TBI Blood-Based Biomarkers: Impacts on diagnosis, prognosis and therapy development

Kevin K. Wang, PhD
### Most promising TBI Biofluid biomarkers

- Biomarkers in blood & CSF have been widely studied in TBI
  - Over 10-12 distinct protein biomarkers have been reported
  - Novel biomarkers (micro-RNAs, metabolomic and lipodomic profiles) are currently under active investigation
- **GFAP, S100β, UCH-L1, Tau/ P-Tau**
- Additional markers that merit further investigation include: SBDP150, SBDP145, SBDP120, SNTF, MBP, NSE, NF-L, NF-H, Neurogranin

<table>
<thead>
<tr>
<th>Protein Biomarker</th>
<th>Biomarker Full Name</th>
<th>Pathophysiologic mechanism</th>
<th>Evidence of robust detection in serum/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animal TBI (severe-mild) rat, mouse, micropig</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial Fibrillary acidic protein</td>
<td>Astrogliosis / CHANGED TO ASTROGLIAL Astroglial injury</td>
<td>✔</td>
</tr>
<tr>
<td>S100b</td>
<td>calcium-binding S100b protein</td>
<td>Astrogliosis/ BBB compromise</td>
<td>✔</td>
</tr>
<tr>
<td>UCH-L1</td>
<td>Ubiquitin C-terminal hydrolase-L1</td>
<td>Neuronal injury</td>
<td>✔</td>
</tr>
<tr>
<td>Tau, P-Tau</td>
<td>Tau protein and Phospho-Tau</td>
<td>Axonal injury / neurodegeneration</td>
<td>✔</td>
</tr>
</tbody>
</table>
Novel TBI Biomarkers Identification

**Acute markers**
- UCH-L1
- GFA-BDP
- SBDP150
- SBDP120

**Subacute markers**
- MBP-frag
- MAP2

**Chronic markers**
- AutoAb

**Cell body damage**
- Gliosis / Glial Injury
- Axonal injury
- Demyelination
- Dendritic injury

**Oligodendrocyte markers**
- CNPase

**Microgliosis-Neuroinflammation Markers**
- EAMP-II

**Synaptic Injury Markers**
- Sypt-1

UCH-L1: A MARKER OF NEURONAL CELL BODY INJURY

- **CNS Specificity:**
  - Highly enriched in neurons
  - Also known as PGP9.5

- **Function:** Protein de-ubiquitinization

- **Subcellular Location:** Cell Body (perikarya)

- **Special feature:** Implicated in familial Parkinsonism

- **Biomarker Protein attributes:**
  - Globular shape
  - Cytosolic
  - M.W. 24 kDa
UCH-L1 – CNS Specificity, Enrichment

mRNA level in mouse tissues: Affymetrix U74A chip analysis

UCH-L1 tryptic peptide tissue distribution (by Mass spect.)

UCH-L1 protein distribution in rat tissues

in human tissues

humanproteomemap.org/batch.php
**GFAP: AS ASTROGLIAL INJURY BIOMARKER**

<table>
<thead>
<tr>
<th>Neuro-disease/Disorder</th>
<th>Species, biofluid type</th>
<th>Temporal profile</th>
<th>Key refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic stroke; ICH</td>
<td>Human serum</td>
<td>&lt; 4 h</td>
<td>[96-100]</td>
</tr>
<tr>
<td>Cerebral ischemia</td>
<td>Human, ischemic stroke</td>
<td>24-72 h</td>
<td>[97-98]</td>
</tr>
<tr>
<td></td>
<td>Rat MCAO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe TBI</td>
<td>Human CSF serum, Rat CSF, serum</td>
<td>6 h – 24 h</td>
<td>[90-93]</td>
</tr>
<tr>
<td>Mild TBI</td>
<td>human serum, plasma</td>
<td>&lt; 24 h</td>
<td>[85-86]</td>
</tr>
<tr>
<td>Spinal cord injury</td>
<td>Human, CSF, serum, rat CSF, serum</td>
<td>&lt; 24 h</td>
<td>[52-53]</td>
</tr>
<tr>
<td>Glioma / Glioblastoma</td>
<td>Human biofluid</td>
<td>-</td>
<td>[105-106]</td>
</tr>
</tbody>
</table>
GFAP Tissue Specificity

GFAP tryptic peptide tissue distribution (by Mass spect.)

