ProTECT III

Second Annual Investigators' Meeting
April 2011

ProTECT III Investigators Meeting Agenda

Thursday, April 28th

5:00 – 6:00	**HSP Working group**	
6:00 – 9:30	**Reception/Dinner Meeting with Cash Bar**	
7:00 – 7:30	**Welcome and Enrollment Update**	Wright
7:30 – 8:00	**Protocol Changes**	Wright
8:00 – 9:30	**BIO-ProTECT**	Frankel

ProTECT III Investigators' Meeting Agenda

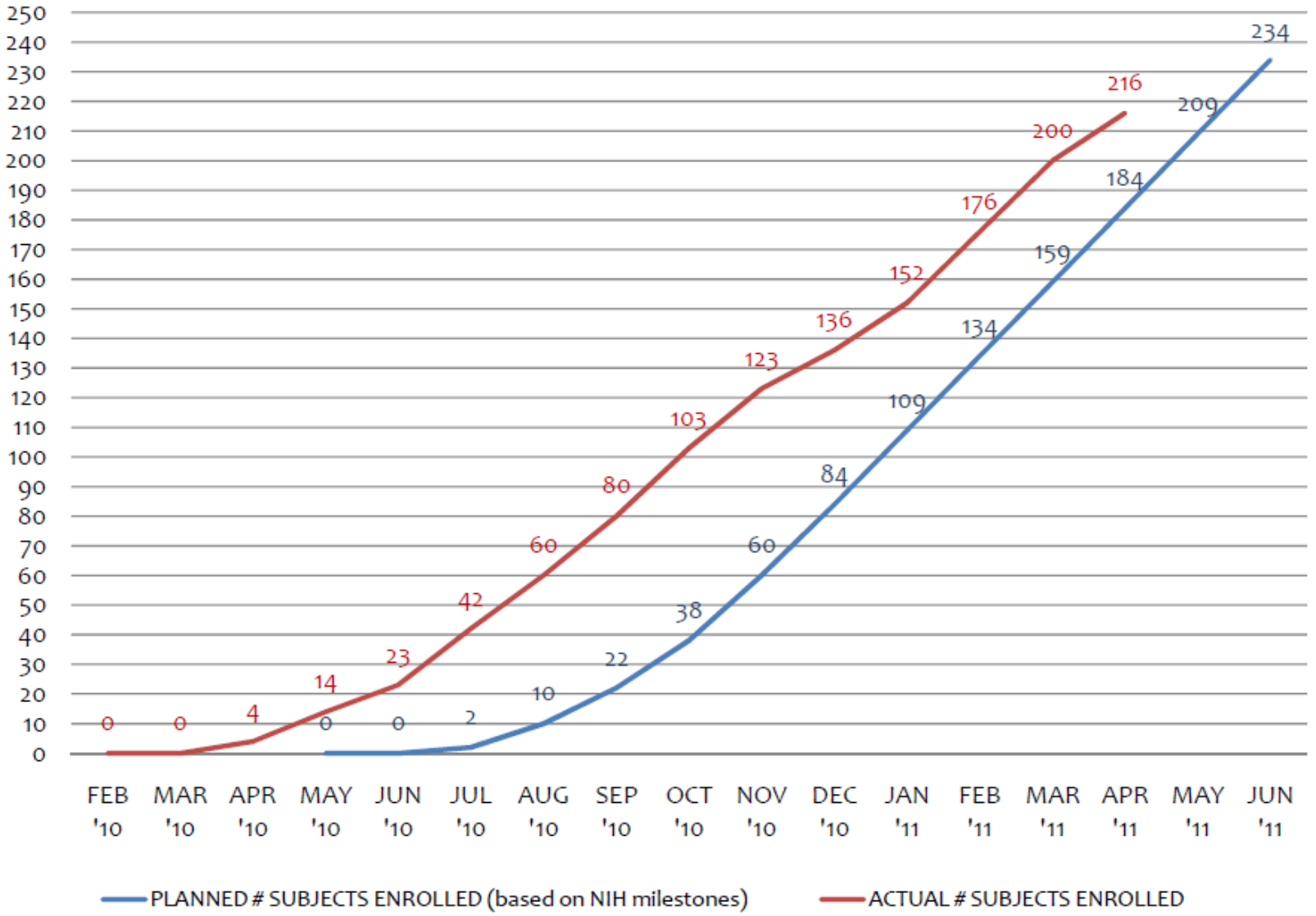
Friday, April 29th

7:15 – 8:00 am	BREAKFAST	ALL
8:00 – 8:45	**Enrollment Reviews**	Wright / Howlett-Smith
8:45 – 9:05	**CST and Transgressions Review**	Howlett-Smith
9:05 – 9:25	**Monitoring and AE's**	DeYempert/Howlett-Smith/Mawocha
9:25 – 9:55	**Patient Tracking**	Brandt/Hermanson/Howlett-Smith Mendoza-Moore/Ottman
9:55 – 10:15	BREAK	
10:15 – 11:45	**Outcomes Review**	Wright / Howlett-Smith
11:45 – 12:00	**Contracts and Payments**	Stevenson/Wright
12:00 – 1:00 pm	LUNCH / Open Discussion	ALL

Trial Updates

- Enrollment 216
- Met NIH Milestones – All sites active April 1
- DSMB, FDA and ProTECT III Annual Reports submitted
- No known safety concerns, tracking phlebitis
- BioProTECT - Approved and pending NOA
- Protocol Version 7 (change incorporates BioProTECT Approved by DSMB, Emory IRB)
- Updated IB v3, SMP v3

ProTECT - SUBJECT ENROLLMENT
(through June 2011)

