from the MASCIS study were the NYU/MASCIS impactor model and the Basso Beattie Bresnahan locomotor outcome scale.[66] In addition, recent "data archeology" studies have digitized and curated all of the MASCIS data, and advanced analysis using machine intelligence has generated novel findings about acute blood pressure management that translated into the clinic.[67,68] This demonstrates the value of FAIR data for driving novel discoveries.

Pre-clinical TBI also has prior examples of multi-center studies. The Department of Defense-funded Operation Brain Trauma Therapy (OBTT) successfully employed a multi-center consortium to screen potential therapeutics across multiple animal models of TBI and demonstrated the importance of conducting cross-institutional studies.[26,69–72] Major differences between OBTT and TOP-NT are the degree of standardization across centers as well as the primary objectives of the consortia (i.e., therapeutic testing in OBTT vs. biomarker evaluation in TOP-NT). OBTT tested pharmacotherapies with a history of pre-clinical success in a series of three distinct TBI models, with each individual laboratory executing its own specialized protocols. In contrast, TOP-NT developed standardized TBI models and protocols that were deployed in parallel across centers. In this sense, TOP-NT mirrors the structure of a multi-center clinical observational study with a high degree of standardization across enrolling centers, whereas OBTT mirrors a phase IV clinical study where post-market drugs are deployed across heterogeneous centers. The OBTT group successfully aggregated datasets collected by independent groups in a way that allows for more broadly encompassing reports than is typically found in the TBI literature (i.e., three injury models, two species, numerous functional outcome assessments).[3,13,24,70–74] While the field continues to adjust to this collaborative mindset, there remains an urgent need for the characterization and multi-site validation of pathophysiological end-points such as neuroimaging and more extensive biomarker panels. Further development and adoption of data management infrastructures that support the option to share data with other TBI researchers may provide new opportunities for data reuse to drive novel discoveries.

TOP-NT reflects a next-generation collaborative pre-clinical project in neurotrauma whereby multiple laboratories work together toward a common goal of biomarker development through standardization and adoption of the same methods and models. The primary outputs will include the development and validation of robust pathophysiological biomarkers of TBI, and high-quality publicly available datasets as well as their synthesized findings that will be reported in a series of forthcoming articles. The transparency in sharing protocols and datasets will contribute to the reproducibility and potential utility of the novel pre-clinical tools we propose are central to this endeavor. As we continue to refine these tools and apply them within the context of TBI research, it will be critical to assess not only their effectiveness in advancing our understanding of TBI but also their practicality and relevance for clinical application. This will involve a concerted effort to validate these tools against established clinical outcomes and to engage in a continuous dialogue with both the research community and clinical practitioners to ensure that our approaches are aligned with the needs and realities of TBI treatment. TOP-NT also represents an early implementation of the data management and sharing policy that is now required for all new NIH awards as of 2023, where datasets themselves are viewed as citable primary work products. More broadly, TOP-NT provides a model of multi-center pre-clinical research that can be adopted by other fields to usher in a new era of collaborative, team-based pre-clinical research to improve scientific rigor, reproducibility, transparency, and bench-to-bedside translation.

Transparency, Rigor, and Reproducibility

The TOP-NT project is a multi-center project specifically designed to improve transparency, rigor, and reproducibility of biomarkers and data for pre-clinical TBI models. TOP-NT promotes transparency by making all resources public, including data, experimental

procedures, instrumentation, and analyses by a range of measurement variables, including MRI protocols, biomarker assays, histopathology, and behavioral testing. All statistical analysis plans and reporting were developed, *a priori* by the external data coordinating center, including a plan to publicly post all data in the odc-tbi.org data repository site and to assign DOIs to provide citable persistent identifiers data, the SOPs, as well as the associated data dictionaries and CDEs. Before the UH3 experiments were initiated, the data from the UG3 phase was used for power calculations to estimate effective power >0.80 with 48 rats for each study. Rare instances of non-survival of laboratory rats were followed up with a replacement to ensure the planned sample sizes. Rigor was a planned component of the research, including close adherence at all research sites to the SOPs and documentation of CDEs, and detailed reporting of all pre-identified variables. Rigor also included random assignment of rodents to the experimental conditions and blinding of investigators to animal treatments as well as exclusion criteria. Reproducibility was an inherent component of the project, where at least two research sites conducted the same pre-clinical TBI model independently, with full transparency and documentation of potential site-specific differences, and planned statistical analyses for determining the degree of such site-specific differences. True replicates were built-in for biomarker assays and GFAP and area histopathology data, which allowed to determine whole cohort coefficients of variation.

