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14. ABSTRACT Operation brain trauma therapy (OBTT) and the related OBTT-extended studies (OBTT-ES) programs represent a multi-center therapy and biomarker screening consortium for the field of traumatic brain injury (TBI). OBTT uses there established models of TBI (Fluid percussion, controlled cortical impact (CCI), and penetrating ballistic-like brain injury) in rats for screening of therapies and evaluation of two biomarkers (glial fibrillary acidic protein [GFAP] and ubiquitin carboxyl-terminal esterase L1 [UCH-L1]) at three different sites (University of Pittsburgh, University of Miami, and WRAIR). Biomarker assessment and interpretation are carried out at Banyan biomarkers, LLC, the University of Florida, and Messina University. OBTT has screened or is currently in screening or protocol optimization on 10 drugs, including nicotinamide, erythropoietin, cyclosporine, simvastatin, levetiracetam, glibenclamide, kollidon VA64, amantadine, and minocycline. Approximately 2000 rats have been studied and >5000 biomarker levels to date. Thus far, of the 8 drugs that have completed testing, levetiracetam has shown significant benefit in two models while glibenclamide has shown benefit in CCI. GFAP has performed well including reproducibility, correlations to outcomes, and theranostic utility.					
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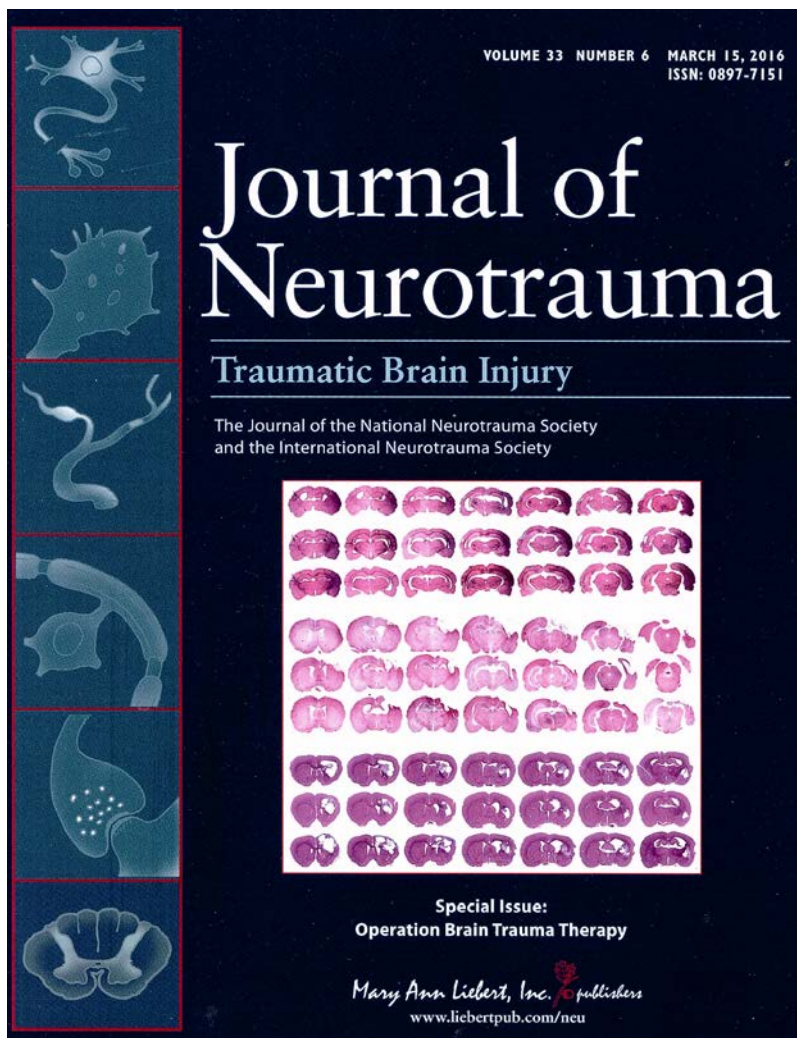
PROGRES REPORT (OBTT and OBTT-ES; Year 5, April 21, 2016)

