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CONTRACTING ORGANIZATION: The University of Pittsburgh
Pittsburgh, PA 15213

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INTRODUCTION

Operation brain trauma therapy (OBTT) is a unique multi-center, pre-clinical, drug screening and brain injury biomarker development consortium for the ultimate translation of the best potential drugs to clinical trials in traumatic brain injury (TBI, Figure 1). OBTT includes investigators at the Safar Center for Resuscitation Research (University of Pittsburgh School of Medicine, Patrick Kochaneck, MD, PI; C. Edward Dixon, Co-I), the Miami Project to Cure Paralysis, (University of Miami School of Medicine, W. Dalton Dietrich, site PI; Helen Bramlett, Co-I), the Neuroprotection program at WRAIR (Frank Tortella, site PI; Deborah Shear, PhD, and Kara Schmid, PhD, Co-I), Virginia Commonwealth University (John Povlishock, PhD, site PI) and Banyan Biomarkers (Kevin Wang, PhD, PI, and Ronald Hayes, PhD, Co-I). Three rodent models (controlled cortical impact (CCI), parasagittal fluid percussion injury (FPI), and penetrating ballistic-like brain injury, PBBI) are used in Pittsburgh, Miami, and at WRAIR, respectively, for primary drug screening with the most promising candidates tested in a micropig model at Virginia Commonwealth University. Additional secondary screening of the most promising drugs is also carried out in more complex rodent models with polytrauma, hemorrhage or advanced monitoring. The principle concept of OBTT is that clinical TBI is a heterogeneous disease process that involves multiple brain injury phenotypes and that success of an agent tested across multiple established TBI models will identify the best candidates for success in clinical trials. Two types of drugs will be screened, low hanging fruit (drugs already FDA approved for other uses, or otherwise ready for clinical translation) and higher risk but potentially high-reward more novel targeted therapies. However, drugs in the latter category should already have at least some track record of success in experimental TBI.

<table>
<thead>
<tr>
<th>Site</th>
<th>Biomarkers</th>
<th>Neuro Exam /Stress</th>
<th>Motor Function</th>
<th>Cognitive Function</th>
<th>Neuropathology</th>
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<tr>
<td>PITTSBURGH</td>
<td>Rat: Blood Samples (0.7 ml; tail artery): 4 h, 24h, at sacrifice</td>
<td>Rat: Righting reflex</td>
<td>Rat: Beam balance and beam walking d1-5</td>
<td>Rat: MWM task: 14-20d hidden (14-18d) and visible platform (19-20d) and probe trial (20d)</td>
<td>Rat: Euthanize d21; serial sections, volumetric analyses + hippocampal neuron counts</td>
</tr>
<tr>
<td>MIAMI</td>
<td>Rat: Blood Samples (0.7 ml) via IV (jugular): 4h, 24h, at sacrifice</td>
<td>Rat: None</td>
<td>Rat: Spontaneous forelimb use, gridwalk task, rotated: 7d</td>
<td>Rat: MWM task: 13-21d (hidden platform, probe, working memory)</td>
<td>Rat: Euthanize d21; serial sections, volumetric analysis + neuron counts &amp; axonal pathology</td>
</tr>
<tr>
<td>WRAIR</td>
<td>Rat: Blood Samples (0.7 ml) via IV (jugular): 4h, 24h, at sacrifice</td>
<td>Rat: Neuroscore Exam: 30, 24h, 72h, 7d, 21d</td>
<td>Rat: Balance Beam/ Rotarod: 7d and 10d</td>
<td>Rat: MWM task: 13-17d (4/5x5); 30m (11); end w/probe trial</td>
<td>Rat: Euthanize at 21d, serial 40 um sections; H&amp;E/Silver</td>
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Table 1: Outcome Metrics: Primary Screening in OBTT

IV=Intravenous; MWM=Morris water maze; ITI=Inter-trial interval; H&E=Hematoxylin & eosin; WRAIR=Walter Reed Army Institute of Research

Dr. Kochaneck also created a detailed manual of operations (Appendix 1) that includes the key facets of the

BODY

Administrative overview of accomplishments in year 1 of funding
Safar Center for Resuscitation Research
(Patrick M. Kochaneck, MD, overall PI)

In year 1 of funding, to address the first milestone, the consortium was established including obtaining all administrative approvals (individual site contracts, IACUC, ACURO).
models, protocols, and outcomes at each site. For example, the outcomes at each site for primary screening of therapies are shown in Table 1. A literature review was also carried out by Dr. Kochanek along with a survey of the site investigators and co-investigators to define putative therapies, and the first two therapies (nicotinamide, vitamin B3 [drug #1] and erythropoietin [EPO, drug #2]) were selected by unanimous consensus of the team. Dr. Kochanek then carried out a comprehensive search to identify all key publications on each of these agents in experimental models of TBI. Comprehensive tables outlining the relevant findings from each study were then put into tabular form and this material was included in the manual of operations (Appendix 1). The literature support for drug #1 nicotinamide is shown as an example of our approach in

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<th>Study/Author</th>
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<th>Study/Author</th>
<th>Therapy</th>
<th>Study/Author</th>
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<tr>
<td>[1] Hoane et al, 2006</td>
<td>50 or 500 mg/kg/IV given at 15 min and 24 h</td>
<td>[2] Hoane et al, 2008</td>
<td>50 mg/kg/IP; at 15 min, 4 h, or 8 h post injury followed by 5 subsequent doses of 50 mg/kg IV at 24 h intervals</td>
<td>[3] Holland et al, 2008</td>
<td>50 or 500 mg/kg/IV given at 15 min and 20 h</td>
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<tr>
<td>[4] Spector</td>
<td>C14 niacinamide</td>
<td>Rabbit</td>
<td>None</td>
<td>None</td>
<td>Rapid transit into CSF after IV administration</td>
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<tr>
<td>[5] Sakakibara et al, 2002</td>
<td>IV dosing of 20-750 mg/kg at 2 h effective in rat MCAO model</td>
<td>MCAO/Male Rat</td>
<td>Marked reductions in infarct volume</td>
<td>None</td>
<td>Tested across numerous strains; Fischer, SHR, diabetic</td>
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<tr>
<td>[6] Sakakibara et al, 2000</td>
<td>IV dosing of 500 mg/kg</td>
<td>MCAO/Female Rat</td>
<td>Marked reductions in infarct volume</td>
<td>None</td>
<td>Effective in both female Sprague Dawley and Wistar rats</td>
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<tr>
<td>[7] Bernier et al, 1998</td>
<td>Oral dosing of 6 grams</td>
<td>Human</td>
<td>None</td>
<td>None</td>
<td>Half-life of 9.3 h with a range of between 4.2 and 25.8 h</td>
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**Table 2.** Therapy 1, Nicotinamide key references

**Figure 2.** Overview showing the injury, blood sampling, outcome testing, and sacrifice for therapy testing for drug #1 (nicotinamide) in rats at the Safar Center site. For this drug two doses were administered at 15 min and 24 hrs, both IV. This general approach was taken at all of the primary screening sites below.

The dosing plan was then developed based on the literature review, and for each agent, 4 groups have been used in primary screening, namely, sham, injury plus vehicle, and injury plus treatment at two doses. The overall approach to primary screening of therapies at the Pittsburgh site is shown in Figure 2. This serves as a general model for all of the primary screening sites, although the exact nature of the injury and the outcome tests varies between centers. The drug, dose, treatment regimen, and biomarker sampling is, however, identical between sites.

Both drugs are in primary screening. The consortium has also held a monthly 1 hour conference call that has included a representative from each site and we held a very productive face-to-face investigators meeting at the 2011 congress of the National Neurotrauma Society in July. In addition, Dr. Kochanek sent the manual of
operations and consortium plans for drugs #1 and #2 to the “Therapy and Oversight Committee” members and received their input. In addition, our consultants on functional outcome assessments (Dr. Robert Hamm) and Biostatistics (Dr. Stephen Wisniewski) were also appraised of our plan and contributed extensive recommendations that were discussed on conference call and incorporated into our operations manual. Specific stationary for the OBTT consortium was also designed by Drs. Dixon and Kochanek. Once OBTT was launched, a comprehensive manuscript on the composition and plans of the consortium was submitted and recently published in the *Journal of Trauma* (see reportable outcome 1). Five abstracts (reportable outcomes 2-6) were submitted to the 2011 ATACCC meeting and this resulted in the development of a specific session at the ATACCC meeting focused on the OBTT consortium where the overall PI and a representative of each site presented their component of the program and updated their findings at approximately the half way mark of year #1 of funding. The session was extremely well received at the congress. Dr. Kochanek also presented updates on OBTT and related TBI research germane to combat casualty care to COL Dallas Hack at grand rounds at Ft. Detrick in September, 2011, and at Uniformed Services University in October, 2011.

Finally, we are currently in discussions regarding the selection of drug #3. Four drugs, Cyclosporine A, Progesterone, Simvastatin, and Keppra are the leading candidates.

**Primary screening in rodent models of TBI**

*Primary Screening Site 1, Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine (Patrick Kochanek, MD, C. Edward Dixon, PhD)*

An overview of the approach that is being taken for primary drug screening, biomarker sampling and outcome testing at the Pittsburgh site was previously shown in Figure 2.

**Drug #1 Nicotinamide:**

Treatment or vehicle was administered at 15 min and 24 h after injury—and this identical dosing approach was used at all primary screening centers. All animals for study drug #1 (nicotinamide) have been completed surgically and have completed all of the outcome testing. The brains from all of these animals are currently being processed for contusion volume and other neuropathological outcomes. At the Pittsburgh site, the TBI model that is being used for Tier A screening is controlled cortical impact (CCI) model in adult rats (1), which was actually developed by OBTT Co-investigator Dr. C. Edward Dixon, who oversees all of the rat studies using this model in Pittsburgh. The model is well established and characterized in Pittsburgh. The number of rats per group and dosing plan for Drug #1 was Sham (n=10), TBI-Vehicle (n=10), TBI-50 mg/kg (n=10), TBI-500mg/kg (n=10).
**Sensorimotor Function**

**Beam Balance:** In the beam balance test, the TBI + Vehicle and TBI + Nicotinamide (50mg/kg) groups differed significantly from the Sham + Vehicle group. The TBI + Nicotinamide (500mg/kg) group did not differ from the Sham group, suggesting a modest benefit from the high dose on nicotinamide on beam balance performance (Figure 3).

**Beam Walking:** In the beam walking test, the TBI + Vehicle and TBI + Nicotinamide (50mg/kg) groups differed significantly from the Sham + Vehicle group, demonstrating an obvious functional deficit. In contrast, the TBI + Nicotinamide (500mg/kg) group did not differ from the Sham group, suggesting a modest benefit from the high dose on nicotinamide treatment on beam walking performance (Figure 4). This is consistent with the published literature on nicotinamide in experimental TBI, where beneficial effects of nicotinamide have been shown, predominantly on motor function and neuropathology.