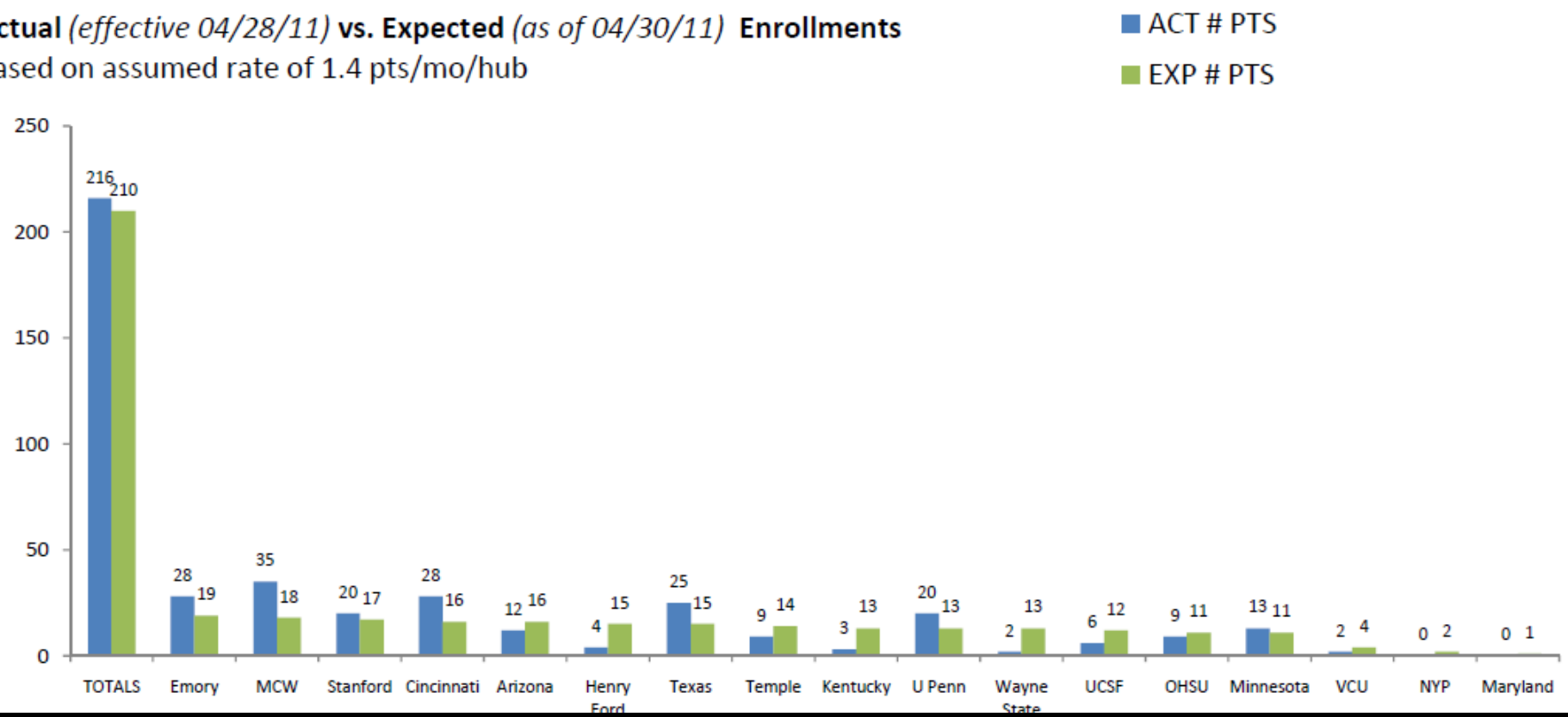


HUB	SPOKE	STATUS	# PTS
UNIVERSITY OF ARIZONA	University of Arizona Medical Ctr	active	10
	Maricopa	active	2
	Banner Good Samaritan	*pending*	-
	Scottsdale Healthcare	*pending*	-
UNIVERSITY OF CINCINNATI	University Hospital	active	28
EMORY UNIVERSITY	Grady Memorial	active	28
	The Med (Memphis)	*pending*	-
HENRY FORD HEALTH SYSTEM	Henry Ford Hospital	active	4
	St. Mary's	*pending*	-
UNIVERSITY OF KENTUCKY	University of Kentucky	active	3
UNIVERSITY OF MARYLAND	University of Maryland	active	0
	Bayview	*pending*	-
	Johns Hopkins	*pending*	-
UNIVERSITY OF MINNESOTA	Hennepin County Medical Center	active	2
	Regions	active	11
	Mayo Clinic	*pending*	-
	North Memorial	*pending*	-
NEW YORK PRESBYTERIAN	Columbia	active	0
	Cornell	*pending*	-
	Winthrop	*pending*	-
OHSU	Oregon Health and Science Univ	active	9
STANFORD UNIVERSITY	Stanford	active	15
	Santa Clara Valley	active	5
TEMPLE UNIVERSITY	Temple University Hospital	active	8
	Hahnemann University Hospital	active	0
	Hershey	active	1
	Jefferson	active	0
	Geisinger	*pending*	-
UNIVERSITY OF TEXAS, HOUSTON	Memorial Hermann	active	25
	UT Houston	*pending*	-
	UT Southwestern	*pending*	-
	Austin Brackenridge	*pending*	-
UC SAN FRANCISCO	San Francisco General Hospital	active	6
UNIVERSITY OF PENNSYLVANIA	St. Luke's	active	18
	Hospital of UPenn (HUP)	active	2
	Cooper	*pending*	-
VIRGINIA COMMONWEALTH	VCU/MCV	active	2
WAYNE STATE UNIVERSITY	Detroit Receiving	active	2
	Sinai Grace	*pending*	-
	Beaumont Royal Oak	*pending*	-
MEDICAL COLLEGE OF WISCONSIN	Froedtert Memorial Hospital	active	35
	Mt. Sinai Chicago	*pending*	-
	St. Johns - St. Louis	*pending*	-
WILFORD HALL	*Wilford Hall*	*pending*	-
17/17	44	24	216
(active / total # of Hubs)	*(# potential spokes)*	*(# active spokes)*	*(# pts)*