This represents a supplemental progress report to the report submitted on October 29, 2015 for the OBTT and OBTT-ES grants (WH81XWH-10-1-0623 and WH81XWH-14). The work of the OBTT consortium is supported by both grants and the work is intimately linked. This approach to the progress report recent for OBTT-ES was taken as recommended by our program officer, since the two grants supporting OBTT have different start dates. Thus, this update outlines accomplishments for the OBTT consortium supported by both grants subsequent to the October 2015 report which was listed as a report for only WH81XWH-10-1-0623. The content of that report actually addressed work supported by both grants. The full report from October 29, 2015, also follows, again at the request of the program officer. We hope that this will be sufficient.

Since the last progress report there have been many new reportable outcomes and continued progress on investigations by the OBTT consortium. **First, we are pleased to report that we published on March 15, 2016 a total of 8 manuscripts of our findings (S1-S8), describing the results of the first 5 therapies tested by OBTT in a special issue of the *Journal of Neurotrauma* (See Supplemental Figure 1).**

In the progress report of October 29 (attached), we presented results from 7 therapies (nicotinamide, erythropoietin, cyclosporine, simvastatin, levetiracetam, glibenclamide, and koloidon VA64) tested by OBTT. We have completed studies with two additional therapies, the aquaporin-4 antagonist AER-271 and the putative cognitive enhancing drug amantadine. AER-271 failed to improve outcomes in any of our models.

Amantadine showed benefit in one of our models, namely on behavioral outcomes in the penetrating ballistic-like brain injury (PBBi) model. We are currently testing the anti-inflammatory and anti-apoptotic agent minocycline and are planning studies with two additional therapies. At present, we plan to test 1) the cell death targeting agent P7C3 A20 and are working with the University of Texas Southwestern on the MTA for that agent and 2) the cathepsin/calpain protease antagonist ALP-495 and are working



Supplemental Figure 1. Cover of the March 15 issue of the *Journal of Neurotrauma* which was completely devoted to the work of OBTT. The cover image depicts serial coronal brain sections from rats in each of the 3 therapy screening models used by the OBTT consortium. **A total of 8 manuscripts were published in the issue (S1-S8) along with an INTRODUCTION by COL Rasmussen and Dr. Alicia T. Crowder.** Of note, the special issue generated a very favorable press release by Mary Ann Liebert publishing that was written by Drs. Ross Bullock and David Brody, highly respected investigators in the fields of TBI.

with American Life Science Pharmaceuticals on that MTA. Both agents are considered promising by OBTT. If one of these MTAs cannot be obtained, we will test another protease inhibitor E64D which is also promising. A full report on AER-271, amantadine, and minocycline, and any additional agents tested by OBTT will be included in subsequent reports. Note that parent OBTT grant WH81XWH-10-1-0623 is in a NCE until September 29, 2016 and the OBTT-ES grant WH81XWH-14 is ending on April 30, 2016 with an anticipated NCE until April 30, 2017. The time taken to obtain MTAs has slowed our progress somewhat; thus the need for NCEs. However, OBTT desires to test the most promising agents—therapies that can be taken to clinical trials, and some of these are proprietary, thus we feel that the inherent delays involved are substantiated.

There have been a remarkable 20 new reportable outcomes since our progress report of October 29, 2015. In addition to the 8 newly published manuscripts described above (S1-S8), OBTT investigators gave several invited presentations at the 2015 meeting of the International Neurotrauma Society in Cape Town SA (S9-S11) including a comprehensive plenary lecture on OBTT by Dr. Kochanek, a panel presentation by Dr. Kochanek on enhancing rigor in pre-clinical investigations as exemplified by the work of OBTT, and an oral presentation by Dr. Mondello on the most recent biomarker work—showing theranostic applications in our studies. These were extremely well received by a large audience. In addition, investigators in the OBTT consortium have submitted 6 abstracts (S12-17) to the upcoming National Neurotrauma Society meeting in Lexington in June, 2016 and three abstracts (S18-20) the annual Military Health System Research Symposium (MHSRS) meeting in August. Manuscripts on each of the additional therapies tested will of course follow and be included in the final report when all work is completed. We anticipate that there will be at least 6-7 additional manuscripts generated from our work.