Acknowledgments

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Author Disclosure Statement

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References

1. Maas AIR, Menon DK, Manley GT, et al. InTBIR Participants and Investigators. Traumatic brain injury: Progress and challenges in prevention, clinical care, and research. Lancet Neurol 2022;21(11):1004–1060; doi: 10.1016/S1474-4422(22)00309-X
2. Maas AIR, Marmarou A, Murray GD, et al. Clinical trials in traumatic brain injury: Current problems and future solutions. Acta Neurochir 2004; 89(Suppl):113–118; doi: 10.1007/978-3-7091-0603-7_16
3. Radabaugh HL, Ferguson AR, Bramlett HM, et al. Increasing rigor of preclinical research to maximize opportunities for translation. Neurotherapeutics 2023;20(6):1433–1445; doi: 10.1007/s13311-023-01400-5
4. RFA-NS-17-023: Translational Outcomes Project in Neurotrauma (TOP-NT) (UG3/UH3). n.d. Available from: https://grants.nih.gov/grants/guide/rfa-files/RFA-NS-17-023.html [Last accessed: September 2, 2023].
5. Lubbers L, Iyengar SS, CURE Infantile Spasms Consortium, CURE Staff. A team science approach to discover novel targets for infantile spasms (IS). Epilepsia Open 2021;6(1):49–61; doi: 10.1002/epi4.12441
6. Wilkinson MD, Dumontier M, Aalbersberg I, et al. The FAIR guiding principles for scientific data management and stewardship. Sci Data 2016; 3(1):160018; doi: 10.1038/sdata.2016.18
7. Chou A, Torres-Espín A, Huie JR, et al. Empowering data sharing and analytics through the Open Data Commons for traumatic brain injury research. Neurotrauma Rep 2022;3(1):139–157; doi: 10.1089/neur.2021.0061
8. A Novel and Non-Invasive fMRI Biomarker of Neurodegenerative Disease. n.d. Available from: https://otc.georgetown.edu/fmri-biomarker/ [Last accessed: September 2, 2023].
9. Halford J, Shen S, Itamura K, et al. New astroglial injury-defined biomarkers for neurotrauma assessment. J Cereb Blood Flow Metab 2017; 37(10):3278–3299; doi: 10.1177/0271678X17724681
10. Villapol S, Loane DJ, Burns MP. Sexual dimorphism in the inflammatory response to traumatic brain injury. Glia 2017;65(9):1423–1438; doi: 10.1002/glia.23171
11. Huh JW, Raghupathi R, Laurer HL, et al. Transient loss of microtubule-associated protein 2 immunoreactivity after moderate brain injury in mice. J Neurotrauma 2003;20(10):975–984; doi: 10.1089/089771503770195821
12. Dong Y, Gu Y, Lu J, et al. Amide proton transfer-weighted magnetic resonance imaging for detecting severity and predicting outcome after traumatic brain injury in rats. Neurotrauma Rep 2022;3(1):261–275; doi: 10.1089/neur.2021.0064
13. Mondello S, Shear DA, Bramlett HM, et al. Insight into pre-clinical models of traumatic brain injury using circulating brain damage biomarkers: Operation brain trauma therapy. J Neurotrauma 2016;33(6):595–605; doi: 10.1089/neu.2015.4132
14. Yang Z, Xu H, Sura L, et al. Combined GFAP, NFL, Tau, and UCH-L1 panel increases prediction of outcomes in neonatal encephalopathy. Pediatr Res 2023;93(5):1199–1207; doi: 10.1038/s41390-022-01994-0
15. Yue JK, Upadhyayula PS, Avalos LN, et al. The role of blood biomarkers for magnetic resonance imaging diagnosis of traumatic brain injury. Medicina (Kaunas) 2020;56(2):87; doi: 10.3390/medicina56020087