ProTECT III

Actual *(effective 04/28/11)* **vs. Expected** *(as of 04/30/11)* **Enrollments**

based on assumed rate of 1.4 pts/mo/hub



Subject Status Update

- 114 Subjects at End of Study
  - 78 Completed Study
  - 6 withdrawals
  - 29 deaths

Enrollment by site by target

PERCENT OF EXPECTED	
MCW	194%
CINCINNATI	175%
TEXAS	167%
PENN	154%
EMORY	147%
MINN	118%
STANFORD	112%
OHSU	82%
ARIZONA	75%
TEMPLE	64%
UCSF	50%
VCU	50%
HENRY FORD	27%
KENTUCKY	23%
WAYNE	15%
NYP	0%
MARYLAND	0%


ProTECT
™

Version 7

PROTOCOL CHANGES

Protocol Update
Version 7

Essentially a few paragraphs

Inclusion Criteria

- Moderate to severe brain injury (iGCS 12-4 or motor response 2-5 if intubated). At any point prior to randomization a motor score of 6 excludes the patient
- Age > 18 years (or developmental stage Tanner 5 in patients where age is not known
- Blunt, traumatic, closed head injury (altered mental status due to brain injury)
- Able to initiate study drug infusion within 4 hours from time of injury

Exclusion criteria

- Non-survivable injury as determined by treating team (e.g. withdrawal of care prior to randomization, no intention for aggressive intervention, on hospice, or DNR order, etc.)
- Bilateral dilated unresponsive pupils
- Spinal cord injury with neurological deficits, pre-injury paralysis (quad/paraplegic)
- Inability to perform activities of daily living (ADL) without assistance
- Cardiopulmonary arrest
- Status epilepticus on arrival or concern for post ictal state
- SBP < 90 for two consecutive readings at least 5 minutes apart anytime prior to randomization
- O_2 Sat < 90 for at least 5 consecutive minutes anytime prior to randomization
- Prisoner or ward of state
- Known active breast or reproductive organ cancers (via medical records or family interview)
- Known allergy to progesterone or Intralipid components (egg yolk) (via medical records or family interview)
- Known history of blood clotting disorder (Protein S or C deficiency, etc.) or history of pulmonary embolism (via medical records or family interview) or active/ongoing thromboembolic event (myocardial infarction, ischemic stroke, pulmonary embolism, deep vein thrombosis).
- Blood or serum ethanol (EtOH) ≥ 250 mg %
- Positive qualitative urine or serum pregnancy test
- Concern for inability to follow up at 6 months (residence in foreign country, homeless with limited contacts, undocumented immigrants, or high likelihood of becoming incarcerated during study period, etc.)
- Patient in Opt Out registry or wearing Opt Out bracelet

Protocol Changes v7

- **2.0 Trial Objectives (pg 11)**
- Ancillary Study – BioProTECT
- The primary aim of this ancillary study is to determine whether elevated levels of serum biomarkers (S100B, GFAP, UCH-L1, SBDP150), measured within 4 hours of TBI or at 24 and 48 hours after randomization, independently predict clinical outcome as measured by the Glasgow Outcome Scale Extended (GOS-E) at 6 months. The secondary aim is to determine, in progesterone treated subjects, if there a correlation between steady state serum progesterone levels and serum levels of S100B, GFAP, UCH-L1, SBDP150 at 24 and 48 hours after randomization, and whether progesterone levels predict those subjects with a favorable clinical response to the experimental treatment as determined by the primary outcome of the study.

Protocol Changes v7

- **3.0 Background (pg 18)**
- Pathophysiology of TBI and Relevance of Biomarkers
- Immediately after moderate or severe TBI, multiple deleterious processes such as excitotoxicity, ischemia, oxidative stress, inflammation and apoptosis are set in motion ultimately leading to the death of neurons and glial cells. Although numerous proteins that reflect injury from these processes are released from the injured brain into the cerebrospinal fluid (CSF) and serum, very few of these proteins have been sufficiently characterized to demonstrate potential clinical utility for predicting long term outcome. Several astroglia and neuronal proteins are released from the injured brain, and could be used as markers of cell damage after TBI. Of these, S100B and Neuron-Specific Enolase (NSE) have been the best characterized, but the clinical utility of these markers remains unclear, due to the absence of large, well-validated prospective human studies. Our preliminary data, obtained from 4 independent cohorts of acute TBI patients, support the hypothesis that serum levels of S100B, GFAP, UCH-L1, and SBDP150 reflect the degree of structural injury and predict clinical outcome, making them highly promising candidate biomarkers for TBI.

Protocol Changes v7

4.0 Objectives (pg 19)

- **Primary Objective:** To determine whether serum biomarkers of structural brain injury are independent predictors of clinical outcome assessed 6 months after moderate or severe acute TBI, and whether the correlation of these biomarkers and progesterone levels predicts favorable response in subjects treated with progesterone.

- **Primary Hypotheses:** Elevated levels of serum biomarkers (S100B, GFAP, UCH-L1, SBDP150), measured within 4 hours of TBI, independently predict clinical outcome as measured by the Glasgow Outcome Scale Extended (GOS-E) at 6 months. Elevated levels of serum biomarkers (S100B, GFAP, UCH-L1, SBDP150), measured at 24 and 48 hours after study randomization, independently predict clinical outcome as measured by the GOS-E at 6 months.

- **Secondary Hypothesis:** In progesterone-treated subjects, steady state serum progesterone levels inversely correlate with serum levels of S100B, GFAP, UCH-L1, SBDP150 (24 and 48 hours after study randomization) and correlate directly with a favorable outcome of progesterone treatment at 6 months.

Protocol Changes v7

- **5.0 & 6.0 Subject Selection - Exclusion Criteria and Pretreatment Evaluation (pg 20-21&25)**
- Spinal cord injury with neurological deficits, pre-injury paralysis (quad/paraplegic)
- Inability to perform activities of daily living (ADL) without assistance

Inability to perform activities of daily living without assistance

- Measure
  - Independent prior to injury
- Rationale
  - Difficult to determine functional status post injury

Protocol Changes v7

- **7.0 Registration/Randomization (pg 27)**

- Pre-intervention Blood Draws
- Prior to initiation of study drug, two 10 cc vials of blood will be obtained from the subject, centrifuged for 15 minutes, aliquoted, and frozen for later analysis of markers of brain injury.