![Water Maze](image)

**Figure 5.** Morris water maze testing of rats treated with drug #1 nicotinamide after CCI demonstrates no beneficial effect of high dose therapy on cognitive outcome, rather a paradoxical detrimental effect of low dose on water maze performance.

**Table 3. Physiology**

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<th>Sham</th>
<th>TBI-Vehicle</th>
<th>TBI-50mg/kg</th>
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<td>pH</td>
<td>7.46 ±0.01</td>
<td>7.45 ±0.01</td>
<td>7.47 ±0.02</td>
<td>7.45 ±0.01</td>
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<tr>
<td>pO2</td>
<td>165.1 ±5.96</td>
<td>155.56 ±7.52</td>
<td>152.0 ±10.39</td>
<td>144.6 ±6.79</td>
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<td>pCO2</td>
<td>39.16 ±0.90</td>
<td>40.3 ±1.32</td>
<td>38.39 ±1.18</td>
<td>39.24 ±0.68</td>
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<td>MAP</td>
<td>132.04 ±2.91</td>
<td>127.72 ±3.48</td>
<td>133.04 ±3.98</td>
<td>129.25 ±3.78</td>
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<td>Brain Temp</td>
<td>36.6 ±0.05</td>
<td>36.7 ±0.06</td>
<td>36.6 ±0.05</td>
<td>36.6 ±0.05</td>
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<tr>
<td>Body Temp</td>
<td>36.7 ±0.14</td>
<td>36.8 ±0.09</td>
<td>36.8 ±0.06</td>
<td>36.9 ±0.08</td>
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|        |            |             |             |               |
| **Post-TBI** |          |             |             |               |
| pH     | 7.46 ±0.01 | 7.46 ±0.01  | 7.47 ±0.01  | 7.46 ±0.01    |
| pO2    | 155.0 ±5.08| 145.33 ±6.63| 132.67 ±6.54| 143.2 ±4.68   |
| pCO2   | 37.76 ±0.82| 37.86 ±0.57 | 38.31 ±0.82 | 39.69 ±1.00   |
| MAP    | 132.83 ±2.14| 126.43 ±3.83| 128.14 ±3.05| 109.78 ±3.43  |
| Brain Temp | 36.7 ±0.05 | 36.7 ±0.04  | 36.7 ±0.05  | 36.62 ±0.03   |
| Body Temp | 36.8 ±0.07 | 36.85 ±0.08 | 36.76 ±0.06 | 36.73 ±0.05   |

**Cognitive Function**

**Morris water maze:** The only statistical group difference in latency to find the hidden platform was between the Sham + Vehicle and TBI + Nicotinamide (50mg/kg) groups (Figure 5). Thus, the 50mg/kg dose of nicotinamide worsened performance in the water maze task. No differences were observed between the other groups, including the Sham + Vehicle and the TBI + Vehicle groups. This indicates the absence of an injury effect in the acquisition phase of the water maze task. The probe trial revealed that the Sham group spent a significantly greater amount of time in the target quadrant than the three TBI groups. There were no differences between the TBI groups. Comparing the sham group to another sham group from another concurrent study indicates that the shams in the nicotinamide study are performing worse than our typical shams that undergo a less invasive surgical plan.

Based on these findings, we also addressed this by increasing our injury level for the drug #2 (EPO) study (see below). There was no difference between the experimental groups in swim speed measured on day 19 post injury.

Serum biomarker data for drug #1 from the Pittsburgh site are presented later in the report from the Banyan Biomarker site.

For Tier B primary screening in Pittsburgh,
we are using an established mouse model of CCI followed by 35 min of hemorrhagic shock (CCI+HS) based on the work of Dennis et al (2), with minor modifications. This model is being used to simulate more complex polytrauma insults commonly seen in combat casualty care. We have carried out 20 studies (10 vehicle and 10 high dose nicotinamide treated mice) and have followed the mice for 17 days. Neuropathology in these mice is currently being processed.

**Drug #2, Erythropoietin (EPO):**
Based on a comprehensive review that identified thirty-one studies in experimental TBI supporting its potential efficacy, EPO was selected by the OBTT consortium as the drug #2 for primary screening. Based on that same review, two doses were selected, namely 5000 or 10,000 IU/kg, by a single IV injection administered at 15 min after injury. This treatment regimen is being used at all of the sites. At the Pittsburgh site, surgery and injury for all of the rats (n=40) for drug #1, EPO have also been completed and the rats have also nearly completed functional testing. The results are being processed.

**Primary Screening Site 2. Miami Project to Cure Paralysis, University of Miami School of Medicine (W. Dalton Dietrich, PhD, Helen Bramlett, PhD)**
At the Miami site, the TBI model used is parasagittal fluid percussion injury (FPI) in adult rats, which is well established and characterized in the laboratory in Miami.

**Drug #1 Nicotinamide:**
All animals for study drug #1 (nicotinamide) have been completed surgically at the Miami site. The number of rats per groups are Sham (n=10), TBI-Vehicle (n=10), TBI-50 mg/kg (n=10), TBI-500mg/kg (n=10), identical to the Pittsburgh site. At the Miami site, all rats receive arterial catheters and thus this site for all drugs is serving an important additional function, namely, to determine the systemic physiological effects of the doses selected for each therapy. In this regard, at the initiation of the study, the injection of the nicotinamide resulted in a decrease in mean arterial blood pressure (MAP) that was below 90mmHg. We thus, using this important information from the Miami group, adjusted the injection protocol at all injury sites to a slower injection over 15 minutes and this attenuated this drastic drop in blood pressure. **Table 3** clearly shows that all animals in the study maintained normal physiology, notably MAP, both pre- and post-TBI at the slower infusion rate and
thus early post injury hypotension would not confound the treatment findings. This would be very important for clinical translation.

Sensorimotor function

Sensorimotor testing was performed using the cylinder task (Figure 6) and the rotarod test (Figure 7). The cylinder task is measured as an asymmetry index. An index of 0.5 indicates equal use of the contra- and ipsilateral forelimbs; scores below 0.5 indicate greater use of the forelimb ipsilateral to the injury (i.e., a contralateral limb deficit). Sham animals exhibited no deficits on this task. All TBI groups had contralateral limb deficits with the TBI-500mg/kg nicotinamide-treated animals demonstrating less of a deficit in the contralateral limb use relative to the other two TBI groups—again suggesting a beneficial effect of nicotinamide on motor function after experimental TBI, similar to what was observed in CCI. For the rotarod test, sham animals were able to stay on the rotarod slightly longer than the other TBI groups. However, there was no statistically significant effect of treatment at either dose on this sensorimotor task.

Cognitive Outcome

Cognitive function was assessed using a simple place task (Figure 8) tested over 4 days followed by a working memory test (Figure 9). Sham animals showed reduced latencies over the four day testing period. All three TBI groups had increased latencies versus sham with the two treated TBI groups exhibiting more cognitive deficits on this task. Thus, nicotinamide treatment did not appear to improve learning and memory using this paradigm—which is similar to what was observed for CCI and also in the PBBI model (please see below). However, on the working memory task, while sham animals as expected showed the greatest improvement in the delay match-to-place task, rats treated with nicotinamide at the 500mg/kg dose did demonstrate improvement on this task as well. This was the only evidence for a potential cognitive benefit from nicotinamide treatment in primary screening—in this case, in the parasagittal FPI model.

Serum samples from drug #1 studies for biomarker assessments were sent to Banyan for processing and the results are pending for the Miami studies. Brain tissues sections are being processed for drug #1 at the University of Miami site and brain injury.

Drug #2, EPO:

At the Miami site, injuries and functional outcome assessments on drug #2, EPO in the parasagittal FPI model are underway. An identical dosing and treatment regimen, namely 5000 or 10,000 IU/kg IV as a single dose at 15 min post injury is being used. Again, as in all primary screening studies, sham and vehicle treatment groups are also being performed.

Tier B primary screening in Miami with agents found to be promising will be carried out in the future using the parasagittal FPI model in rats with
superimposed infusion of IL-1β, again to simulate a polytrauma setting that is commonly seen in combat casualty care.

**Primary Screening Site 3. WRAIR (Frank Tortella, PhD, Deborah Shear, PhD, Kara Schmid, PhD).**
WRAIR serves as the primary screening center using a military-relevant model of PBBI in rats. The basic PBBI model is used to screen therapies on neurobehavioral (motor and cognitive) and histopathological outcomes, and the advanced (EEG) model will be used to target injury-induced changes in higher level cortical function with the most promising agents. Tier A of the study consists of screening each agent in the PBBI model using 2 doses on neurofunctional (motor and cognitive), neuropathological outcome metrics (compared to appropriate vehicle-treated and sham injury groups), and serum biomarker profiles (Banyan), as described above for the other OBTT sites.

**Drug #1 Nicotinamide:** Nicotinamide (50 or 500mg/kg), as carried out in the other OBTT primary screening sites, was delivered via a 10-min IV infusion at 15 min and 24h post-PBBI. Neuroscore assessments of neurofunctional outcome were conducted (prior to dosing) at 15m, 24h, 72h, 7d, 14d, and 21d post-injury. Motor abilities were assessed on a rotarod task at 7 and 10 days post-PBBI and cognitive abilities were assessed in the MWM task on post-injury days 13-17 (see Shear et al. 2010 for additional details). Again, consistent with the other OBTT primary screening sites, serum blood draws were taken at 4h and 24h post-PBBI (prior to administration of the 24h dose) and at the terminal endpoint and were sent to Banyan for further processing of biomarkers results. All animals were euthanized at 21 days post-PBBI and the brains were sent to FuDu Neurotechnologies for processing with H&E (lesion reconstruction) and silver staining (axonal damage).

**Sensorimotor function**
WRAIR has completed all neurobehavioral assessments for nicotinamide in the PBBI model. The number of animals per group were Sham (n=9), PBBI-vehicle (n=14), PBBI-50mg/kg (n=15), PBBI-500mg/kg (n=16). PBBI groups displayed significant motor abnormalities on the rotarod task at both 7 and 10 days post-PBBI. No significant treatment effect was detected on this task (Fig. 10A).

**Cognitive Outcome**
Cognitive outcome was assessed using a spatial learning (hidden platform) task tested over 5 days followed by a retention (missing platform) test. All three PBBI groups showed significant cognitive impairment indicated by longer latencies and swim distances to the hidden platform. No significant treatment effect was detected in either the spatial learning paradigm or in the retention (missing platform) test (Figure 10B, C). Quantification of lesion volume and axonal damage are ongoing at the WRAIR site and are targeted for completion by November 2011.

Biomarker data from serum samples from the rats subjected to PBBI in the drug #1 study are presented and discussed later in this report.

**Figure 10 (A-C).** Administration of nicotinamide (50 or 500 mg/kg IV) failed to produce a significant treatment effect on PBBI-induced motor abnormalities on the rotarod task (A) or cognitive impairment in the MWM task (B and C). Please see text for details.
Agent 2 – Erythropoietin (EPO): EPO (Procrit; provided by Dr. Kochanek; 50 or 100ml/kg) is being administered via IV infusions at 15 min post-PBBI using the same dosing and treatment protocol that is being used at the other primary screening sites. Outcome metrics are again identical to those used for nicotinamide. The WRAIR EPO study was initiated in September 2011 and is currently ongoing. The number of animals that have been injured/dosed to date are Sham (n=6), PBBI+Veh (n=10), PBBI+50ml/kg (n=12), PBBI+100ml/kg (n=12). Neurobehavioral assessments are targeted for completion in November 2011 and neuropathological assessments are targeted for completion in January 2012.