16. Osier N, Dixon CE. The controlled cortical impact model of experimental brain trauma: Overview, research applications, and protocol. Methods Mol Biol 2016;1462:177–192; doi: 10.1007/978-1-4939-3816-2_11
17. Radabaugh HL, LaPorte MJ, Greene AM, et al. Refining environmental enrichment to advance rehabilitation based research after experimental traumatic brain injury. Exp Neurol 2017;294:12–18; doi: 10.1016/j.expneurol.2017.04.013
18. Osier N, Dixon CE. Mini review of controlled cortical impact: A well-suited device for concussion research. Brain Sci 2017;7(7):88; doi: 10.3390/brainsci7070088
19. Radabaugh HL, Carlson LJ, O'Neil DA, et al. Abbreviated environmental enrichment confers neurobehavioral, cognitive, and histological benefits in brain-injured female rats. Exp Neurol 2016;286:61–68; doi: 10.1016/j.expneurol.2016.09.015
20. Kohama SG, Goss JR, McNeill TH, et al. Glial fibrillary acidic protein mRNA increases at proestrus in the arcuate nucleus of mice. Neurosci Lett 1995;183(3):164–166; doi: 10.1016/0304-3940(94)11141-5
21. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: Some helpful considerations. Braz J Biol 2002;62(4A): 609–614; doi: 10.1590/s1519-69842002000400008
22. Alder J, Fujioka W, Lifshitz J, et al. Lateral fluid percussion: Model of traumatic brain injury in mice. J Vis Exp 2011(54):3063; doi: 10.3791/3063
23. Lifshitz J, Rowe RK, Griffiths DR, et al. Clinical relevance of midline fluid percussion brain injury: Acute deficits, chronic morbidities and the utility of biomarkers. Brain Inj 2016;30(11):1293–1301; doi: 10.1080/02699052.2016.1193628
24. Radabaugh H, Bonnell J, Schwartz O, et al. Use of machine learning to re-assess patterns of multivariate functional recovery after fluid percussion injury: Operation brain trauma therapy. J Neurotrauma 2021;38(12): 1670–1678; doi: 10.1089/neu.2020.7357
25. Bramlett HM, Dietrich WD. Long-term consequences of traumatic brain injury: Current status of potential mechanisms of injury and neurological outcomes. J Neurotrauma 2015;32(23):1834–1848; doi: 10.1089/neu.2014.3352
26. Kochanek PM, Bramlett HM, Dixon CE, et al. Operation brain trauma therapy: 2016 update. Mil Med 2018;183(suppl_1):303–312; doi: 10.1093/milmed/usx184
27. Namjoshi DR, Cheng WH, McInnes KA, et al. Merging pathology with biomechanics using CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration): A novel, surgery-free model of traumatic brain injury. Mol Neurodegener 2014;9(1):55; doi: 10.1186/1750-1326-9-55
28. Vonder Haar C, Martens KM, Bashir A, et al. Repetitive closed-head impact model of engineered rotational acceleration (CHIMERA) injury in rats increases impulsivity, decreases dopaminergic innervation in the olfactory tubercle and generates white matter inflammation, tau phosphorylation and degeneration. Exp Neurol 2019;317:87–99; doi: 10.1016/j.expneurol.2019.02.012
29. Feng Y, Li K, Roth E, et al. Repetitive mild traumatic brain injury in rats impairs cognition, enhances prefrontal cortex neuronal activity, and reduces pre-synaptic mitochondrial function. Front Cell Neurosci 2021; 15:689334; doi: 10.3389/fncel.2021.689334
30. Vonder Haar C, Wampler SK, Bhatia HS, et al. Repeat closed-head injury in male rats impairs attention but causes heterogeneous outcomes in multiple measures of impulsivity and glial pathology. Front Behav Neurosci 2022;16:809249; doi: 10.3389/fnbeh.2022.809249