Reportable outcomes Since the October 29, 2015 report

- S1.** Kochanek PM, Bramlett HM, Dixon CE, Shear DA, Dietrich WD, Schmid KE, Mondello S, Wang KKW, Hayes RL, Povlishock JT, Tortella FC: Operation Brain Trauma Therapy: Approach to modeling therapy evaluation, drug selection, and biomarker assessments, for a multi-center pre-clinical drug screening consortium for acute therapies in severe traumatic brain injury. *J Neurotrauma* 33:513-522, 2016.
- S2.** Shear DA, Dixon CE, Bramlett HM, Mondello S, Dietrich WD, Deng-Bryant Y, Schmid KE, Wang KKW, Hayes RL, Povlishock JT, Kochanek PM, Tortella FC: Operation Brain Trauma Therapy: Nicotinamide treatment in traumatic brain injury. *J Neurotrauma* 33:523-537, 2016.
- S3.** Bramlett HM, Dietrich WD, Dixon CE, Shear DA, Schmid KE, Mondello S, Wang KKW, Hayes RL, Povlishock JT, Tortella FC, Kochanek PM: Operation Brain Trauma Therapy: Erythropoietin treatment in traumatic brain injury. *J Neurotrauma* 33:538-552, 2016.
- S4.** Dixon CE, Bramlett HM, Dietrich WD, Shear DA, Yan HQ, Deng-Bryant Y, Mondello S, Wang KKW, Hayes RL, Empey PE, Povlishock JT, Tortella FC, Kochanek PM: Operation Brain Trauma Therapy: Cyclosporine treatment in traumatic brain injury. *J Neurotrauma* 33:553-566, 2016.
- S5.** Mountney A, Bramlett HM, Dixon CE, Mondello S, Dietrich WD, Wang KKW, Caudle K, Empey PE, Poloyac SM, Hayes RL, Povlishock JT, Tortella FC, Kochanek PM, Shear DA: Operation Brain Trauma Therapy: Simvastatin treatment in traumatic brain injury. *J Neurotrauma* 33:567-580, 2016.
- S6.** Browning M, Shear DA, Bramlett HM, Dixon CE, Mondello S, Schmid KE, Poloyac SM, Dietrich WD, Hayes RL, Wang KKW, Povlishock JT, Tortella FC, Kochanek PM: Operation Brain Trauma Therapy: Levetiracetam treatment in traumatic brain injury. *J Neurotrauma* 33:581-594, 2016.
- S7.** Mondello S, Shear DA, Bramlett HM, Dixon CE, Schmid Maj KE, Dietrich WD, Wang KK, Hayes RL, Glushakova O, Catania M, Richieri S, Povlishock J, Tortella FC, Kochanek PM: Insight into preclinical models of traumatic brain injury using circulating brain damage biomarkers: Operation Brain Trauma Therapy. *J Neurotrauma* 33:595-605, 2016.
- S8.** Kochanek PM, Bramlett HM, Shear DA, Dixon CE, Mondello S, Dietrich WD, Hayes RL, Wang, KKW, Poloyac SM, Empey PE, Povlishock JT, Mountney A, Browning M, Deng-Bryant Y, Yan HQ, Jackson TC, Catania M, Glushakova O, Richieri SP, Tortella FC: Operation Brain Trauma Therapy:

Synthesis of findings, current investigations, and future directions. J Neurotrauma 33:606-614, 2016.