In Tier B, the 3 highest rated agents will be assessed on the advanced PBBI model (using electrophysiological monitoring) in rats. Studies in the advanced PBBI model may include factors important to therapy optimization, including assessments of effects on regional cerebral blood flow (rCBF), blood brain barrier (BBB) permeability, and brain edema formation.

Serum Biomarker Development and Application to the primary screening studies

Banyan Biomarkers (Kevin Wang, PhD; Ronald Hayes, PhD)
For the biomarker studies, a rigorous sampling, shipping, and processing protocol was followed across the OBTT sites.

Figure 11. Schematic based on Mondello et al (6) of the Banyan biomarker portfolio. For OBTT in this report, the first two biomarkers UCHL-1 and GFAP were evaluated across models in the consortium. Please see text for details.

temperature for 60 min. Tubes were centrifuged at 5,000xg at room temperature for 5 min. Serum was collected, snap frozen on dry ice, and stored at -80°C until shipped. Each sample was coded for rat number followed by a -4 h, -24 h, or final (-F) designation. Sampling for biomarkers that coincided temporally with drug dosing was done prior to drug administration. Samples were shipped on dry ice and Banyan was notified of the shipment. This approach, as shown below, produced high quality serum biomarker data across the OBTT consortium.
Initial analysis focused on two biomarkers, a neuronal cell body damage marker UCHL1 and a glial injury marker glial fibrillary acidic protein (GFAP) (Figure 11) at the 4 and 24 h time points. Based on prior experience, it was anticipated that the 4 h sample might represent largely the response to primary injury in the various models while the 24 h sample would reflect the evolution of secondary injury— influenced by model and potentially by treatment. Thus, several principle analyses were carried out, namely: 1) comparison of biomarker levels across models (injury vehicle vs sham for the respective models) was a unique and novel comparison for the field of TBI, 2) comparison of the effect of treatment on biomarker levels at the individual time points, and 3) comparison of the delta in serum biomarker level between the peak at 4 h and the magnitude of the increase remaining at 24 h— reflecting the impact of treatment on the evolution of secondary damage. Thus far, all of these analyses have revealed interesting and exciting findings.

As shown in Figure 12, the biomarker response to the PBBI model revealed an increase in serum GFAP level to ~300-400 pg/mL at 4 h after injury. This decreased to levels of ~200-250 pg/ML by 24 h. Remarkably, treatment with high dose but not low dose nicotinamide reduced serum GFAP levels at 24 h vs. vehicle, suggesting that this agent attenuates secondary injury at the high dose. This is a potentially important finding and suggests that the serum biomarker GFAP may represent a very sensitive means to accomplish drug screening. We are awaiting completion of the neuropathology (lesion volume, etc) to determine if there is a correlation between biomarkers and neuropathology including GFAP immunohistochemistry. Of note, UCHL1
levels increased at 4 and 24 h after injury vs. sham, again with the 4 h levels somewhat higher than the 24 h levels, however, nicotinamide treatment did not have an effect on GFAP levels at either time point after injury.

As shown in Figure 13, the biomarker response in the CCI model revealed a much greater increase (4-fold higher) in serum GFAP and UCHL-1 levels than in the PBBI model. This was a surprising finding given the neuropathology—particularly lesion volume response that is seen in each of the models. PBBI produces a large cavitory lesion at the injury level used in our studies, certainly larger in volume than CCI. In contrast, CCI may produce a more diffuse injury with shearing forces related to the impact velocity as suggested by the work of Hall et al (7). In CCI for UCHL1, even sham levels were higher than in PBBI injury rats, and at 4 h there was no increase in TBI vs. sham. However, by 24 h, there was a trend toward increased levels of UCHL1, again, with substantially higher levels in CCI vs. PBBI. We are also awaiting the data from the parasaggital FPI model.

Figure 13. Serum biomarker data from the CCI model for GFAP (top) and UCHL-1 (bottom). Note difference in scales at 4 h and 24 h for GFAP and UCHL1. A marked initial increase in GFAP was seen vs. sham in all injury groups at 4 h. At 24 h after injury high dose nicotinamide treatment produced a trend toward a reduction in GFAP levels. UCHL1 was not increased at 4 h after injury, but serum levels tended to be higher than sham at 24 h and there was no treatment effect. Please see text for details.
in Miami to see how the three injury models compare. Study of the differences between serum biomarker levels across models is a very unique facet of OBTT and should provide exciting insight for the general field of experimental TBI. Figure 13 also demonstrated a trend in reduction of GFAP levels by nicotinamide treatment at the high dose. Demonstration of an effect of treatment on the evolution of secondary injury after TBI was better supported by examining the difference between 4 h and 24 h serum GFAP levels (GFAP decay) in the CCI-treated rats (Figure 14). A significant increase in the decay of serum GFAP levels was seen in rats treated with 500 mg/kg of nicotinamide. This is an exciting finding and again suggests that nicotinamide treatment is blunting the secondary glial response after TBI—either indirectly by attenuating secondary damage—with a resultant down-regulation of the glial response, or by directly blunting the glial response. Other possibilities include alteration of BBB permeability or altering systemic clearance of GFAP. There was no significant effect of treatment on either UCHL1 clearance or GFAP clearance in the PBBI model, although there was a modest trend toward increased GFAP clearance again with high dose nicotinamide (p=0.2, data not shown). Further study of this important and unique potential utility of serum biomarkers is needed, and is an integral component of the OBTT consortium.

**Secondary Screening**

**Large animal model of TBI in micropigs**

Virginia Commonwealth University site (J. Povlishock, PhD)

The purpose of this component of the application is to provide a gyrencephalic model of TBI for subsequent screening of those agents found to be most efficacious in the proposed rodent model systems. Specifically, micropigs will be subjected to TBI and therein, traumatically-induced microvascular dysfunction and axonal damage will be assessed in both sham and drug treated animals. Based on the specific structure of the OBTT consortium, the milestone for year one in the large animal work was to re-establish our large animal model and set the stage for testing, in years 2-5, of the most promising drugs that have been identified through the rodent screening studies that are underway. We have accomplished that goal in year 1 of funding.

During the period of time in which the contractual agreements and IACUC/ACURO approvals were submitted and approved, we reviewed over 14 cases of previously generated micropig brain injury studies to evaluate the brain loci that display the maximal burden of traumatically-induced axonal damage as a prelude to the therapeutic assessments proposed in this funded application.

An important aspect of investigation highly relevant to blast TBI that represents a key component of the large animal model is assessment of therapeutic effects on vascular dysfunction and axonal injury. Related to this important goal within the milestone, a select population of micropigs is currently being prepared for TBI with emphasis on refining the placement of cranial windows for assessing concomitant microvessel dysfunction through the use of various physiological challenges in both sham and injured animals.

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*Figure 14. Decay in serum UCHL1 (left) and GFAP (right) levels between 4 and 24 h after CCI, sham, or CCI plus treatment with either low or high dose nicotinamide in rats studied at the Pittsburgh site. Treatment with nicotinamide at 500 mg/kg increased the decay in serum GFAP level after CCI suggesting a reduction in the secondary damage that is evolving in the brain after injury. No significant change in decay of HCHL1 was seen by treatment.*
We are also working with Dr. Wang from Banyan on the details involved in blood sampling to generate parallel serum biomarker studies in our model, given the high level of success in this regard that has been seen for this aspect of the consortium across the rodent models.

Collectively, these approaches should leave us well positioned to begin the proposed drug screening studies in the second year of this grant.

**KEY RESEARCH ACCOMPLISHMENTS**

1. IACUC and ACURO Approval at all sites along with necessary updates
2. Creation of a Manual of Operations for the OBTT consortium by Dr. Kochanek
3. Monthly consortium investigator conference calls
4. TBI drug therapy literature review, investigators survey, and selection of the first two therapies to be evaluated by the OBTT consortium
5. Comprehensive review of the TBI literature for the first two drugs, nicotinamide and erythropoietin by Dr. Kochanek to facilitate updating the manual of operations with regard to the protocols for the first two drugs.
6. Submission of a manuscript on the OBTT concept to the *Journal of Trauma* which was accepted for publication and published in 2011 (1)
7. Submission of five abstracts on the individual components of OBTT to the 2011 ATACCC meeting. Those abstracts were accepted and served as the basis of a symposium at the conference.
8. Report sent by Dr. Kochanek on the launching of OBTT to the Therapy and Oversight Committee and Consultants
9. Therapeutic testing and serum biomarker assessments for drug #1 (nicotinamide)—primary screening of the drug across three rodent models Controlled cortical impact, parasagittal fluid percussion, and penetrating ballistic-like brain injury, at the University of Pittsburgh Safar Center for Resuscitation Research, The University of Miami (Miami Project), and WRAIR, respectively.
10. Investigators meeting held on July 12th at the 2011 National Neurotrauma Society Meeting
11. Presentation of an afternoon symposium on OBTT by the PI and site PIs at the 2011 ATACCC conference.
12. Therapeutic testing and serum biomarker assessments for drug #2 (erythropoietin)—primary screening of the drug across three rodent models.
13. Secondary screening of drug #1, nicotinamide in a murine model of TBI plus hemorrhage at the University of Pittsburgh Safar Center for Resuscitation Research,
14. Re-establishment and refinement of the large animal micropig model of fluid percussion TBI at Virginia Commonwealth University
15. Identification of three promising candidates to be selected as drug #3 for primary screening in OBTT.

**REPORTABLE OUTCOMES**


CONCLUSION

The unique multicenter pre-clinical drug screening consortium OBTT has been launched and is successfully screening drugs across three established rodent models of TBI. In addition, exciting biomarker applications have also been successfully launched and the large animal model is being refined for testing of the most promising agent identified in year 2. Overall, no significant problems have been encountered.

REFERENCES


APPENDICES

Appendix 1. Manual of operations for OBTT updated October 17, 2011
Appendix 2. Peer reviewed publication from year 1 in the Journal of Trauma.
OPERATION BRAIN TRAUMA THERAPY
Operations manual; Prepared by P. Kochanek, MD and C.E. Dixon, PhD
Updated October 17, 2011

Primary Screening

1. **Modeling and injury protocol**

Drug therapies will be tested in three rat traumatic brain injury (TBI) models, controlled cortical impact (CCI; Pittsburgh), parasagittal fluid percussion injury (FPI; Miami), and penetrating ballistic-like brain injury (PBBI; WRAIR). The protocol to be used will represent the standard approach in each model at each site (see Table 1) with the minor modifications needed to incorporate the following standardized parameters at each site.