31. Bashir A, Abebe ZA, McInnes KA, et al. Increased severity of the CHIMERA model induces acute vascular injury, sub-acute deficits in memory recall, and chronic white matter gliosis. Exp Neurol 2020;324:113116; doi: 10.1016/j.expneurol.2019.113116
32. Tucker LB, Fu AH, McCabe JT. Hippocampal-dependent cognitive dysfunction following repeated diffuse rotational brain injury in male and female mice. J Neurotrauma 2021;38(11):1585–1606; doi: 10.1089/neu.2021.0025
33. Zhou J, Heo H-Y, Knutsson L, et al. APT-weighted MRI: Techniques, current neuro applications, and challenging issues. J Magn Reson Imaging 2019;50(2):347–364; doi: 10.1002/jmri.26645
34. Zhou J, Payen J-F, Wilson DA, et al. Using the amide proton signals of intracellular proteins and peptides to detect pH effects in MRI. Nat Med 2003;9(8):1085–1090; doi: 10.1038/nm907
35. Zhang H, Wang W, Jiang S, et al. Amide proton transfer-weighted MRI detection of traumatic brain injury in rats. J Cereb Blood Flow Metab 2017;37(10):3422–3432; doi: 10.1177/0271678X17690165
36. O'Leary LA, Davoli MA, Belliveau C, et al. Characterization of vimentin-immunoreactive astrocytes in the human brain. Front Neuroanat 2020; 14:31; doi: 10.3389/fnana.2020.00031
37. Yang Z, Arja RD, Zhu T, et al. Characterization of calpain and caspase-6-generated glial fibrillary acidic protein breakdown products following traumatic brain injury and astroglial cell injury. Int J Mol Sci 2022;23(16): 8960; doi: 10.3390/ijms23168960
38. Willson VJ, Thompson RJ. Human brain aldolase C4 isoenzyme: Purification, radioimmunoassay, and distribution in human tissues. Ann Clin Biochem 1980;17(3):114–121; doi: 10.1177/000456328001700303
39. Levine J, Kwon E, Paez P, et al. Traumatically injured astrocytes release a proteomic signature modulated by STAT3-dependent cell survival. Glia 2016;64(5):668–694; doi: 10.1002/glia.22953
40. Wanner IB, Anderson MA, Song B, et al. Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal

cord injury. J Neurosci 2013;33(31):12870–12886; doi: 10.1523/JNEUROSCI.2121-13.2013
41. Papa L, Brophy GM, Welch RD, et al. Time course and diagnostic accuracy of glial and neuronal blood biomarkers GFAP and UCH-L1 in a large cohort of trauma patients with and without mild traumatic brain injury. JAMA Neurol 2016;73(5):551–560; doi: 10.1001/jamaneurol.2016.0039
42. Pelinka LE, Kroepfl A, Schmidhammer R, et al. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J Trauma 2004;57(5):1006–1012; doi: 10.1097/01.ta.0000108998.48026.c3
43. Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): A multicentre observational study. Lancet Neurol 2018;17(9):782–789; doi: 10.1016/S1474-4422(18)30231-X
44. Sainio MT, Rasila T, Molchanova SM, et al. Neurofilament light regulates axon caliber, synaptic activity, and organelle trafficking in cultured human motor neurons. Front Cell Dev Biol 2021;9:820105; doi: 10.3389/fcell.2021.820105
45. Shahim P, Politis A, van der Merwe A, et al. Neurofilament light as a biomarker in traumatic brain injury. Neurology 2020;95(6):e610–e622; doi: 10.1212/WNL.0000000000009983