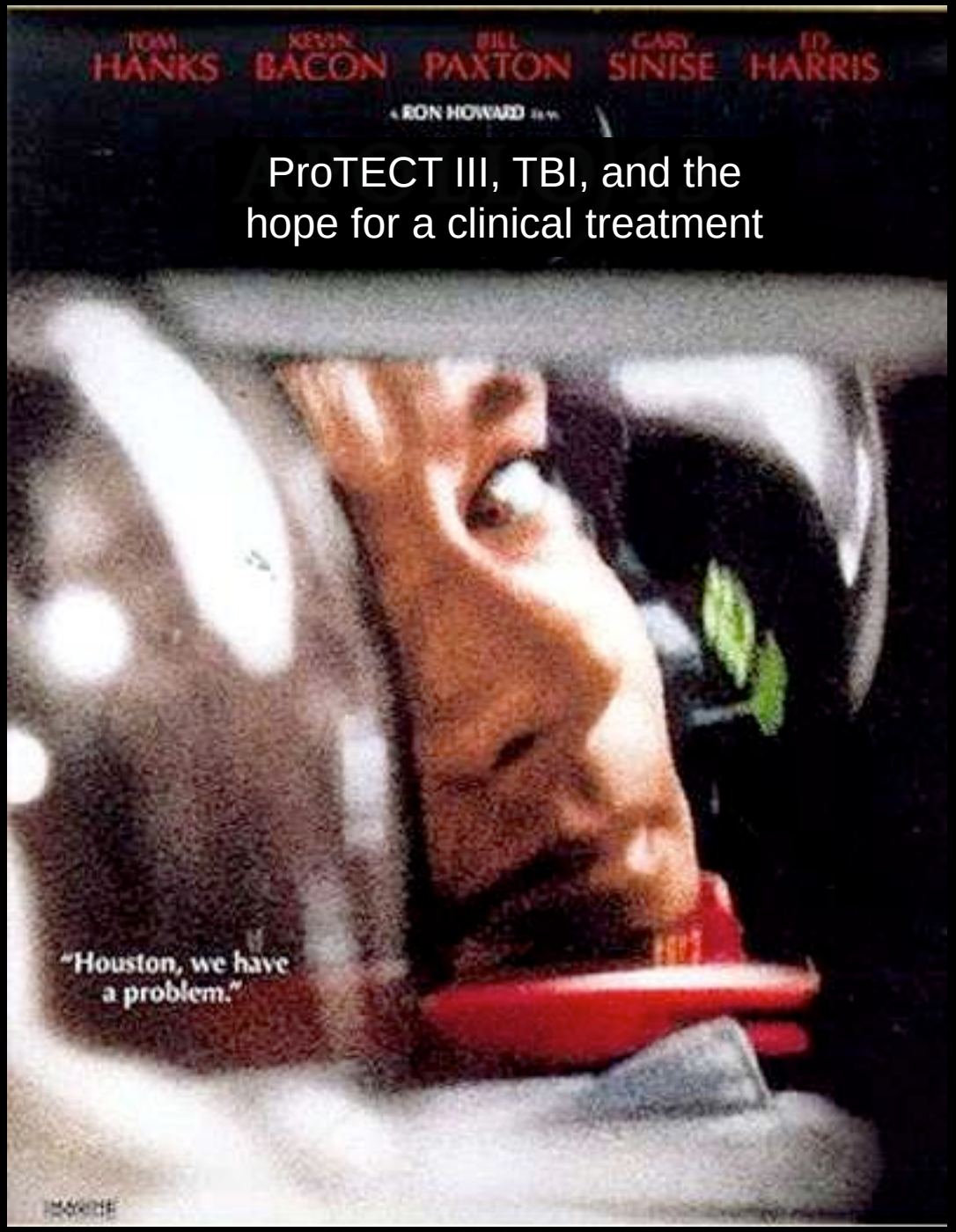
Protocol Changes v7

- **9.0 Patient Assessment (pg 30)**

- Blood samples will be obtained at 24 and 48 hours from the time of randomization for the BioProTECT Ancillary study.

Protocol Changes v7

- **TABLE: Per Visit Activities / Requirements (pg 31)**
- Blood Draws required at Enrollment, Day 1, and Day 1