PRESENTATIONS AT THE INTS MEETING, February 2016 Cape Town

- S9.** Kochanek PM: Operation Brain Trauma Therapy (plenary presentation)
- S10.** Kochanek PM: OBTT: Logistical challenges in multi-centered laboratory studies (panel presentation)
- S11.** Mondello S: The potential utility of brain injury biomarkers in pre-clinical drug screening by Operation Brain Trauma Therapy (platform presentation)

Abstracts - National Neurotrauma Society meeting 2016

- S12.** Bramlett HB, Furones-Alonso O, Sanchez-Molano J, Truettner J, Sequiera D, Moreno W, Treu R, Dietrich WD: Evaluation of AER-271 in the Miami fluid percussion model of traumatic brain injury: An OBTT consortium study. National Neurotrauma Society meeting, July 2016. (submitted)
- S13.** Bramlett HB, Furones-Alonso O, Sanchez-Molano J, Truettner J, Sequiera D, Moreno W, Treu R, Dietrich WD: Dose-response evaluation of amantadine in the Miami fluid percussion model of traumatic brain injury: An OBTT consortium study. National Neurotrauma Society meeting, July 2016. (submitted)
- S14.** Wallisch J, Yan H, Ma X, Empey P, Poloyac S, Feldman K, Kochanek PM, Dixon CE: Evaluation of AER-271 in the controlled cortical impact model of traumatic brain injury: An OBTT consortium study. National Neurotrauma Society meeting, July 2016. (submitted)
- S15.** Dixon CE, Yan H, Ma X, Empey P, Poloyac S, Feldman K, Kochanek PM: Dose-response evaluation of amantadine in the controlled cortical impact model of traumatic brain injury: An OBTT consortium study. National Neurotrauma Society meeting, July 2016. (submitted)
- S16.** Mondello S, Shear DA, Bramlett HM, Dixon CE, Schmid K, Dietrich WD, Wang KK, Hayes RL, Tortella FC, Kochanek PM: Time course of brain injury biomarkers across TBI models: findings from Operation Brain Trauma Therapy. National Neurotrauma Society meeting, July 2016. (submitted)
- S17.** Jenny R. Browning, Ying Deng-Bryant, Sindhu K. Madathil, Rebecca Pedersen, Justin Sun, Justin Hahn, Janice Gilsdorf, Frank Tortella, Stefania Mondello, and Deborah Shear. Evaluation of AER-271 in the WRAIR PBBI Model: Studies from the Operation Brain Trauma Therapy Consortium. National Neurotrauma Society meeting, July 2016. (submitted)

Abstracts – MHSRS 2016

- S18.** Mondello S, Shear DA, Bramlett HM, Dixon CE, Dietrich WD, Wang H, Hayes RL, Tortella FC, Kochanek PM: Differential release kinetics of brain injury biomarkers across TBI models: findings from Operation Brain Trauma Therapy. MHSRS 2016. (submitted)
- S19.** Okada-Rising S, Pedersen R, Sun J, Browning J, Gilsdorf J, Tortella F, Mondello S, Shear D: Dose-response evaluation of amantadine in the WRAIR PBBI model: studies from the Operation Brain Trauma Therapy (OBTT) consortium. MHSRS 2016. (submitted)
- S20.** Kochanek PM, Bramlett H, Dixon CE, Shear D, Dietrich WD, Mondello S, Hayes RL, Wang KKW, Povlishock JT, Lafrenaye A, Tortella FC: Operation Brain Trauma Therapy: Synopsis of the first nine therapies evaluated by a pre-clinical multi-center drug and biomarker screening consortium for traumatic brain injury. MHSRS 2016. (submitted)

INTRODUCTION

As outlined in the grant proposals and the prior reports Operation Brain Trauma Therapy (OBTT) and OBTT-extended studies (OBTT-ES) represent truly unique and highly productive DoD supported multi-center pre-clinical drug screening and biomarker development/ evaluation programs for the field of