46. Graham NSN, Zimmerman KA, Moro F, et al. Axonal marker neurofilament light predicts long-term outcomes and progressive neurodegeneration after traumatic brain injury. Sci Transl Med 2021;13(613):eabg9922; doi: 10.1126/scitranslmed.abg9922
47. O'Brien WT, Pham L, Brady RD, et al. Temporal profile and utility of serum neurofilament light in a rat model of mild traumatic brain injury. Exp Neurol 2021;341:113698; doi: 10.1016/j.expneurol.2021.113698
48. Li X, Pierre K, Yang Z, et al. Blood-Based brain and global biomarker changes after combined hypoxemia and hemorrhagic shock in a rat model of penetrating ballistic-like brain injury. Neurotrauma Rep 2021;2(1):370–380; doi: 10.1089/neur.2021.0006
49. Montalto G, Ricciarelli R. Tau, tau kinases, and tauopathies: An updated overview. Biofactors 2023;49(3):502–511; doi: 10.1002/biof.1930
50. Alonso A, Zaidi T, Novak M, et al. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci U S A 2001;98(12):6923–6928; doi: 10.1073/pnas.121119298
51. Kulbe JR, Hall ED. Chronic traumatic encephalopathy-integration of canonical traumatic brain injury secondary injury mechanisms with tau pathology. Prog Neurobiol 2017;158:15–44; doi: 10.1016/j.pneurobio.2017.08.003
52. Rubenstein R, Chang B, Yue JK, et al. the TRACK-TBI Investigators. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. JAMA Neurol 2017;74(9):1063–1072; doi: 10.1001/jamaneurol.2017.0655
53. Yang Z, Wang P, Morgan D, et al. Temporal MRI characterization, neurobiochemical and neurobehavioral changes in a mouse repetitive concussive head injury model. Sci Rep 2015;5:11178; doi: 10.1038/srep11178
54. Rubenstein R, Sharma DR, Chang B, et al. Novel Mouse Tauopathy Model for Repetitive Mild Traumatic Brain Injury: Evaluation of Long-Term Effects on Cognition and Biomarker Levels After Therapeutic Inhibition of Tau Phosphorylation. Front Neurol 2019;10:124; doi: 10.3389/fneur.2019.00124
55. Hausser N, Johnson K, Parsley MA, et al. Detecting behavioral deficits in rats after traumatic brain injury. J Vis Exp 2018(131):56044; doi: 10.3791/56044
56. Ndode-Ekane XE, Santana-Gomez C, Casillas-Espinosa PM, et al. Harmonization of lateral fluid-percussion injury model production and post-injury monitoring in a preclinical multicenter biomarker discovery study on post-traumatic epileptogenesis. Epilepsy Res 2019;151:7–16; doi: 10.1016/j.eplepsyres.2019.01.006
57. Shiotsuki H, Yoshimi K, Shimo Y, et al. A rotarod test for evaluation of motor skill learning. J Neurosci Methods 2010;189(2):180–185; doi: 10.1016/j.jneumeth.2010.03.026
58. Cleal M, Fontana BD, Ranson DC, et al. The Free-movement pattern Y-maze: A cross-species measure of working memory and executive function. Behav Res Methods 2021;53(2):536–557; doi: 10.3758/s13428-020-01452-x
59. Zou H, Brayer SW, Hurwitz M, et al. Neuroprotective, neuroplastic, and neurobehavioral effects of daily treatment with levetiracetam in experimental traumatic brain injury. Neurorehabil Neural Repair 2013;27(9):878–888; doi: 10.1177/1545968313491007
60. Shepherd JK, Grewal SS, Fletcher A, et al. Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology (Berl) 1994;116(1):56–64; doi: 10.1007/BF02244871
61. NOT-OD-21-013: Final NIH Policy for Data Management and Sharing. n.d. Available from: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-21-013.html [Last accessed: September 2, 2023].