Factors standardized across models

- Adult, Male, Sprague Dawley rats (specific vendor and weights per site).
- Blood sampling (0.7 mL whole blood) obtained at 4 h, 24 h, and at sacrifice from each rat in primary screening (jugular venous catheter or tail artery sampling method per site). Blood removed will be replaced with an equal volume of sterile normal saline given either IV or SQ to limit hemodynamic consequences of phlebotomy.
- Motor function testing performed at each site (specific tools and methods per site).
- Cognitive testing using MWM at each site (specific MWM paradigm per site).
- Euthanasia at 21 days after injury.
- Histopathology includes volumetric analysis (specific approach/ancillary outcomes per site).

**Table 1: Outcome Metrics: Primary Screening**

<table>
<thead>
<tr>
<th>Site</th>
<th>Biomarkers</th>
<th>Neuro Exam /Stress</th>
<th>Motor Function</th>
<th>Cognitive Function</th>
<th>Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PITTGBURGH</td>
<td>Blood Samples (0.7 mL; tail artery): 4 h, 24 h, at sacrifice</td>
<td>Rat: Righting reflex</td>
<td>Rat: Beam balance and beam walking d1-5</td>
<td>Rat: MWM task: 14-20d hidden (14-18d) and visible platform (19-20d) and probe trial (20d)</td>
<td>Rat: Euthanize d21; serial sections volumetric analyses + hippocampal neuron counts</td>
</tr>
<tr>
<td>MIAMI</td>
<td>Blood Samples (0.7 mL) via IV (jugular): 4h, 24h, at sacrifice</td>
<td>Rat: None</td>
<td>Rat: Spontaneous forelimb use, gridwalk task, rotarod: 7d</td>
<td>Rat: MWM task: 13-21d hidden platform, probe, working memory</td>
<td>Rat: Euthanize d21; serial sections, volumetric analysis + neuron counts &amp; axonal pathology</td>
</tr>
<tr>
<td>WRAIR</td>
<td>Blood Samples (0.7 mL) via IV (jugular): 4h, 24h, at sacrifice</td>
<td>Rat: Neuroscore Exam: 30m, 24h, 72h, 7d, 21d</td>
<td>Rat: Balance Beam/ Rotarod: 7d and 10d</td>
<td>Rat: MWM task: 3-17d (4x/dx5d; 30m ITI; end w/ probe trial)</td>
<td>Rat: Euthanize at 21d serial 40 um sections; H&amp;E/Silver</td>
</tr>
</tbody>
</table>

IV=Intravenous; MWM=Morris water maze; ITI=Inter-trial interval; H&E=Hematoxylin & eosin; WRAIR=Walter Reed Army Institute of Research
2. **Approach to treatment**

Drug treatment will be identical between sites and will employ an IV route whenever feasible and optimal. However, strategies involving other routes may be used in selected situations (i.e., nutraceuticals, rehabilitation-related therapies). For each agent, two doses will be screened, again based on the best available literature evidence. Treatments that are selected will be agreed upon unanimously by the site PIs involved in each given study. For each drug screened, four groups will be studied with 10 rats in each group. Specifically, two treatment groups (two doses) along with a single vehicle group and sham group (no treatment) will be studied for each therapy. Results of the primary and secondary outcomes (MWM performance and volumetric analysis, respectively) at each site will not be revealed until all sites have completed work with each agent in primary screening. However, if an agent at a given dose produces greater than 20% mortality, the site PI will notify the other primary screening site PIs since this could trigger reconsideration of dosing at all of the sites.

Specific considerations relevant to each therapy will be generated for each agent that is selected to move forward in primary screening. Thus, the information in this section will build as the program moves forward.

- **Therapy 1: Nicotinamide**

  Vitamin B3 has shown dramatic beneficial effects on all aspects of outcome evaluated including function, neuropathology, and blood-brain barrier damage, with several positive reports in TBI, including CCI and FPI (1-3) ([Table 2](#table2)). Most of the reports showing benefit of nicotinamide in TBI are from a single laboratory. Nicotinamide has been shown to attenuate several mechanisms that are important in TBI, including poly-ADP-ribose polymerase activation, inflammation, and replenishing NADPH levels with resultant increases in glutathione. Doses of 50-500 mg/kg have shown efficacy and with a promising 4 h time window (1). Nicotinamide is commercially available as vitamin B3. It represents an example of an agent that could be readily moved forward if found to show benefit across models and could also be used as a nutritional supplement in a pre-treatment approach particularly in light of the ability to provide dietary neuroprotective additives in theater.

  Regarding dosing, route of administration and pharmacology, Evidence suggests that nicotinamide rapidly reaches high levels in brain related to the presence of a specific uptake mechanism (4). In addition to the aforementioned key references outlining efficacy of doses ranging between 50 and 500 mg/kg in experimental TBI, two references in the stroke literature are relevant to dosing. Sakakibara et al (5) demonstrated that IV administration is effective in male rats in transient MCAO with administration at 2 h, given immediately before reperfusion. In this study, a variety of rat strains including Fischer, SHR, and diabetic were studied and benefit on infarct volume was seen in all strains. Similarly, in permanent MCAO, nicotinamide (500 mg/kg) given by the IV route at 2 h attenuated infarct volume in both Sprague Dawley and Wister female rats, and reduction in infarct size was larger with IV administration than in prior reports using IP administration (6). This supports the proposed IV use in our studies in TBI. Regarding half-life, it has been reported that nicotinamide has a long half-life when administered PO in humans, where it averaged 9.3 h (7).

  For the proposed studies in OBTT, nicotinamide (MW 122.12) will be purchased from Sigma (catalog number N3376). Dosing will be 50 mg/kg or 500 mg/kg given IV at 15 min and 24 h after injury. For administration, the drug should be prepared fresh daily by dissolving it in sterile...
0.9 normal saline (NS). The doses of 50 mg/kg or 500 mg/kg will be prepared for delivery for each rat in 1 mL/Kg of sterile NS, respectively. Thus, as an example, for a 400 gram rat, for each 500 mg/kg dose, 200 mg of nicotinamide would be dissolved in 0.4 ml of sterile NS. Vehicle treated rats will also receive 1 mL/kg of sterile NS. Room temperature saline should be used rather than cold saline to dissolve the nicotinamide, and agitation of the solution may be needed particularly for the 500 mg/kg dosing (personal communication per Dr. Michael Hoane). Once dissolved, the solution should not be refrigerated since cooling to 4 °C can result in precipitation. Each dose should be prepared by an individual who is different from the one performing the surgery and injury and/or carrying out the primary or secondary outcome assessments, namely, MWM or lesion volume analyses. A specific coding system must be carefully developed and used between the technicians preparing the drug, performing, and carrying out the outcome assessments for each rat in the 4 groups particularly given the use of two injections over 2 days and the assessment of outcomes over 21 days.

### Table 2. Therapy 1, Nicotinamide

#### Key references

<table>
<thead>
<tr>
<th>Drug: Nicotinamide</th>
<th>Dose</th>
<th>Model/Species</th>
<th>Outcomes</th>
<th>Function</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study/Author</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Hoane et al, 2006</td>
<td>50 or 500 mg/kg/IP Given at 15 min and 24 h</td>
<td>FPI/Rat</td>
<td>Lesion volume reduced by both doses</td>
<td>MWM improved by 500 mg/kg</td>
<td></td>
</tr>
<tr>
<td>(2) Hoane et al, 2008</td>
<td>50 mg/kg/IP; At 15 min, 4 h, or 8 h post injury followed by 5 subsequent doses of 50 mg/kg IV at 24h intervals</td>
<td>CCI/Rat</td>
<td>Lesion volume improved by 15 min initial dose</td>
<td>MWM improved most by 15 min dose Tactile/Placing improved by all doses</td>
<td>Drug levels were monitored</td>
</tr>
<tr>
<td>(3) Holland et al, 2008</td>
<td>50 or 500 mg/kg/IP Given at 15 min and 20 h</td>
<td>FPI/Rat</td>
<td>Reduction in cortical tissue loss and FJ expression; Slightly better effect at 500 mg/kg dose</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>(4) Spector</td>
<td>C14 niainamide</td>
<td>Rabbit</td>
<td>None</td>
<td>None</td>
<td>Rapid transit into CSF after IV administration</td>
</tr>
<tr>
<td>(5) Sakakibara et al, 2002</td>
<td>IV dosing of 20-750 mg/kg at 2 h effective in rat MCAO model</td>
<td>MCAO/Male Rat</td>
<td>Marked reductions in infarct volume</td>
<td>None</td>
<td>Tested across numerous strains; Fischer, SHR, diabetic</td>
</tr>
<tr>
<td>(6) Sakakibara et al, 2000</td>
<td>IV dosing of 500 mg/kg</td>
<td>MCAO/Female Rat</td>
<td>Marked reductions in infarct volume</td>
<td>None</td>
<td>Effective in both female Sprague Dawley and Wister rats</td>
</tr>
<tr>
<td>(7) Bernier et al, 1998</td>
<td>Oral dosing of 6 grams</td>
<td>Human</td>
<td>None</td>
<td>None</td>
<td>Half-life of 9.3 h with a range of between 4.2 and 26.8 h</td>
</tr>
</tbody>
</table>
Therapy 2: Erythropoietin

Review of the experimental TBI literature suggests that erythropoietin (EPO) is one of the most promising future therapies available. A search of PubMed revealed a remarkable 24 studies all showing efficacy of EPO in rodent models of TBI (Table 3). A single center clinical trial of EPO in severe TBI is ongoing at the Baylor College of Medicine (Claudia Robertson, MD, PI), and Dr. Kochanek has discussed this treatment possibility for OBTT with Dr. Robertson. A pleiotropic cytokine involved in erythropoiesis, EPO has a number of beneficial effects that could be important in TBI such as attenuation of glutamate and NO toxicity, anti-apoptotic, antioxidant, and anti-inflammatory effects, stimulation of neurogenesis and angiogenesis, protection of mitochondria and beneficial effects across many CNS insults such as global and focal ischemia, kainite toxicity, and intracranial hemorrhage (8-32). The exact mechanism of benefit is unclear. Although classical EPO receptors are seen in many cell types in the CNS, these receptors are up-regulated by hypoxia (30), and EPO receptor null mice have a worse outcome than wt after CCI (18), EPO receptors surprisingly do not appear to be needed to mediate the benefit of exogenously administered EPO therapy (19). In the aforementioned 24 studies on TBI, work has included papers in both rats and mice and across models including CCI, FPI, impact acceleration, focal closed head injury, Feeney weight drop, and combined injury (8-31) (Table 3). Studies in large animal models of TBI, however, were not identified.