OBTT OVERVIEW

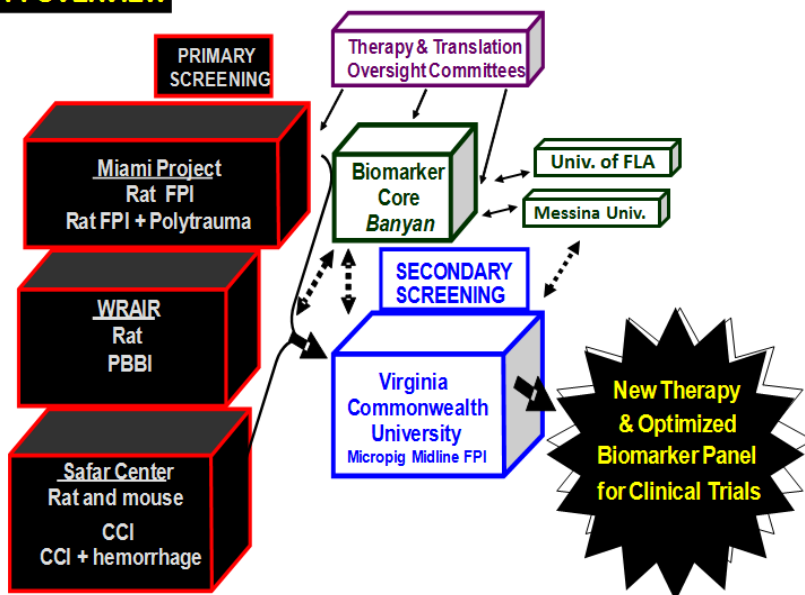


Figure 1. Schematic of the components and goals of OBTT and OBTT-ES.

Commonwealth Univ. (John Povlishock, site PI; Audrey Lafrenaye, Co-I) and biomarker experts at

Models used for primary screening therapies in OBTT

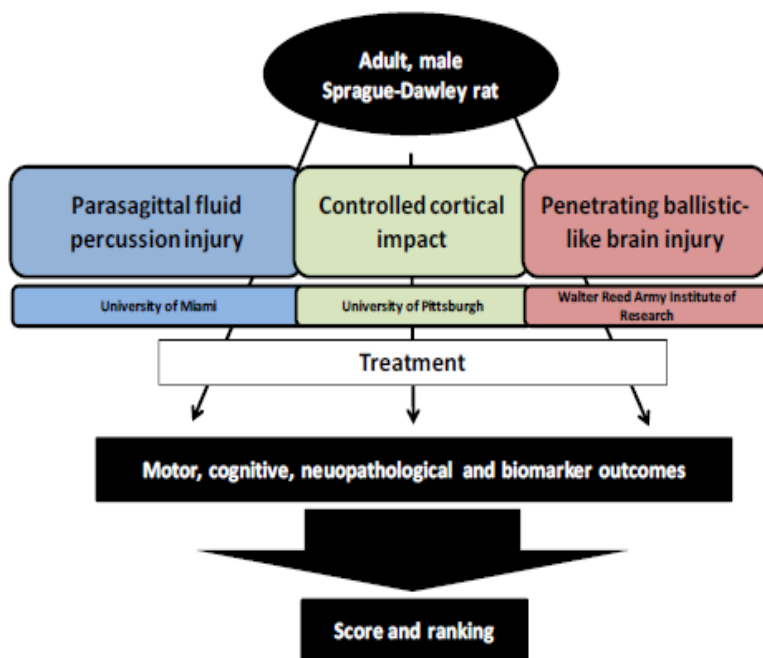


Figure 2. Primary Screening of therapies in established rat models of TBI used in both OBTT and OBTT-ES.

models or with advanced monitoring, as appropriate.

traumatic brain injury (TBI) that are being carried out by a consortium that represents a partnership between civilian and military academic centers and industry. **(Figure 1).** OBTT includes TBI investigators at the Safar Center for Resuscitation Research (Univ. of Pittsburgh School of Medicine, Patrick Kochanek, PI; C. Edward Dixon, Co-I), the Miami Project to Cure Paralysis, (Univ. of Miami School of Medicine, W. Dalton Dietrich, site PI; Helen Bramlett, Co-I), the Neuroprotection program at WRAIR (Frank Tortella, site PI; and Deborah Shear Co-I), Virginia

Banyan Biomarkers (Ronald Hayes, site PI), The University of Florida (Kevin Wang), and Messina University (Stefania Mondello).

Three rodent models (controlled cortical impact [CCI], parasagittal fluid percussion injury [FPI], and penetrating ballistic-like brain injury [PBB]) are used in Pittsburgh, Miami, and WRAIR, respectively, for primary drug screening **(Figure 2)** with the most promising candidates tested in a micropig TBI model at Virginia Commonwealth Univ. Additional screening of promising drugs is also carried out in more complex rodent

The **overall hypothesis** is that clinical TBI is a heterogeneous disease involving multiple brain injury phenotypes and that success of an agent tested across multiple established TBI models using an approach with unprecedented rigor and blinding across centers will identify the best candidates for clinical trials.