*Based on GFAP tryptic peptide
QQVHVELDVALKPLTAALK, MH+ 2075.1495, m/z 1038.0784
humanproteomemap.org/batch.php
**TAU (MAPT): MICROTUBULE ASSOCIATED PROTEIN TAU: A TAUOPATHY BIOMARKER**

- Subjected to multiple kinase phosphorylation
  - GSK3β, TTBK1, casein kinase1, cdk-5, PKA

- Subjected to proteolysis by calpain & caspase

**Tauopathy diseases** (chronic traumatic Encephalopathy, AD, FTD-17)
- Pair-helical filament (PHF) tau + Neurofibrillary tangle (NFT) formation
Tau (MAPT): Microtubule Associated Protein in Axons

Human Tau

Shortest fetal form

Longest adult form

Six isoforms in human brain: 2-3-10-; 2-3-10-; 2-3-10-; 2-3-10+; 2-3-10+

Microtubule-binding repeat domain:
MT1: 252-282; MT2: 283-313; MT3: 314-344; MT4: 345-376

TAU (MAPT) tryptic peptide tissue distribution (by Mass spect.)

Human tissue – WB
* TBI BIOMARKERS AS IN VITRO DIAGNOSTIC (IVD) TEST

(REFER TO DR. R. HAYES’ TALK NEXT)
* TBI BIOMARKERS AS THERAPY DEVELOPMENT TOOLS

CONTEXT OF USE (COU) BIOMARKERS

+ BIOMARKER QUALIFICATION PROGRAM (BQP)

EXAMPLES: GFAP, TAU/P-TAU
Theranostic describes the parallel use of a new Therapy and Diagnostic test for a human disease or disorder so as to facilitate drug development and clinical trials and to achieve optimal clinical outcomes in a population of patients.
"Theranostic" TBI Studies in rat models

**Operations Brain Trauma Therapy (OBTT) study:**
P. Kochanek, E Dixon, (UPMC) - **CCI**
D. Shear, F. Tortella (WRAIR) - **PBBI**
D. Dietrich, H. Bramlett (U Miami) - **FPI**
J Povlishock (VUC) - **Micropig**
K. Wang (UF)/Hayes (Banyan), Mondello (U Messina) - biomarkers analysis

- More than 10 promising experimental TBI drugs tested
  - Histopathology
  - Biomarker reduction
  - Functional outcome

**CCI model + Keppra**
(Levetiracetam)

**PBBI model + Nicotinamide**

# Current Data Supporting TBI therapeutic-supporting use of biofluid-based Biomarkers

## Randomized Clinical Trials: Progesterone; INTREPID2566

<table>
<thead>
<tr>
<th>Study name</th>
<th>Study identifier</th>
<th>Summary of study</th>
<th>Outcome Measures</th>
<th>Biofluid Biomarkers used:</th>
<th>Imaging Biomarker(s) used:</th>
<th># of Subjects</th>
<th>Duration</th>
<th>Meeting proceeding/Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of NNZ-2566 in Patients With Traumatic Brain Injury (INTREPID2566)</td>
<td>ClinicalTrials.gov Identifier: NCT00805818</td>
<td>A Phase 2 study examining neuroprotective effects of peptidic drug (NNZ-2556) in severe non-penetrating TBI patients (moderate-severe, GCS 4-12)</td>
<td>Primary: Reduce adverse events (AEs) and serious adverse events (SAEs); Secondary: modifying global outcome; improvement in cognitive and neuropsychological functioning; modification of acute physiological processes (by EEG) and biomarker levels.</td>
<td>GFAP, UCH-L1</td>
<td>EEG</td>
<td>~260</td>
<td>April 2010 - Aug. 2015 (projected)</td>
<td>Mondello et al. Int. Neurotrauma Symposium Poster (Budapest, HU, 2013).</td>
</tr>
</tbody>
</table>
FDA-defined Biomarker types and potential contexts of use (COU) in drug development process

(i) “Diagnostic Biomarker” to distinguish TBI patients with pathoanatomic lesions (on NCCT and MRI) from those without

(ii) “Prognostic Biomarker” to provide information on the likely course of TBI; to select TBI patients that are more likely to progress to TBI-induced disability