Route of administration, dosing and therapeutic window appear to be tantalizingly favorable. The studies outlined in Table 3 have suggested that ANY parenteral route of administration shows efficacy including IV, IP or SQ—without obvious differences in this regard. A dose of 5000 IU per kg appear to be best, with doses of 1000 and 3000 also showing efficacy. Reports testing the higher doses were not identified. The therapeutic window is controversial, with some studies suggesting benefit with first dose as late as 24h (9). However, the most comprehensive study of time window examined 5 min, 3h, 6h, 9h, and 12h dosing and identified 6h as the latest time point for successful initial dosing. Studies have shown benefit from a single dose, two doses, three doses, of daily treatment for 14d (8-31). The initial report of efficacy with EPO in TBI used dosing a 1h and 24h and showed benefit across many outcomes (8). The most detailed study of single vs multiple dosing showed that 3 daily doses were better than a single dose (28). Of note, all of these studies used 5000IU/kg as the dose.

There are some special caveats with regard to EPO therapy in TBI. A concern for potential use of this agent in stroke has resulted from the fact that it increases hematocrit (HCT) and increased mortality (37) in clinical testing. Using single dose regimens in rat TBI models, HCT increased from baseline values of ~45% to between 52 and 60% with increases most prominent on d4-14 after administration (15, 19, 27). An elegant study by Zhang et al (19) from Dr. Chopp’s group showed that benefit was independent of HCT by using post-injury isovolemic hemodilution to normalize HCT. This side effect of EPO may not be a major concern in severe TBI or polytrauma, since HCT is typically reduced in patients suffering these conditions, and some level of erythropoiesis could reduce transfusion risk. For mild TBI, this would not be the case and hyperviscosity could be a concern. Recently, carbamylated EPO analogs (CEPO) that have no effect on HCT but show benefit in TBI have been developed (29). CEPO analogs do not bind to the EPO receptor, yet show similar efficacy in CCI (29). Another EPO analog darbepoietin—which has a longer half-life than EPO, has been tested in CCI and shown to be beneficial. Finally, with special relevance to Banyan, there has been a study of the effect of EPO on serum levels of S100B and IL6 assessed between 6h and 7d after TBI in rats—which showed reductions with treatment (25).

The studies identified in Table 3 are focused on work in TBI models with conventional outcomes—specifically to guide the approach in OBTT. For EPO, there are many papers in
other models related to TBI such as hippocampal slices, and intracerebral hemorrhage, among others, that suggest benefit; they are beyond the scope of this manual of operations (32-34). A recent review on potential benefit of EPO in experimental TBI was published and suggests effects via JAK-2 and downstream effects on NFkB, AKT, and ERK and MAPK pathways, resulting in anti-apoptotic effects (35). However, that review suggests involvement of EPO receptor, which based on recent work, may be incorrect. Finally, a review by Nichol and Cooper (36) discusses relevant issues related to EPO and suggests the need for a multicenter RCT—specifically, the EPO study investigators within the ANZICS Clinical Trials group.

For the proposed studies in OBTT, EPO (PROCRIT, Amgen, preservative free) is likely available through your hospital pharmacy. Prices for PROCRIT vary considerably between vendors. Cost at the UPMC Pharmacy in Pittsburgh is approximately $87 per 10,000 IU/mL vial. Thus, it will cost less than $20 per rat for the low dose and $40 per rat at the high dose. The clinical grade preparation contains tiny amounts of albumin and bicarbonate that would not be expected to have any effect in a TBI model. It must be kept refrigerated. If it is not available to you at a similar price, it can be ordered by us and shipped to you for use. Dosing will be 5000 IU/kg IV or 10,000 IU/kg IV administered at 15 min after injury. Thus, 0.5 mL/kg or 1.0 mL/kg of the 10,000 IU/mL solution is given IV for the low and high dose groups respectively. It can be infused over 5 min safely. The vehicle that we propose is sterile NS. As an example, for a 400 gram rat, for each rat in the 10,000 U/kg high dose group, 0.4 mL of PROCRIT solution (i.e., 1 mL/kg) would be given IV over 5 min. Vehicle treated rats will also receive an equal volume of sterile NS. We will have a single vehicle group using the higher dose volume (i.e., 1 mL/kg). We have experience with the low dose and have used it in preliminary studies in our mouse model of CCI plus hemorrhage, and mice tolerate 5000 IU/kg with no major change in MAP (a modest increase) when it is given the drug during hemorrhagic shock with a MAP of 25-20 mmHg—as tested in our combined injury model. We would suggest using a new vial of PROCRIT each day since it is preservative free.

Thus, for EPO treatment:

Treatments:

PROCRIT—10,000IU/mL vial;

Or

Sterile Normal Saline

Groups

1. Sham (surgery but no treatment)
2. CCI plus Sterile Normal Saline at 1.0 mL/kg given at 15 min after injury
3. CCI plus PROCRIT 0.50 mL/kg (Low dose; equates to 5000 IU/kg) given at 15 min after injury
4. CCI plus PROCRIT 1.00 mL/kg (high dose; equates to 10,000 IU/kg) given at 15 min after injury

Please remember that blood sampling and outcomes must remain identical to the studies carried out with Drug #1, as defined in the modeling and injury protocol.
<table>
<thead>
<tr>
<th>Study/Author</th>
<th>Drug/Method</th>
<th>Dose</th>
<th>Model/Species</th>
<th>Outcomes</th>
<th>Function</th>
<th>Other Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/Yatsiv et al 2005 (Shohami group)</td>
<td>5000 IU/kg IP in 1 mL of sterile PBS at 1h and 24h vs PBS</td>
<td>Shohami weight drop closed TBI with unilateral insult in mice and rats</td>
<td>TUNEL markedly reduced, axonal injury-Bielochowski reduced, C3, CD11b, and GFAP reduced</td>
<td>Neuroscore improved from d 3-14; Novel object recog improved on d 3</td>
<td>Focus on late treatment; Limited number of outcomes</td>
<td></td>
</tr>
<tr>
<td>9/Lu et al 2005 (Chopp group)</td>
<td>5000 IU/kg IP at 24h and then daily for 14d</td>
<td>CCI in rat; chloral hydrate anesthesia</td>
<td>Increased BRDU labeling in ipsi DG</td>
<td>Improved probe trial on d 14</td>
<td>Also improved LDF &amp; NO (NO electrode) with EPO treatment; Supports 6 h window</td>
<td></td>
</tr>
<tr>
<td>10/Cherian et al 2006 (Robertson group)</td>
<td>5000 IU/kg SC vs NS</td>
<td>Thermal window for single dose at 3min, 3h, 6h, 9h, 12h</td>
<td>CCI in rat</td>
<td>Histo at 2 wks CA1 and contusion volume improved with Rx from Smin-6h. No effect on CA3</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>11/Chen et al 2007</td>
<td>5000 IU/RH in 4 mL/kg saline IP at 1h 24h, 48h and 72h</td>
<td>Male rat focal wt drop—Feeney; pentobarb anesth</td>
<td>NFkB, ICAM-1, TUNEL, Evan blue, water content all attenuated at 3 days</td>
<td>NONE</td>
<td>Unclear when d3 dose was given relative to d3 sacrifice; Modest effects of 10-20%</td>
<td></td>
</tr>
<tr>
<td>12/Ozturk et al 2007</td>
<td>5000IU/kg IP +/- propofol 100mg/kg IP at 10 min</td>
<td>Variant of impact accel in ether anesth female Wistar rats</td>
<td>At 24h EPO reduced CO, MDA and NO levels—no additive effect with propofol</td>
<td>NONE</td>
<td>Restricted to only markers of oxidative stress</td>
<td></td>
</tr>
<tr>
<td>13/Ozisik et al 2007</td>
<td>1000IU/kg RH at 5 min IP</td>
<td>Wistar rats anesth with ketamine + xylazine; Feeney type wt drop</td>
<td>At 24h EPO reduced histo damage score on EM along with increase in BCL2</td>
<td>NONE</td>
<td>Very restricted outcomes; no effect on TBARS</td>
<td></td>
</tr>
<tr>
<td>14/Verdonc et al 2007</td>
<td>5000IU/kg RH at 30 min—IV in 0.5 mL saline</td>
<td>Male Wistar rats; TBI by impact acceleration</td>
<td>MRI assessments over 6h showed improved ADC and T1 and %BW by EPO</td>
<td>NONE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/Xiong et al 2008 (Chopp group)</td>
<td>5000IU/kg rh at 6h, 3d, and 7d IP</td>
<td>Male C57 mice; CCI</td>
<td>50% reduction in lesion volume at 35d; Inc BRDU and neuron counts in DG</td>
<td>EPO improved probe trial and foot faults</td>
<td>HCT increased from 45 to 55% at d 7 &amp; 14</td>
<td></td>
</tr>
<tr>
<td>16/Hartley et al 2008</td>
<td>5000IU/kg RH IP at 30 min</td>
<td>Sprague Dawley rats FPI</td>
<td>Reduced lesion volume at 10h</td>
<td>NONE</td>
<td>Microdialysis glucose, lactate and pyruvate improved for 10h</td>
<td></td>
</tr>
<tr>
<td>17/Lieutaud et al 2008</td>
<td>1000, 3000 or 5000IU/kg given IV or IP</td>
<td>Male Sprague Dawley rats FPI</td>
<td>IL-1B and MIP-2 concentrations reduced</td>
<td>NONE</td>
<td>Brain conc at 4, 8, 12, or 24h; Dose response seen; 5000 IV gave highest brain conc</td>
<td></td>
</tr>
<tr>
<td>18/Xiong et al 2008 (Chopp group)</td>
<td>NONE</td>
<td>Adult female C57 mice null for CNS EPO receptor; CCI model- chloral hydrate anesth</td>
<td>EPO null mice surprisingly did not No exacerbation of lesion volume or cell counts in null vs wt; less neurogenesis</td>
<td>EPO null mice surprisingly did MWM in EPO null not worse than wt; worse motor funct</td>
<td>EPO receptor responsible for only part of recovery benefit via EPO; No baseline effects</td>
<td></td>
</tr>
<tr>
<td>19/Zhang et al</td>
<td>5000IU/kg RH</td>
<td>Male Wistar rat</td>
<td>Hemodilution did not</td>
<td>Hemodilution did</td>
<td>HCT to 60 in</td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Group</td>
<td>Intervention</td>
<td>Outcome Measures</td>
<td>Conclusion</td>
<td></td>
<td></td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Chopp group</td>
<td>IP d1, d2 and d3; +/- isovolemic hemodilution</td>
<td>CCI; chloral hydrate anesthesia</td>
<td>not alter benefit of EPO on cell loss in DG or CA3 or BDRU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Xiong et al</td>
<td>1000, 3000, or 5000 IU at 30 min-6h after CCI IP</td>
<td>CCI in Sprague Dawley rats</td>
<td>Mitochondrial function improved at all doses; benefit to 7d by 2000 or 5000;6h window</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Xiong et al</td>
<td>5000 IU/kg IP 6h, 3d and 7d</td>
<td>CCI in EPO null and wt mice—rescued with EPO expression in hematopoietic tissue</td>
<td>Better effect of EPO on functional outcome in wt than null</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Zhu et al</td>
<td>5000 IU/kg IP at 30 min</td>
<td>Sprague Dawley rat modified Feeney model</td>
<td>Functional outcome figs may be mislabeled; EPOR may not mediate EPO effect in TBI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Valable et al</td>
<td>5000 IU/kg IV at 30 min</td>
<td>Male Wistar Rat Impact acceleration</td>
<td>Also showed reduced zinc accumulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Liao et al</td>
<td>5000 IU/kg IP daily for 7d</td>
<td>Male Wistar rats Feeney wt drop</td>
<td>Did not indicate time of first dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Bian et al</td>
<td>1000, 3000, or 5000 IU/kg, or citocoline or NS IP immediately after TBI</td>
<td>Male Wistar rats Feeney wt drop</td>
<td>Unusual paper shows decrease of serum biomarkers by EPO—relevant to Banyan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Zhang et al</td>
<td>5000 IU/kg IP on d1, 2 and 3 after CCI</td>
<td>Male Wistar rat CCI; chloral hydrate anesthesia</td>
<td>EPO Improved foot faults &amp; neuroscore; Correlation with CST crossings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Chauhan and Gatto</td>
<td>5000 IU/kg IP at 6h, 3 and 7d after CCI; Simvastatin 2 mg/kg in feeds</td>
<td>Male C57 mice; CCI; ketamine and xylazine anesthesia</td>
<td>HCT to 52 with EPO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Xiong et al</td>
<td>5000 IU/KG IP at d1 vs d1,2 and 3</td>
<td>Male Wistar rat CCI;</td>
<td>Day 35 histo was improved in both doses but the 3 dose best</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Xiong et al</td>
<td>Carbamylated EPO (CEP01)/50 μg/kg IP at either 6h or 6, 24 and 48h</td>
<td>CCI in rat</td>
<td>Functional outcome improved in both doses but 3 dose best</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Cherian et al</td>
<td>Darbepoetin alfa (darbEPO) 2.5, 5, 10, 25 or 50 μg/kg SQ at 5 min after</td>
<td>CCI in Long Evans rat</td>
<td>Modified EPO that does not affect HCT which is also the case for novel EPO peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Claudia Robertson Group</td>
<td>DarbEPO Closely related to EPO bit has a longer half-life; does increase HCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The most relevant studies to OBTT are highlighted in gray background; DG = dentate gyrus; SC = subcutaneous