ProTECT III, TBI, and the hope for a clinical treatment

Update

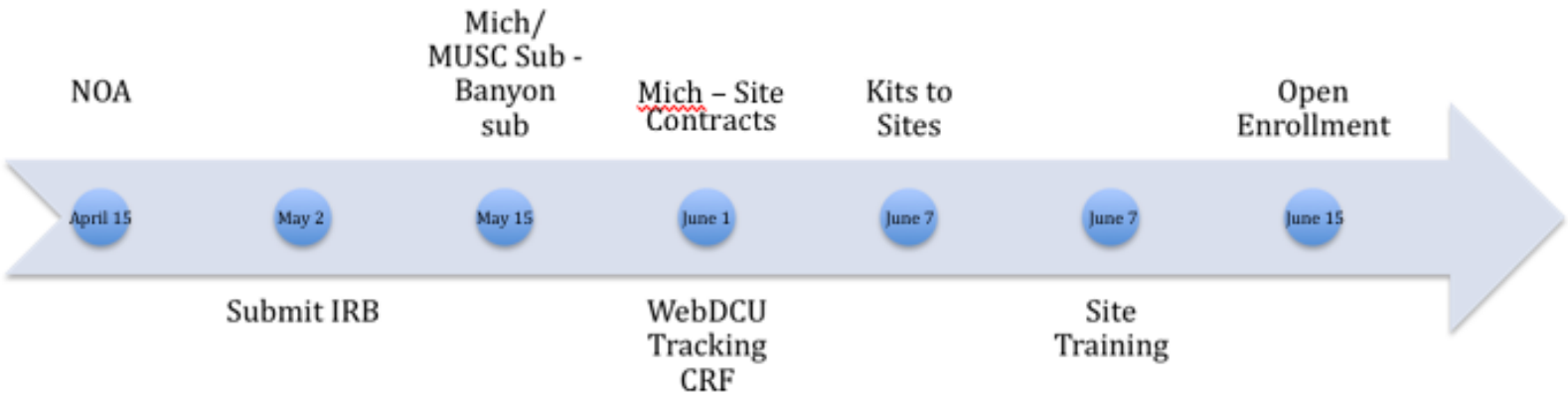
PEER ProTECT

Neal Dickert, MD
Principal Investigator

BIO-ProTECT

Mike Frankel, MD
Principal Investigator

BIO-ProTECT


NOA
April 15
Submit IRB
May 2
Mich/ MUSC Sub - Banyon sub
May 15
Mich – Site Contracts
June 1
WebDCU Tracking CRF
Kits to Sites
June 7
Site Training
June 7
Open Enrollment
June 15

BIO-ProTECT

Biomarkers of Injury and Outcome in ProTECT III

NIH/NINDS: 1R01NS071867
PI: Michael Frankel
Emory University

Prognosis in moderate (GCS 9-12) or severe TBI (GCS 4-8)

- Prior studies
  - Retrospective design
  - Small cohorts
  - Combining multiple studies
  - No prospective validation of outcome

Structrual Biomarkers in TBI

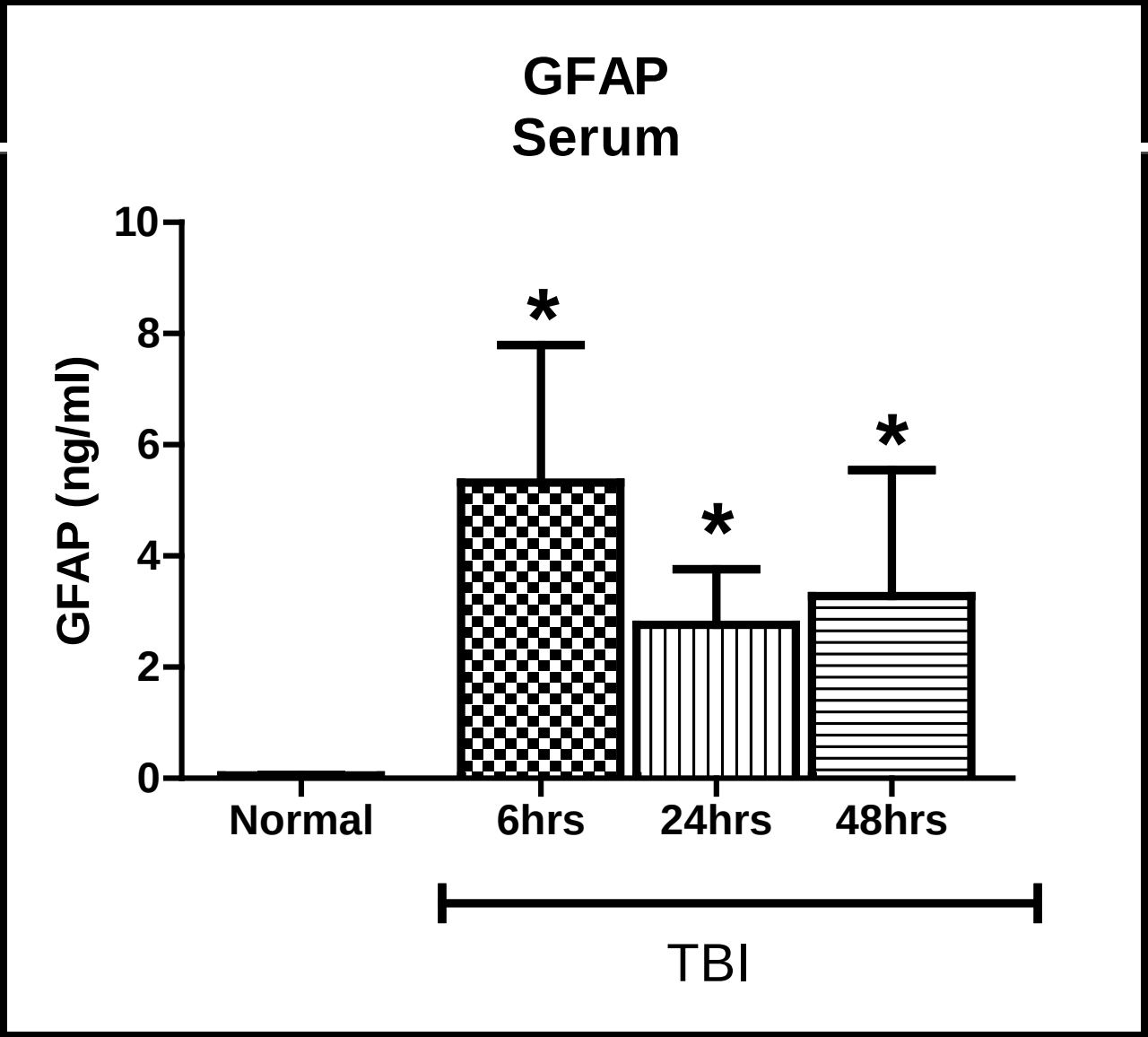
- Release of structural proteins into the bloodstream
  - S100B protein (S100B)
  - Glial Fibrillary Acidic Protein (GFAP)
  - Alpha-2 Spectrin Breakdown Products (SBDP150)
  - Ubiquitin C-hydrolase (UCH-L1)
- Serum blood levels may correlate with extent of brain injury
- Could serve as useful markers of the severity of the injury
- May provide useful information about response to treatment.
- None of these biomarkers have been prospectively validated to assess their clinical utility.