(iii) “Predictive Biomarker” for stratification of TBI patients who are more likely to progress to a clinically meaningful improvement in response to a TBI therapy in a clinical trial setting

(iv) “Pharmacodynamic biomarker” is a response biomarker to indicate the intended activity of a drug; and to confirm that a biological response has occurred in response to a therapy

(v) “Efficacy Response Biomarker” is a surrogate measure of specific disease-related clinical outcome, thus, as a marker of efficacy response of a TBI therapy
GFAP as a Diagnostic Biomarker: (predicting CT neuroanatomical pathology)

A. Serum GFAP-89 Levels (ng/ml)

B. ROC Curve: Plasma [GFAP-89] vs CT +/−

C. ROC Curve: all TBI Patients – CT Positive vs CT Negative

AUC = 0.79 (95% CI 0.69-0.89)

N = 117

Papa et al. 2012

N = 215

Okonkwo et al., 2013 J Neurotrauma 30:1490
GFAP as Prognostic Biomarker: Acute post-TBI GFAP levels predict poor functional outcome

Okonkwo et al., 2013 *J Neurotrauma* 30:1490–1497


<table>
<thead>
<tr>
<th>Biomarker</th>
<th>TBI vs. Healthy control</th>
<th>CT positive vs. CT negative</th>
<th>Incomplete Recovery (GOSE &lt; 8 vs. GOSE = 8)</th>
<th>Poor outcome (GOSE &lt; 4 vs. GOSE &gt; 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP</td>
<td>0.91 (0.88 – 0.94)</td>
<td>0.88 (0.84 – 0.93)</td>
<td>0.65 (0.55 – 0.74)</td>
<td>0.74 (0.61 – 0.87)</td>
</tr>
</tbody>
</table>

N= 120  
N= 168
T-Tau, P-Tau, Tau & P-Tau/T-Tau ratio as diagnostic biomarkers: (identifying mild TBI versus controls)

### All acute TBI (n=195) vs. controls (n=20),

<table>
<thead>
<tr>
<th>Area under the ROC curve</th>
<th>T-Tau</th>
<th>P-Tau</th>
<th>P-Tau/T-Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>0.9194</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.036</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.8488 to 0.9899</td>
<td>1.000 to 1.000</td>
<td>1.000 to 1.000</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

### mTBI (GCS 13-15) (n=162) vs. controls (n=20),

<table>
<thead>
<tr>
<th>Area under the ROC curve</th>
<th>T-Tau</th>
<th>P-Tau</th>
<th>P-Tau/T-Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>0.916</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Std. Error</td>
<td>0.03682</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.8439 to 0.9882</td>
<td>1.000 to 1.000</td>
<td>1.000 to 1.000</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Plasma Total Tau, P-Tau & P-Tau/T-Tau ratio as Diagnostic Biomarkers: (predicting CT neuroanatomical pathology)

<table>
<thead>
<tr>
<th></th>
<th>ROC Area Under the Curve</th>
<th>Area</th>
<th>Std. Error</th>
<th>Asymptotic Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Tau</td>
<td>.921</td>
<td>.023</td>
<td>.0001</td>
<td>.876 - .966</td>
<td></td>
</tr>
<tr>
<td>T-Tau</td>
<td>.646</td>
<td>.039</td>
<td>.0001</td>
<td>.569 - .723</td>
<td></td>
</tr>
<tr>
<td>P-Tau/T-Tau</td>
<td>.923</td>
<td>.021</td>
<td>.0001</td>
<td>.882 - .964</td>
<td></td>
</tr>
</tbody>
</table>
Plasma T-Tau, P-Tau & P-Tau/T-Tau as Prognostic Biomarkers: (predicting TBI patients’ 6 mo. outcomes)

( GOSE =7-8 vs. GOSE ≤ 6)

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Std. Error</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>P-Tau</td>
<td>.663</td>
<td>.048</td>
<td>.569</td>
</tr>
<tr>
<td>Total Tau</td>
<td>.552</td>
<td>.051</td>
<td>.451</td>
</tr>
<tr>
<td>P-Tau/Total Tau</td>
<td>.658</td>
<td>.049</td>
<td>.562</td>
</tr>
</tbody>
</table>

( GOSE ≤ 4 vs GOSE = 5-8)