**Biomarker sampling processing**

Blood sampling will be carried out as described above. For the early time points, 0.7 mL will be obtained as described above. The final time point at sacrifice will include sampling of 2-3 mL of blood obtained from the left cardiac ventricle via a 20-gauge needle. Blood will be placed immediately in microcentrifuge tubes (1.5 mL Eppendorf brand, colorless) and allowed to clot at room temperature for 60 min. Tubes will be centrifuged at 5,000xg at room temperature for 5 min. The serum is collected into 1.5 mL microcentrifuge tubes or cryotubes (screw cap-type preferred) with “Tough-tag label” (freezer proof) – use with permanent fine black marker for label writing. Serum samples after labeling will be snap frozen on dry ice and stored at -80°C in waxed cardboard freezer boxes (#C5520)/81 cell divider (#CD81) [http://www.crystalgen.com/wax.php](http://www.crystalgen.com/wax.php) until used or shipped. Each sample will have a code number for the specific rat followed by a -4 h, -24 h, or final (-F) designation. Also note that any sampling for biomarkers that coincides temporally with drug dosing should be done prior to drug administration. For example, if a drug dose is scheduled for 24 h, obtain the biomarker sample first and then administer the drug. Samples will be shipped (FedEx overnight on Monday or Tuesday) in large Styrofoam boxes (taped) with extra dry ice that can last 3 days (~4-6 kg). Prior to shipping please notify Banyan and provide tracking number.

**Shipping contact:**

Ms. Olena Glushakova, 386-518-6762; oglushakova@banyanbio.com
Backup: Danny Johnson, 386-518-6763; djohnson@banyanbio.com

**References**

36. Nichol AD, Cooper DJ. POLAR Study Investigators on behalf of the ANZICS-Clinical Trials Group; EPO Study Investigators on behalf of the ANZICS-Clinical Trials Group. Can we improve neurological outcomes in severe traumatic brain injury? Something old

A Novel Multicenter Preclinical Drug Screening and Biomarker Consortium for Experimental Traumatic Brain Injury: Operation Brain Trauma Therapy

Patrick M. Kochanek, MD, FCCM, Helen Bramlett, PhD, W. Dalton Dietrich, PhD, C. Edward Dixon, PhD, Ronald L. Hayes, PhD, John Povlishock, PhD, Frank C. Tortella, PhD, and Kevin K. W. Wang, PhD

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality in Operation Iraqi Freedom largely due to the emergence of blast-injury from attacks with improvised explosive devices (IEDs) along with continued importance of ballistic injury.\(^1,2\) The pathology resulting from these insults is complex, spans the spectrum from mild-to-severe TBI and is often complicated by polytrauma, hemorrhagic shock (HS), and burns.\(^3\) Current therapy of severe TBI includes supportive care, but brain-oriented therapy is limited to approaches targeting intracranial pressure (ICP) such as mannitol or surgical decompression.\(^4\) In blast-induced TBI, vasospasm, neuronal death, cognitive disability, and axonal injury are key targets. There is also no current therapy for mild TBI, which represents a source of morbidity and may be linked to posttraumatic stress disorder (PTSD).

Research to date in the field of TBI has focused on the study of pathomechanisms. This National Institutes of Health–driven approach has helped to identify and characterize many mechanisms of secondary damage. However, focus on mechanism has not led to the rapid advancement of new therapies to the bedside. This approach also has not encouraged cross-talk between laboratories and has unfortunately failed to build consensus in the field regarding the efficacy of new therapies and move them forward to clinical trials. A recent search of the terms “TBI” and “therapy” on PubMed produced over 21,000 citations including many positive results. Yet, few therapies have been tested in Phase III clinical trials and no new therapy for TBI has emerged.

As with the need for new therapies, there is a parallel need for the development of serum biomarkers of brain injury. Research has identified several potential biomarkers through clinical studies and limited work in TBI models.\(^5\)–\(^7\) The performance of biomarkers of brain injury across the key contemporary TBI models, species, injury levels, and secondary insults such as hypoxemia or HS; however, has not been systematically evaluated. Studies of the ability of serum biomarkers to confirm neuroprotection are also lacking, and a comparison of the effect of therapies on conventional outcomes (function/neuropathology) versus serum biomarker levels remains to be carried out. Given that specific serum biomarkers have been developed to identify specific aspects of brain injury such as neuronal death, axonal injury, or glial injury, there is the potential to better understand the effect of therapies on these various cellular components. Such studies could advance the potential utility of biomarkers in both their translation to clinical applications and utility in drug screening.

This review article discusses a consortium called operation brain trauma therapy (OBTT) that was recently established in attempt to address both the need for novel therapies and biomarkers in TBI. OBTT was designed to serve as a high-throughput therapy screening research consortium that identifies the most promising therapies and compares them across a spectrum of the state-of-the-art models and injury levels. The most promising therapies will be moved up the phylogenetic scale to a large animal model and ultimately to clinical trials.

WHY HAVE THERAPIES IN EXPERIMENTAL TBI FAILED TO TRANSLATE TO CLINICAL EFFICACY?

A key question in designing a research consortium to evaluate new therapies for TBI is “why have therapies failed to translate from the lab to the clinical in TBI?” Many reasons have been suggested to explain this failure, ranging from the...
complexity of the disease, difficulties in stratifying and evaluating outcome in humans with TBI, lack of knowledge of brain pharmacodynamics for new therapies, the fact that patients with TBI are already treated with various drugs and interventions that are not incorporated into preclinical studies, and the fact that the timing of drug administration in pre-clinical models is often chosen for proof-of-concept and maximal effect rather than clinical relevance. A common criticism of pre-clinical work in TBI has been the lack of a "proven definitive model" that has demonstrated success in translating a therapy from the bench to the bedside. However, it is being increasingly recognized that TBI, particularly severe TBI, is an extremely complex and heterogeneous disease. The importance of heterogeneity in TBI was highlighted in a recent review by Saatman et al. who have suggested the need to consider the myriad forms of TBI such as contusion, diffuse axonal injury, diffuse swelling, and subdural hemorrhage, and their combinations, as individual conditions, that may require individualized therapy including stratification in clinical trials. This suggests that optimized preclinical therapeutic screening will require the use of multiple models. This concept represents a key element of the approach that was developed for OBTT.

COMPONENTS OF OBTT

Figure 1 outlines the components of the consortium including the institutions, principal investigators (PIs), and models that will be used. Therapies will be screened first in rodents across a spectrum of established TBI models in two species, mice and rats, using clinically relevant paradigms. Outcomes in the primary screening models will include lesion volume, neuronal death, and axonal injury along with motor and cognitive outcomes (including Morris water maze performance). Drugs that show benefit in the first phase of primary screening in established TBI models will move to testing in more complex TBI models, namely, those with superimposed secondary insults such as HS or an inflammatory insult (IL-1β infusion). Therapies showing benefit in these screening models will also be studied across injury levels from mild to severe and will also be evaluated for effects on brain edema and cerebral blood flow (CBF) after TBI. The most promising therapies will be evaluated in a large animal TBI model—specifically, midline fluid percussion injury (FPI) in micropigs. Outcomes in the micropig model will include assessments for axonal injury, cerebrovascular dysfunction, and ICP—outcomes that have been shown to be highly relevant to blast injury, where axonal injury, vasospasm, and malignant brain swelling have been recently described. The time line for therapy development in this multiterior approach includes screening of each drug in the primary models (controlled cortical impact (CCI), parasagittal FPI, and penetrating ballistic-like brain injury) in rats over a 2- to 3-month period at each center, secondary evaluation of promising agents in the more complex rodent models such as TBI plus hemorrhage over a 6-month period, and finally, advancement of the single most promising agent tested each year to the micropig TBI model. Serum biomarker assessments will also be incorporated into the study designed.