62. Chou A, Lee S, Krukowski K, et al. Aggregated animal subject metadata from 11 UCSF preclinical TBI publications and 1 ODC-TBI published dataset. Odc-Tbi n.d:566; doi: 10.34945/F51P49
63. Chou A, Krukowski K, Morganti J, et al. Peripheral monocyte and resident microglia number and cytokine expression at subchronic timepoints after controlled cortical impact TBI in adult and aged mice. Odc-Tbi n.d:376; doi: 10.34945/F5T595
64. Chou A, Morganti J, Jopson T, et al. Cytokine expression and peripheral monocyte infiltration time course and effect of CCR2-antagonist treatment on chronic behavioral outcome in wildtype adult mice after controlled cortical impact TBI. Odc-Tbi n.d:159; doi: 10.34945/F5PC77
65. Young W. Chapter 17 Spinal Cord Contusion Models. In: Progress in Brain Research. (McKerracher L, Doucet G, Rossignol S. eds). Spinal Cord Trauma: Regeneration, Neural Repair and Functional Recovery Elsevier; 2002; pp. 231–255; doi: 10.1016/S0079-6123(02)37019-5
66. Basso D M, Beattie M S, Bresnahan J C, et al. MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. J Neurotrauma 1996;13(7):343–359; doi: 10.1089/neu.1996.13.343
67. Almeida CA, Torres-Espin A, Huie JR, et al. Excavating FAIR Data: The Case of the Multicenter Animal Spinal Cord Injury Study (MASCIS), blood pressure, and neuro-recovery. Neuroinformatics 2022;20(1):39–52; doi: 10.1007/S12021-021-09512-Z
68. Torres-Espín A, Haefeli J, Ehsanian R, et al. TRACK-SCI Investigators. Topological network analysis of patient similarity for precision management of acute blood pressure in spinal cord injury. Elife 2021;10:10; doi: 10.7554/ELIFE.68015
69. Browning M, Shear DA, Bramlett HM, et al. Levetiracetam treatment in traumatic brain injury: Operation brain trauma therapy. J Neurotrauma 2016;33(6):581–594; doi: 10.1089/neu.2015.4131
70. Bramlett HM, Dietrich WD, Dixon CE, et al. Erythropoietin treatment in traumatic brain injury: Operation brain trauma therapy. J Neurotrauma 2016;33(6):538–552; doi: 10.1089/neu.2015.4116
71. Kochanek PM, Dixon CE, Mondello S, et al. Multi-Center pre-clinical consortia to enhance translation of therapies and biomarkers for traumatic brain injury: Operation brain trauma therapy and beyond. Front Neurol 2018;9:640; doi: 10.3389/FNEUR.2018.00640
72. Shear DA, Dixon CE, Bramlett HM, et al. Nicotinamide treatment in traumatic brain injury: Operation brain trauma therapy. J Neurotrauma 2016;33(6):523–537; doi: 10.1089/neu.2015.4115
73. RadabaughHL, Bonnell J, Dietrich WD, et al. Development and evaluation of machine learning models for recovery prediction after treatment for traumatic brain injury. In: Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS Institute of Electrical and Electronics Engineers Inc.; 2020; pp. 2416–2420; doi: 10.1109/EMBC44109.2020.9175658
74. Lafrenaye A, Mondello S, Povlishock J, et al. Operation brain trauma therapy: An exploratory study of levetiracetam treatment following mild traumatic brain injury in the micro pig. Front Neurol 2020;11:586958; doi: 10.3389/FNEUR.2020.586958/BIBTEX
75. Wanner I-B, McCabe JT, Russell Huie J, et al. The paper prospective harmonization, common data elements, and sharing strategies for multicenter preclinical TBI research in the translational outcomes project in neurotrauma (TOP-NT) consortium. *J. Neurotrauma* in press. (2024)