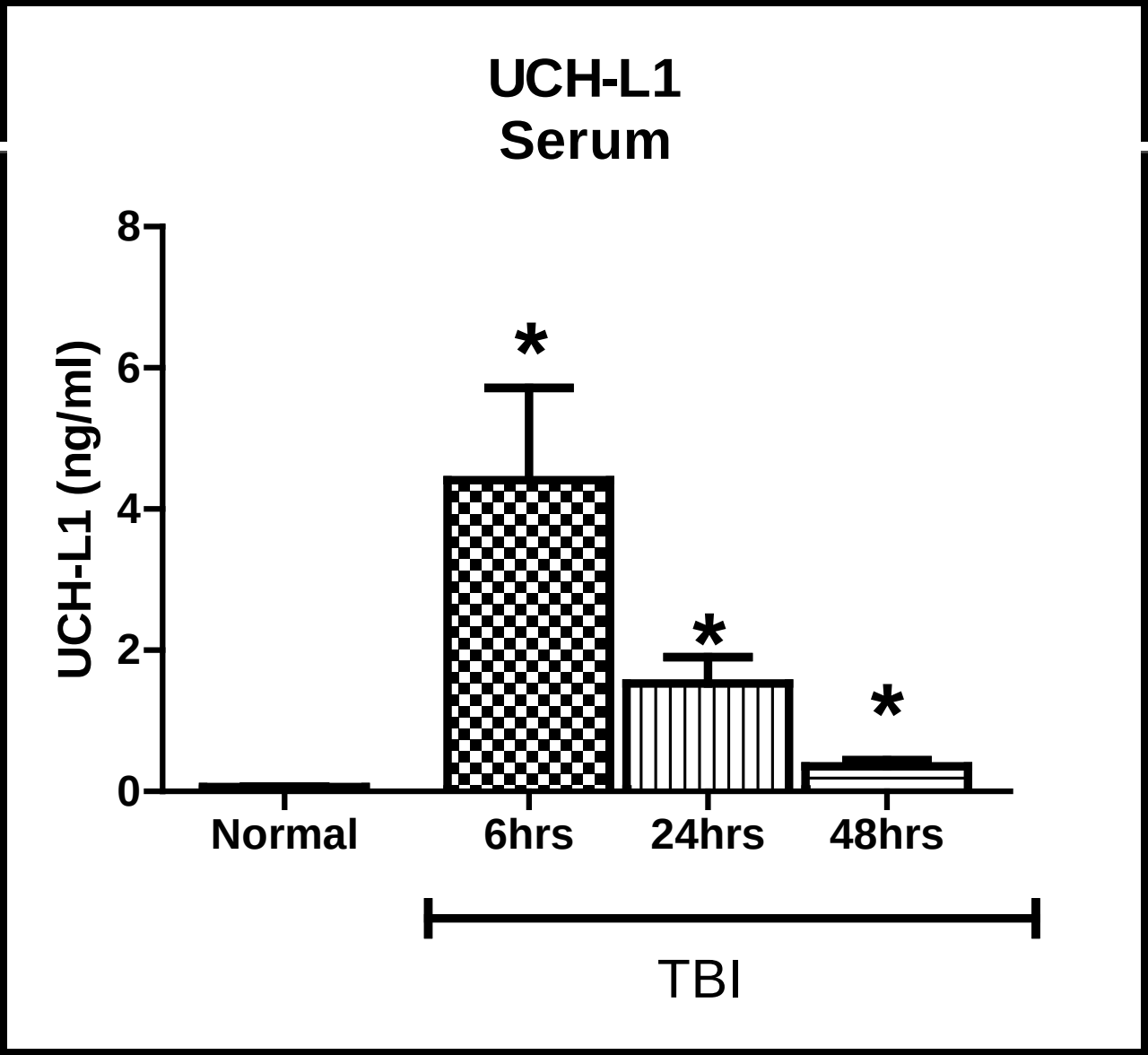
S100B, GFAP, UCH-L1

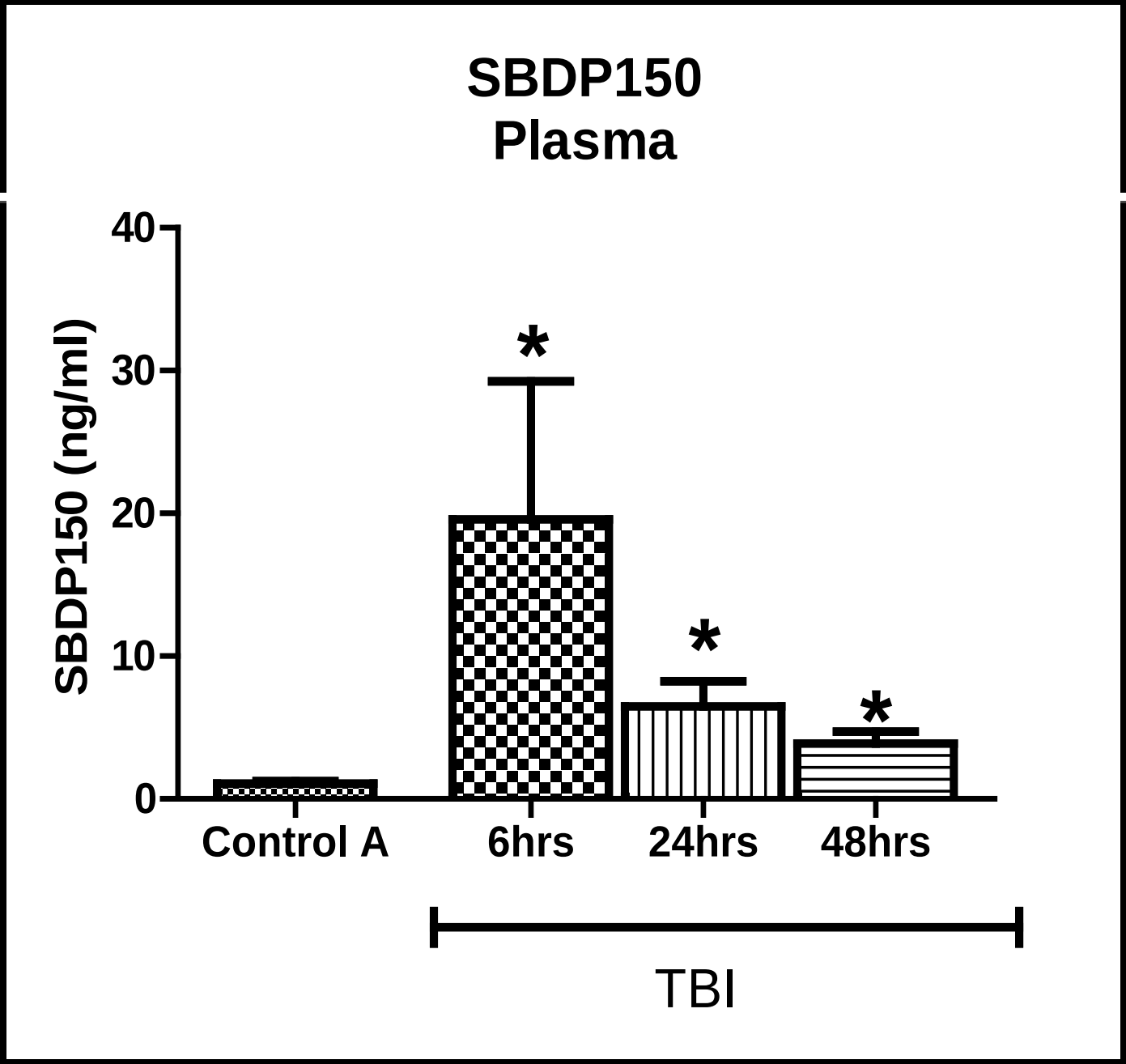
- S100B is a calcium binding protein in astroglia, oligodendrocytes, and Schwann cells.
- GFAP is a monomeric intermediate filament protein derived from the astroglial cytoskeleton
- Ubiquitin C-hydrolase (UCH-L1) is a protein that is almost exclusively found in neurons. It maintains ubiquitin homeostasis and is released into the blood stream after TBI.

αII-Spectrin

- αII-Spectrin is a structural protein in the neuronal cytoskeleton. It is found in neuronal cell bodies, axons and pre-synaptic terminals. Calpain and caspase-3 degrade αII-spectrin into breakdown products via necrosis and apoptosis cell death pathways, respectively. In TBI, calcium entry into neurons triggers calpain mediated proteolysis of αII-Spectrin creating two αII-Spectrin breakdown products (SBDPs) with molecular weights of 150 and 145 kDA. Caspase-3 also produces two SBDPs with molecular weights of 150 and 120 kDA.


GFAP
Serum
GFAP (ng/ml)
10
8
6
4
2
0
*
*
*
Normal
6hrs
24hrs
48hrs
TBI


UCH-L1
Serum
UCH-L1 (ng/ml)
8
6
4
2
0
*
*
*
Normal
6hrs
24hrs
48hrs
TBI


SBDP150
Plasma
SBDP150 (ng/ml)
40
30
20
10
0
*
*
*
Control A
6hrs
24hrs
48hrs
TBI

BIO-ProTECT

Biomarkers of Injury Outcomes in the ProTECT III Clinical Trial

Michael Frankel (PI)

- **Goal: Examine the utility of serum biomarkers as a tool to reliably assess injury severity and to guide treatment decisions in acute TBI patients.**

- **Primary Aim: To determine whether serum biomarkers of structural brain injury (S100B, GFAP, UCH-L1, SBDP150) are independent predictors of clinical outcome assessed 6 months after moderate or severe acute TBI.**