We also have a **secondary hypothesis** that has developed over the course of our investigations, namely that by evaluating therapies using identical protocols across model, we may be able to identify therapies that should be targeted in specific TBI phenotypes within clinical trials.

Parallel hypotheses could also be generated for the investigation of circulating biomarkers of brain injury within the framework of OBTT and OBTT-ES. Two types of drugs are screened, 1) low hanging fruit (drugs that are already FDA approved for other uses or that are otherwise ready for clinical translation [these types of drugs are being assessed with funding from the parent grant OBTT]) and 2) higher risk but potentially high reward novel therapies (these types of drugs are being assessed—as requested by the DoD in prior reviews—with funding from OBTT-ES). Drugs in the latter category should have at least some track record of success in experimental brain injury.

BODY

Administrative overview of accomplishments in year 5 of funding in OBTT and OBTT-ES: Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine (Patrick M. Kochanek, MD, overall PI)

Disclaimer: Please recognize that this report can only present a small fraction of the findings to date from the consortium. We have studied >2000 rats and measured >5000 serum and/or plasma biomarker levels. Thus, only the highlights of the work of OBTT are presented below.

Synopsis of major findings and accomplishments this funding period

Year 5 of funding has been highly productive for OBTT and OBTT ES. Please note that this is the final year of support on the current OBTT grant, although we have requested a NCE. There is also 6 months of funding remaining on OBTT-ES—so work is continuing as will be described in this report.

First, we submitted 8 manuscripts to the *Journal of Neurotrauma* to comprise a special issue of that journal devoted to OBTT. The 8 manuscripts addressed the first 5 therapies tested in OBTT along with an introductory and concluding manuscript, and a manuscript focused on the utility of circulating biomarkers across the consortium. All manuscripts have been accepted and are already *in press*. We also published a manuscript in the *Journal of Neuroinflammation* that characterized the neuro-inflammatory response to TBI in our micropig FPI model. That paper sets the stage for therapy testing in that model which is underway.

OBTT and OBTT-ES were also featured this year as a panel at the National Neurotrauma Society (NNTS) conference in Santa Fe, NM, and this included three presentations. In addition, this year OBTT investigators presented 12 abstracts of the ongoing work with additional therapies and biomarkers at the NNTS and MHSRS conferences. Remarkably, since inception, OBTT investigators have presented 50 plenary, panel, platform, and poster presentations on the findings of OBTT and OBTT-ES.

With regard to therapy testing, this funding period, OBTT and OBTT-ES also continued to screen therapies across the three rat models and this included testing of the anti-edema SUR-1 antagonist glibenclamide, the novel membrane re-sealing agent Kollidon-VA64, the aquaporin-4 blocker AER-271, and the rehabilitation targeting dopamine-augmenting agent amantadine. We have also completed all of the necessary PK studies with minocycline and have chosen to launch that drug next. We also anticipate testing on OBTT-ES the novel aminopropyl carbazole agent P7C3-A20 targeting enhancement of neurogenesis and neuroprotection

Finally, OBTT and OBTT-ES this year also received additional national and international acclaim and exposure. OBTT was mentioned in the *New England Journal of Medicine* as an important tool for the

future of the field to identify promising drugs for TBI to test in clinical trials (Wright et al, *New Engl J Med* 371:2457-66, 2014). OBTT will be the topic of a plenary lecture (by Dr. Kochanek) at the 2016 meeting of the International Neurotrauma Society (INTS) in Capetown, South Africa. That will afford additional international exposure and prestige to the DoD sponsored OBTT investigations. Finally, Dr. Mondello is submitting an invited manuscript to the *Journal of Trauma* based on her presentation of the theranostic applications in OBTT of biomarkers for pre-clinical drug screening that she made at the 2015 MHSRS. We believe that all of these accomplishments reflect highly on the OBTT program.