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>Std. Error</th>
<th>Asymptotic 95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>T-Tau</td>
<td>.516</td>
<td>.068</td>
<td>.382</td>
</tr>
<tr>
<td>p-Tau</td>
<td>.771</td>
<td>.048</td>
<td>.678</td>
</tr>
<tr>
<td>p-Tau/T-Tau</td>
<td>.777</td>
<td>.055</td>
<td>.670</td>
</tr>
</tbody>
</table>
A CRITICAL & OPPORTUNE JUNCTURE

TBI public and private stakeholders and FDA are now committed to a partnership in which employing TBI biomarkers as therapy development tools is becoming a reality!
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Linda Papa
### Current Observational Studies with Biomarker Components

#### Observational Cohorts: CENTER, TRACK-TBI, CARE Consortium, Project Head to Head II

<table>
<thead>
<tr>
<th>Location</th>
<th>Project Name (Abbrev.)</th>
<th>Patient Population</th>
<th>Main data collected</th>
<th>Imaging Biomarkers</th>
<th>Biofluid Biomarkers</th>
<th>Study period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Europe (60+ hospitals)</strong></td>
<td>Collaborative Research on Acute Traumatic brain injury in intensive care medicine in Europe (CENTER-TBI) European Commission- funded</td>
<td>S400 adult and pediatric TBI of all levels of severity</td>
<td>• CER (Comparative Effectiveness Research) • Classification • Imaging data • Biofluid samples (serum) • Outcome &amp; prognosis</td>
<td>• CT • Multi-modal 3T MRI • MPRAGE 3D T1 • 3D FSE T2 FLAIR • 2D FSE T2 • DTI/DKI • Resting state fMRI • ASL • SWI</td>
<td>GFAP S100b UCH-L1 Tau P-Tau</td>
<td>2013 - 2020</td>
</tr>
<tr>
<td><strong>USA (11 Level 1 Trauma Center ERs)</strong></td>
<td>Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) NIH- funded AND TBI Endpoint Development (TED) DoD-funded</td>
<td>2,700 adult and pediatric TBI, all levels of severity 300 controls</td>
<td>• CER (Comparative Effectiveness Research) • Classification • Imaging data • Biofluid samples (serum, plasma, CSF) • Outcome &amp; prognosis</td>
<td>• CT • Multi-modal 3T MRI • MPRAGE 3D T1 • 3D FSE T2 FLAIR • 2D FSE T2 • DTI/DKI • Resting state fMRI • ASL • SWI</td>
<td>GFAP UCH-L1 Tau Phospho-Tau</td>
<td>2013- 2018 / 2014 - 2019</td>
</tr>
<tr>
<td><strong>USA (Div I colleges)</strong></td>
<td>NCAA-DoD Grand Alliance: Concussion Assessment, Research and Education (CARE) Consortium NCAA &amp; DoD-funded</td>
<td>1400 male and female NCAA student-athletes and service academy cadets 100 concussed athletes 100 contact controls 100 non-contact controls</td>
<td>• Neurocognitive assessments • Head impact sensor data • MR Imaging data • Biofluid samples (plasma, serum, RNA) • Psych Health measures • Outcome &amp; prognosis</td>
<td>• Multi-modal 3T MRI • MPRAGE 3D T1 • 3D FSE T2 FLAIR • 2D FSE T2 • DTI/DKI • Resting state fMRI • ASL • SWI</td>
<td>UCH-L1 GFAP SBDP150 S100B MAP-2 CNPase Micro RNA</td>
<td>2014-2017</td>
</tr>
<tr>
<td><strong>USA (high schools and Div III colleges)</strong></td>
<td>Comprehensive study of acute effects and recovery after concussion (Project Head to Head II) DoD-funded</td>
<td>1,200 athletes with baseline data 50 concussed athletes 50 contact sport controls, 50 non-contact sport controls</td>
<td>• Neurocognitive assessments • Head impact sensor data • MR Imaging data • Biofluid samples (plasma, serum, DNA) • Psych Health measures • Outcome &amp; prognosis</td>
<td>• Multi-modal 3T MRI • SPGR FLAIR SWI DTI &amp; DKI rs-fMRI pCASL</td>
<td>UCH-L1 GFAP SBDP150 S100B MAP-2 CNPase</td>
<td>2014-2018</td>
</tr>
</tbody>
</table>