- **Secondary Aim: To define the relationship between steady state serum progesterone levels and treatment effect after moderate or severe acute TBI.**

Hypotheses

- Hypothesis 1: Elevated levels of serum biomarkers (S100B, GFAP, UCH-L1, SBDP150), measured **within 4 hours of TBI**, are independent predictors of outcome as measured by the Glasgow Outcome Scale Extended (GOS-E) at 6 months.

- Hypothesis 2: Elevated levels of serum biomarkers (S100B, GFAP, UCH-L1, SBDP150), measured **24 and 48 hours after study randomization**, are independent predictors of outcome as measured by the GOS-E at 6 months.

Hypotheses

- Hypothesis 3: In progesterone-treated subjects, steady state serum progesterone levels measured **24 hours after study randomization** will:
  - a) inversely correlate with biomarker levels at 24 hours and
  - b) directly correlate with a favorable outcome as measured by the GOS-E at 6 months.

Analysis: Hypothesis 1

- Using a two-step analysis plan to develop and validate a predictive model that incorporates baseline biomarker and clinical data (obtained within 4 hours of TBI), we will develop a prognostic model (using half of the subjects within the treatment and control arms of ProTECT III) to predict outcome on the GOS-E, and to validate this model using data from the other half of the subjects within each arm of ProTECT III.

Analysis: Hypothesis 2

- Using a two-step analysis plan to develop and validate a predictive model that incorporates baseline biomarker and clinical data (obtained at 24 and 48 hours after TBI), we will develop a prognostic model (using half of the subjects within the treatment and control arms of ProTECT III) to predict outcome on the GOS-E, and to validate this model using data from the other half of the subjects within each arm of ProTECT III.