Figure 1. Administrative structure of operation brain trauma center (OBTT) including overview of the screening centers, biomarker core, Therapy and Translational Oversight Committee, and path to clinical trials. Therapies were identified by an initial literature search at the time of grant preparation along with a monthly formal search of the literature by the PI. Additional potential agents for testing are also suggested by the site PIs and members of the Translational Oversight Committee on monthly conference calls and in response to the annual report, respectively. Each agent tested is determined by a vote of the site PIs—which must be unanimous. Route of administration and dosing is then addressed in collaboration with input from faculty at the University of Pittsburgh School of Pharmacy. In addition, the overall PI contacts communicating authors of key manuscripts for the drugs to be tested to identify any potential issues or points not clear to the consortium investigators based on review of the published literature. Primary screening is carried out using multiple established models in both rats and mice, at three centers, the Miami Project to Cure Paralysis University of Miami Miller School of Medicine (W. Dalton Dietrich, PhD, site PI), the Department of Applied Neurobiology at the Walter Reed Army Institute of Research (WRAIR) (Frank Tortella, PhD, site PI), and the Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine (Patrick Kochanek, M.D., overall PI). The parasagittal FPI, CCI plus HS, and penetrating ballistic-like brain injury (PBI) models will be used for primary screening at the Miami Project, the Safar Center, and WRAIR, respectively, with behavioral and histopathological (lesion volume, neuronal and axonal injury assessments) end points. For each agent that is evaluated, drug administration and outcome testing are carried out in a blinded fashion. The results from each center are not revealed until all of the centers have completed their evaluations. Promising drugs will undergo additional more advanced secondary screening in the parasagittal FPI injury model with secondary insult simulating polytrauma (IL-1β infusion), CCI plus HS, and PBI with electrophysiological assessments. Quantification of serum biomarkers in these models is superimposed upon these standard outcome assessments using a rigorous protocol for blood sampling and sample processing. The most promising agents across screening models will be evaluated in secondary screening in an established micropig model of lateral FPI at the Commonwealth Center for the Study of Brain Injury at Virginia Commonwealth University (John Pavlishock, PhD, site PI). Biomarker testing of serum samples will be carried out at Banyan Biomarkers (please see text for details).
with serial assessments layered upon the therapy screening (for biomarker assays to be used, Table 1). OBTT will then deliver agents that are either U.S. Food and Drug Administration (FDA) approved (for other uses) or in clinical development that are found to be effective across models for rapid clinical translation to the Defense Advanced Projects Agency Prevent Blast program and other investigative teams working with blast TBI models, and the clinical TBI and PTSD consortia that have been established by the US Army. OBTT will, for the first time, allow a direct comparison of therapies across TBI models in multiple centers. It will also include comparison of therapies across highly relevant combat casualty care scenarios (TBI plus polytrauma).

OBTT includes five internationally recognized centers: (1) the Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine (Patrick Kochanek, MD; overall PI); (2) the Miami Project to Cure Paralysis, University of Miami School of Medicine (W. Dalton Dietrich, PhD; site PI); (3) the Neuroprotection program at Walter Reed Army Institute of Research (Frank Tortella, PhD; site PI); (4) Virginia Commonwealth University (John Povlishock, PhD; site PI); and (5) Banyan Biomarkers Inc. (biomarker core directed by Ronald Hayes, PhD).

**APPROACH TO DRUG SELECTION IN OBTT**

In OBTT, we will consider testing therapies that could be implemented at any point in the continuum of care for the TBI victim in combat casualty care (Fig. 2). However, the majority of the expertise of the screening centers, along with the experimental TBI field as a whole, is in the setting of acute neuroprotection. Severe TBI produces direct parenchymal disruption (primary injury) and sets into motion many secondary injury processes (Fig. 3) including disturbances in CBF resulting in a cascade of mechanisms related to ischemia, excitation, oxidative stress, mitochondrial failure, proteolysis, and disturbances in cell signaling, among other mechanisms, triggering neuronal death cascades from necrosis, apoptosis, and autophagy.\(^{17}\) Cascades contributing to brain swelling are also produced and can result in intracranial hypertension.\(^{17}\) Axonal, dendritic, and synaptic damage also occur\(^{18}\) as does inflammation, which may either contribute to secondary injury or signal the regenerative response.\(^{17,19,20}\)

**TABLE 1. Proposed Biomarkers to Be Used for Operation Brain Trauma Therapy**

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBDP150/SBDP145</td>
<td>Axonal injury: neural necrosis</td>
<td>94–96</td>
</tr>
<tr>
<td>SBDP120</td>
<td>Axonal injury: neural apoptosis</td>
<td>94–96</td>
</tr>
<tr>
<td>UCH-L1</td>
<td>Neuronal cell body injury</td>
<td>96–98</td>
</tr>
<tr>
<td>MAP2</td>
<td>Dendritic injury</td>
<td>99, 100; Wang et al. unpublished</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glialosis</td>
<td>98</td>
</tr>
<tr>
<td>EMAP-II/IL-6</td>
<td>Microgliosis</td>
<td>101</td>
</tr>
</tbody>
</table>

Figure 2. Schematic of the continuum of care for severe TBI in combat casualty care. In OBTT, potential therapies that could be translated to clinical care are considered for use at any point along this continuum. CASH, combat advanced support hospital; OR, operating room; ICU, intensive care unit; Rehab, rehabilitation facility.

Figure 3. Schematic outlining the mechanisms involved in the evolution of secondary damage after TBI (see text for details). Primary injury (green), CBF disturbances (purple), excitation and oxidative stress (gray), cell death cascades (black), brain swelling (blue), axonal, synaptic and dendritic damage (red), and inflammation (yellow) occur simultaneously and interact to exacerbate damage and initiate repair. The secondary injury mechanisms culminate in neuropathological damage and/or behavioral deficits. Many of these important secondary injury mechanisms and outcome targets will be assessed in the various models used in the consortium. See text for details. BBB, blood-brain barrier; CBV, cerebral blood volume; EAA, excitatory amino acid; \(O_2^-\), superoxide anion; AA, arachidonic acid; PKC, protein kinase C; ER, endoplasmic reticulum.
Two key therapeutic targets have served as the primary endpoints in testing of therapies in experimental TBI, namely some aspect of neuropathology (contusion volume, cortical lesion volume, neuronal death, or axonal damage), and functional outcome (neuroscore, motor testing, cognitive testing). Review of the experimental TBI literature identifies a number of therapies that have favorably affected these two outcome categories in individual laboratories.21–74 Many of these therapies reduce lesion volume by at least 20%, and some as much as 60% in experimental TBI. Various therapies targeting mitochondria and neuro-inflammation have shown robust effects in various individual laboratories.27–32,39,40,72,74 In contrast, therapies targeting neurotransmitter systems have generally shown the greatest effects on cognitive outcome reviewed in ref. 75. Nevertheless, for just these two targets, neuropathology and functional outcome, there are a number of promising acute therapy candidates.

PROMISING THERAPEUTIC STRATEGIES FOR TRANSLATION IN TBI

Two categories of drugs have been identified for screening in OBTT. These include (1) “low hanging fruit” representing agents that are FDA approved for other uses and/or otherwise readily available that have shown promise in experimental TBI in multiple published reports and (2) novel but potentially high impact therapies that have a more limited publication track record. A number of agents in each of these categories are provided below, based on literature review and suggestions of the site PIs and overall PI of the consortium.21–74 In addition (Table 2), these drugs are also classified with regard to their putative primary mechanistic targets. Each of these targets, if appropriately addressed, has potential to reduce secondary damage and improve functional outcome.

We anticipate that a number of these therapies will be evaluated by the consortium; however, the specific drugs to be tested and the sequence of testing are currently being debated by the consortium investigators. An oversight committee will also evaluate and contribute recommendations and review results annually. This list does not, in any way, reflect a complete menu of potential agents for evaluation in OBTT, rather it reflects selected promising therapies across a number of categories. The following brief discussions of these therapies provide insight into the basic rationale for therapy selection by the consortium investigators.

POTENTIAL “LOW HANGING FRUIT” THERAPIES FOR TBI TRANSLATION

Nicotinamide

Vitamin B3 has shown dramatic beneficial effects on all aspects of outcome evaluated including function, neuropathology, and blood-brain barrier damage, with several positive reports in TBI, including CCI and FPI.21,22 Most of the reports showing benefit of nicotinamide in TBI are from a single laboratory. Nicotinamide has been shown to attenuate two mechanisms that are important in TBI, including poly-ADP-ribose polymerase activation (resulting in acute energy failure) and inflammation. Doses of 50 to 500 mg/kg have shown efficacy and with a promising 4-hour time window. Nicotinamide is commercially available as vitamin B3. It represents an example of an agent that could readily move forward if found to show benefit across models and could also
be used as a nutritional supplement in a pre-treatment approach particularly in light of the ability to provide dietary neuroprotective additives in theater.

Choline

Chronic pre- and posttreatment with the nutritional supplement choline may offer substantial benefit for TBI. Rats fed a diet supplemented with 2% choline exhibited improved functional outcome, reduced contusion volume, and reduced neuro-inflammation at 2 weeks after injury.23 Chronically after TBI there is a well-recognized reduction in high affinity choline uptake sites; thus, choline may represent a prototype agent for both chronic pre-treatment to attenuate neuroinflammation, and as rehabilitation therapy to serve in neurotransmitter replacement. Cytidine diphosphate-choline, in a posttreatment approach, is in clinical trials.

Atorvastatin

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, also referred to as “statins,” not only reduce serum cholesterol but also have potent inhibitory effects on neuroinflammation and possible effects on CBF and trophic factor production. They confer benefit in experimental TBI.24-25 Atorvastatin, simvastatin, and lovastatin all show promise after TBI in rats. Impressively benefit was seen with atorvastatin therapy by Wang et al.24 where improved performance on rotorod and Morris water maze, reduced hippocampal neuronal death, and attenuated microglial proliferation and cytokine production in the brain were seen after TBI. Equal doses (20 mg/kg) of atorvastatin outperformed simvastatin. Atorvastatin is FDA approved and a logical candidate to study in our consortium. Either pre- or posttreatment paradigms could be evaluated.

FK506

There have been several reports of beneficial effects of the FK 506 (Tacrolimus) in experimental TBI.26,27 It is an immunophilin ligand that inhibits the protein phosphatase calcineurin. Benefit from this agent has been shown mostly in models focusing on axonal injury—which could be a special relevance to blast TBI.26,27 Immunomodulatory effects of this agent could also contribute to potential benefit after TBI.28 Another factor that raises the interest in cross model evaluation of this agent is that it may have fewer propensities to initiate seizure activity than another promising calcineurin antagonist cyclosporine A28—which is currently in clinical trials for TBI. However, unlike cyclosporine A, FK 506 does not appear to inhibit mitochondrial permeability transition. FK 506 is an FDA-approved immunosuppressant. Because the related agent, cyclosporine A, is already in clinical trials for TBI it would be logical to explore this agent in our consortium.

Aniracetam

This agent is an allosteric potentiator of AMPA-specific glutamate receptors and has shown promise in improving cognitive outcome across central nervous system (CNS) injury models including ischemia and TBI.42-44 Transmitter supplementation (norepinephrine and dopamine) is often used in TBI rehabilitation, but a similar strategy has not been developed for glutamatergic neurotransmission. AMPA receptor desensitization is seen chronically after TBI.42 Thus, aniracetam may serve that purpose. It also enhances glucose availability and acetylcholine synthesis, pathways that are similarly disturbed in TBI.42 It has an exceptionally broad therapeutic window being equally effective whether started at 24 hours or 11 days after injury in rats.42 Daily oral doses of 25 to 50 mg/kg were used. It will thus serve as a prototype for potential use as a rehabilitation therapy that is focused on functional rather than neuropathological outcomes. It also has shown benefit in models of anxiety and insomnia.45,46 both of cerebral hemorrhage, and other models.30-32 Minocycline has shown benefit after TBI in mice,32 where it also reduced IL-1β levels in brain. A dose of 45 mg/kg at 30 minutes after TBI, and continued every 12 hours for 3 days decreased lesion volume. A therapeutic window of 2.5 hours has been reported.30 Minocycline is FDA approved for other uses, and available for clinical trials. It is a prototype multifaceted anti-inflammatory candidate that could be rapidly translated into clinical trials. Its antimicrobial effects could also be of benefit in polytrauma.