Analysis: Hypothesis 3

- In progesterone-treated subjects, determine whether steady state serum progesterone levels inversely correlate with serum levels of S100B, GFAP, UCH-L1, SBDP150 (24 and 48 hours after study randomization) and correlate directly with a favorable outcome of progesterone treatment at 6 months

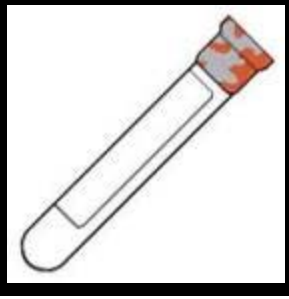
Clinical Utility

- Improve risk stratification
- Improve treatment decisions
- Surrogate or proxy marker of treatment response
- Gain further insight into the pathophysiology of TBI

Consent

- All ProTECT III subjects
- Single consent form
- EFIC
- Withdrawl of participation

Lab Kits



- Lab kits will include the following supplies necessary for all 3 blood draws, as well as for specimen transport via FedEx.
  - Insulated Shipping Box x 1
  - Corrugated cardboard box x 1
  - Disposable Styrofoam rack for sample storage and transport x 1
  - Disposable transfer pipette x 4
  - 10 ml Tiger Top tubes x 6 (2 per draw x 3 draws)
  - Aliquot tubes (cryovials) x 24 (8 per draw x 3 draws)
  - Shipping bags x 4
  - Specimen labels x 30
  - Core Laboratory Shipment Form
  - FedEx Airbill

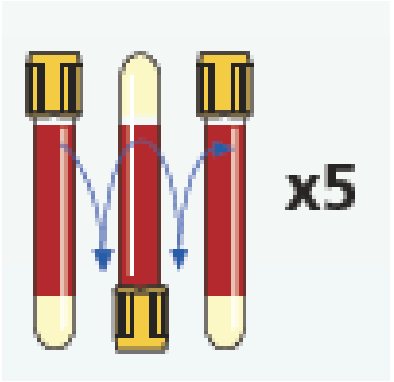
Lab Kits - Supplies

- To obtain additional lab kits:
  - Kit supplies will automatically be replenished based upon enrollment activities, but if you are down to only two kits, send an e-mail request to protectsupplies@emory.edu to ensure that you have at least one box on hand at all times.

Specimen Handling (at Baseline, 24 and 48 hours)

- Fill two10ml tiger top tubes

- **Gently invert the tube 5 times** to provide adequate mixing with the clotting activator. **Avoid hemolyzing the blood.**

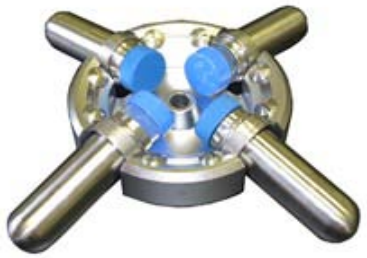


- Put patient study ID, date, time of draw, and your initials on label for the tiger top tubes and the aliquot tubes.
- **Place specimen tube upright in the disposable Styrofoam tube holder provided and allow to clot at room temperature for a minimum of 30 minutes and maximum of 120 minutes.**

- Transport blood sample in Styrofoam tube holder to processing lab NOTE: Keep samples upright at all times

Specimen Processing

- Centrifuge specimen at room temperature.
  - Spin sample at 1200 RCF (g) for:
    - 15 minutes if fixed angle centrifuge rotor or
    - 10 minutes if using a horizontal (sling) rotor



- Carefully remove tube from the centrifuge. The serum (clear yellowish liquid) will be on top of the clotted red cells. **DO NOT shake or invert specimen tube after centrifuging to avoid disturbing the cell palate on the bottom of the tube.**

- Using the pipette, very carefully remove the clear serum, avoiding any of the red cells. If you accidently get red cells in the pipette, gently return the contents of the pipette into the specimen tube, re-spin for 15 minutes, and pipette again.

Divide the serum equally between the 8 aliquot tubes (4 aliquot tubes for each tiger top tube of blood obtained) and put caps on the aliquot tubes tightly .

Place all aliquot tubes into the corrugated cardboard box and **freeze specimens immediately at - 80˚ C,** until ready to ship.

Sample Tracking

- Sample Documentation and tracking log is under development
- (WebDCU Tracking form is under development)
- It is key to properly label TUBES (Patient ID, Date, Time, etc) through out the process so that tubes do not get mixed up.

Packaging for Shipment

- Once all three sets of specimens (Baseline, 24 and 48 hours) are thoroughly frozen, prepare for shipment as follows:
  - Secure the lid on corrugated cardboard box with tape or rubber band.
  - Place corrugated cardboard box into the specimen bag and seal.
  - Fill Styrofoam shipping box with *dry ice*.
  - Place the specimen bag into the insulated Styrofoam shipping box with dry ice.
  - Place the Styrofoam box into FedEx box and seal.
  - Include Forms in shipment (retain copy for file at local site)

Shipping

- Complete a Core Laboratory Shipment Form making sure the tracking number from the FedEx shipping label is completed.
- Place the Core Laboratory Shipment Form and the Fed-ex airbill into the pouch and adhere to outside of box.
- Send **Priority Overnight** Monday through Wednesday only (we want to avoid Saturday deliveries). If specimens are drawn on Thursday or Friday, keep frozen over the weekend and ship the following Monday.
- FedEx shipping label will indicate shipment to:
  - Banyan Biomarker, Inc
  - Attn : Jixiang Mo Seany
  - 13709 Progress Blvd.
  - Alachua, FL 32615
  - 386-518-6736 (office)
  - 386-518-6794 (fax)

BANYAN BIOMARKERS®

- All specimens must be spun, separated, aliquoted and frozen within 2 hours of collection.
- Samples can be stored in a -80 freezer for up to one month, but should be mailed to the Core Laboratory at Banyan Biomarker, Inc, at the earliest possible opportunity (ship on Monday, Tues, or Wednesday)
- Multiple subject specimens can be shipped together, but a separate corrugated cardboard box and specimen bag should be used for each subject

- Problems
  - Contact Harriet Howlett-Smith, ProTECT III Project Manager at hhowlet@emory.edu or send an e-mail to protectsupplies@emory.edu. If urgent, call the ProTECT III Hotline at 888-359-2221.

BIO-ProTECT

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ProTECT III sites

NETT Steering Committee