Lithium

Lithium treatment down-regulates pro-apoptotic mechanisms33,34 and upregulates cell survival factors and markers of plasticity.35,36 It also improves synaptic plasticity, as measured by enhanced long-term potentiation in the hippocampus37 and spatial memory and retention in a T-maze paradigm.38 As evidenced by recent publications on lithium,37,38 there is renewed interest in its therapeutic potential. Thus, lithium has multiple targets that may favorably influence both acute and chronic TBI pathophysiology. This, together with the fact that lithium is approved for human use as a treatment for bipolar disorder, makes it an excellent translational research candidate for the treatment of TBI-induced cognitive and affective dysfunction.

Rolipram

The type IV phosphodiesterase (PDE) inhibitor rolipram has shown benefit in experimental TBI and spinal cord injury.39,40 It blocks PDE-IV-mediated breakdown of cAMP resulting in increased PKA activation, enhancing cell survival pathways, and inhibiting pro-inflammatory NFκb activation.39 Rolipram and other PDE IV and V inhibitors have also shown promise in reducing memory impairment in dementia and improving CBF and are in clinical trials in dementia.31 Although nausea and vomiting can be limiting with the use of rolipram in conscious patients, if beneficial, second generation type IV PDE inhibitors, with better side effect profiles, are in clinical trials for lung injury.
which are behavioral sequelae of TBI. It is available as an
over-the-counter nutritional supplement.

**Pentostatin**

Adenosine is an endogenous neuroprotectant with CBF
promoting, anti-excitotoxic and anti-inflammatory properties. Local injection of the adenosine-2A (A2a) receptor agonist
CGS 21680 increases CBF after CCI in rats.47 Adenosine A1 receptor activation confers anti-excitotoxic effects and A1 receptor knock-out mice develop lethal status epilepticus
after CCI.48 Adenosine effects at A2a and A3 receptors may
also be anti-inflammatory.49,50 Thus, adenosine augmentation could be useful in TBI. A limitation of systemic use of
adenosine agonists (i.e., acadesine, ATL-146e) is hypoten-
sion. An alternative strategy is to enhance or sustain local
increases in adenosine level in brain, where they occur after
injury. An alternative strategy is to enhance or sustain local
increases in adenosine level in brain, where they occur after
injury. Administration of the adenosine deaminase inhibitor
EHNA increased brain adenosine levels after TBI.51 The
adenosine deaminase inhibitor pentostatin, which is 10 times
more potent than EHNA52 is effective in many ischemia
models, and at low doses (0.2–2.0 mg/kg).54,55 It has not been
tested in experimental TBI but is FDA approved and used in
cancer therapy. It could have multiple benefits including
reversal of vasospasm, anti-inflammatory actions, and anti-
excitotoxic effects. Each of these mechanisms is felt to be
important in blast and penetrating TBI.

**Progesterone**

A large body of research from several laboratories sup-
ports the putative beneficial effects of progesterone in experi-
mental TBI is reviewed in ref. 56. Favorable effects across
diverse mechanisms have been shown including excitotoxicity,
inflammation, and brain swelling, among others.57,58 Two
single-center clinical trials have suggested beneficial effects on
ICP and outcome, and a large multicenter randomized controlled
trial is currently underway.59,60 Progesterone thus represents a
logical agent for evaluation by our consortium which could
provide additional insight into issues such as efficacy across
injury severity and complex secondary insults.

**Docosahexanoic Acid**

Fish oil, or one of its constituents, docosahexanoic acid
(DHA), has recently been shown to confer beneficial effects in
experimental TBI.61–63 Notably, attenuation of axonal injury by DHA has been suggested to represent the major
target of its protective effects. Given the important role of
axonal injury recently shown in blast TBI14,15 and the ability
to provide this agent as a nutritional supplement, DHA given
either as pre- or posttreatment represents a logical candidate
to consider for testing in OBTT.

**POTENTIAL HIGH RISK-HIGH REWARD THERAPIES FOR TBI**

**XJB-5–125**

XJB-5–125 is a nitroxide, with multifaceted effects against
oxidative stress, that is conjugated with a gramicidin S fragment.64 The gramicidin S fragment exhibits high affinity for the inner mitochondrial membrane, greatly increasing
its ability to concentrate in mitochondria, enhancing speci-
cificity. XJB-5–125 protects cells against apoptosis.65 The
mechanism(s) of the nitroxide component’s protective effects
may be associated with its superoxide dismutase mimicking
activity, radical scavenging effects, or its electron acceptor
propensities preventing superoxide generation during dys-
regulated electron transport. These properties suggest a spe-
cial opportunity for XJB-5–125 in TBI.66 XJB-5–125 also
exhibits beneficial systemic effects in HS and may be val-
uable in blast polytrauma. A library of hemigramicidin tempol
conjugates has been developed.64,65

**Anti-ASCab**

Recent work67 suggests a pivotal role of a molecular
platform NALPI1 inflammasome consisting of caspase-1,
caspase-11, and apoptosis-associated speck-like protein con-
taining a caspase-activating recruitment domain (ASC) that is
assembled in neurons subjected to experimental spinal cord
injury. Treatment with an antibody against ASC (Anti-ASC),
either intraperitoneal (IP) or intravenous (IV), produced
pluri-potent anti-inflammatory effects (against IL-1β, IL-18,
and caspase-1) with tissue sparing and functional improve-
ment. Effects on multiple pathways make this an attractive
highly novel strategy.

**Necrostatin-1**

Recent work has identified a novel cell death pathway
called “necroptosis” that involves the Fas/TNF receptor path-
way but exhibits both a necrotic phenotype and autophagy.68 A
subsequent study revealed that this pathway is involved
after TBI in mice subjected to CCI. Improvements in func-
tional outcome, cell permeability, and inflammation were
seen with treatment with necrostatin-1, a specific inhibitor of
necroptosis. Necrostatin-1 is commercially available (Biomol)
and a family of necrostatins has been developed. This new cell
death pathway is a worthy potential therapeutic target for
exploration by our consortium.

**Poloxamer-188**

Recent studies in CNS injury models have shown marked
benefit of surfactant poloxamer-188.69–70 It has multifaceted
effects against apoptosis, necrosis, and cell membrane injury.
It attenuates P38-MAP kinase-mediated apoptosis, blunts
neuro-inflammation, attenuates axonal injury, and exhibits a
unique membrane resealing effect.71 It attenuated lesion vol-
ume after intracerebral hemorrhage in rats.69 It is FDA
approved as an indirect food additive in a variety of products,
has been in clinical trials with IV use in conditions such as
sickle cell disease72 and is commercially available. A limita-
tion of this intriguing agent is that it has shown efficacy in
brain injury only with intracisternal administration; thus, we
have characterized this agent speculative despite FDA ap-
proval for other uses.

**Anti-CD11dab**

Recent studies in FPI have revealed a 50% reduction in
leesion volume in rats treated with an antibody to the alpha
chain CD11d of the integrin heterodimer CD11d/CD18.73 In
addition, a marked reduction in CD68 immuno-positive in-
flammatory cell influx into brain was seen with treatment. It
was effective despite a 30 minutes delay in treatment. It was also shown to be highly effective in experimental spinal cord injury. \(^74\) Unlike more broad-spectrum therapies targeting the B2 integrin family, this approach may offer greater selectivity against infiltrating inflammatory cells, blood-brain barrier injury, and tissue destruction.

**GENERAL APPROACH TO BIOMARKER SCREENING IN OBTT**

Banyan Biomarkers, Inc., has established state-of-the-art capability and expertise in tooling and configuring Good Laboratory Practice-level sandwich ELISA assays for a variety of serum biomarkers including glial fibrillary acid protein, an astrocyte marker, ubiquitin C-terminal hydrolase-L1, a neuronal marker, and α-II spectrin degradation products, among others. \(^6,7,76–78\) Proposed brain injury biomarker assays to be run are outlined in Table 1. Assessment of serial samples across models in OBTT will be used both to compare the biomarker profile produced in each of the models and to probe the ability of these biomarkers to be used to assess therapeutic efficacy. OBTT thus represents a unique opportunity to examine biomarkers across simple and complex models, injury levels, and species in experimental TBI.

**DISCUSSION**

Despite the unique potential of the consortium, there are a number of potential challenges and limitations to the approach that is proposed in OBTT. First, in the 1990s, the National Institutes of Health/National Institute of Neurologic Disorders and Stroke sponsored a multicenter preclinical drug screening consortium in the field of spinal cord injury called Multicenter Animal Spinal Cord Injury Study (MASCIS). \(^79\) The approach made important contributions to modeling and outcomes assessments in experimental spinal cord injury, but did not bring new therapies to clinical trials. We selected our overall consortium design to benefit from the experience of the MASCIS consortium. Specifically, a stumbling block in MASCIS resulted from the plan to have all centers learn and use the same experimental model to test therapies. It became difficult for centers to replicate the benefits of methylprednisolone across the consortium sites. This led to a prolonged period of model development and validation. As previously discussed, we have designed our consortium to use the established models at each site to reflect the heterogeneity in clinical TBI, take advantage of the established track records for each of the models at each site, and limit the many well-recognized challenges in model development and modification.

Second, an important facet of TBI in combat casualty care is repeated injury, particularly repeated mild TBI. \(^13\) Given that few established models of repeated TBI exist, \(^80\) addressing this important issue would require considerable model development which we believe is currently outside the scope of our consortium.

Third, it is often suggested that given the multifaceted nature of TBI, combination therapy will be required. \(^81\) Although results of some studies have challenged this notion, \(^82\) the use of combined therapy with the most promising agents identified in years 4 or 5 could be included in our ultimate consortium plan, our primary goal is to advance individual agents to clinical trials.

Fourth, we recognize that all therapies may not produce a simple linear pathway from primary to secondary screening. An agent may be effective only in mild TBI, or only in the advanced TBI plus HS and polytrauma models. Such an agent would not be dismissed as ineffective; rather, it could suggest the need for clinical testing either in mild TBI or in polytrauma. Similarly, an agent showing benefit only in the FPI models might suggest a predominant effect on axonal injury since that mechanism is highlighted in FPI. Our approach could, thus, produce a paradigm shift in the field of TBI and suggest the need for therapies targeting specific types of injury, rather than across all injuries.

Finally, we recognize that PTSD is an important therapeutic target in combat casualty care that may in some cases be linked to TBI. \(^83\) However, given the expertise of the individual members of the consortium, we are focusing on traditional outcomes that have been developed in experimental TBI. We will, of course, communicate our findings with the most promising agents to laboratories studying PTSD in civilian and blast TBI models.

**CONCLUSION**

We have launched a multicenter preclinical drug and biomarker screening consortium, OBTT, for the field of TBI. This approach is unique and has a specific focus on drug development for TBI in combat casualty care. We believe that as it develops, the findings of this consortium have the potential to provide special insight for field of experimental and clinical TBI.

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