An overview of the findings on drug screening carried out to date by OBTT and OBTT-ES is provided in

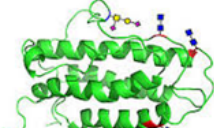
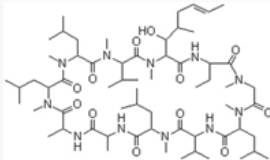
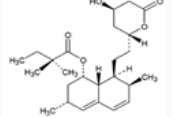
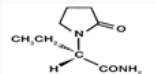
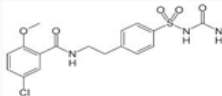
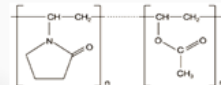
Chemical structure	Dose and treatment regimen utilized	Literature support in TBI at time of selection	Therapeutic target(s)
	50 or 500 mg/kg IV at 15 min and 24 h after injury	Nicotinamide 5+ studies in TBI	PARP Oxidative stress NADPH depletion
	5000 or 10,000 IU/kg IV at 15 min after injury	Erythropoietin 24+ studies in TBI	Apoptosis Inflammation Neurogenesis Angiogenesis
	10 or 20 mg/kg IV at 15 min and 24 h after injury	Cyclosporine A 17+ studies in TBI	Mitochondrial Permeability Transition Calcineurin Inflammation
	1 or 5 mg/kg PO—first dose at 3 h after injury and then daily for 14 d	Simvastatin 14+ studies in TBI	HMGCo-A reductase Neuro-inflammation Cell death CBF Trophic factors
	54 or 170 mg/kg IV at 15 min after injury	Levetiracetam 1+ study in TBI	Excitotoxicity Seizures SV2-A GABA potentiation
	10 µg/kg IP at 10 min after injury followed by SQ infusion of 200 ng/h for 7d by Alzet pump	Glibenclamide 2+ studies in TBI	SUR-1 Cerebral Edema
	0.4 or 0.8 g/kg IV over 5 min at 15 min after injury	Kollidon VA64 1+ study in TBI	Cell membrane re-sealing BBB

Figure 3. Chemical structure, dose and treatment regimen utilized, literature support in TBI at the time of selection for testing, and purported therapeutic targets for the first 7 therapies selected for testing by OBTT and/or OBTT-ES. Please not that for some therapies, additional literature support and dosing information became available during and/or after testing began by our consortium.

manuscripts 4-11 in the Reportable Outcomes for details of the OBTT scoring matrix). This includes all findings from the initial 5 therapies and all but the circulating biomarker data from therapies 6 and 7. Those biomarker results have been obtained and are being analyzed. Therapies 8 and 9 (AER-271 and amantadine) are in various stages of investigation across the consortium. All of the injuries and behavioral testing have been completed for AER 271 and data analysis is ongoing as are the histological assessments. Injuries and behavioral testing are nearly complete for amantadine.

To date, two drugs studied by the OBTT and OBTT-ES consortium have shown some promise. **Of note, as described in manuscripts (4-11) and in our prior reports, the approach taken by OBTT is extremely rigorous. All sites use an identical treatment protocol in all regards, and all data are collected in a blinded manner. The identification codes for the results of each therapy are**

the previously mentioned 8 manuscripts, (see **Reportable Outcomes 4-11**) along with **Figures 3 and 4**. In **Figure 3**, a synopsis of the chemical structure, doses, treatment protocol, literature support at the time of selection and purported therapeutic target(s) and/or mechanisms of action for the first 7 therapies tested in OBTT is provided. **Figure 4** then provides a synopsis of the numerical scoring based on the OBTT scoring matrix (see prior reports and

broken simultaneously by the PI across the sites—eliminating any knowledge of a given site as to the effect of a therapy on a given outcome at any other site.

The most highly rated therapy and the only therapy to produce substantive positive effects in more than one model is the anti-convulsant and anti-excitotoxic agent levetiracetam (Keppra), which was the 5th drug tested by OBTT. As shown in **Figure 4**, it generated a net 10 overall positive points across models, showed significant efficacy in two of the three models (FPI and CCI), was the only therapy that

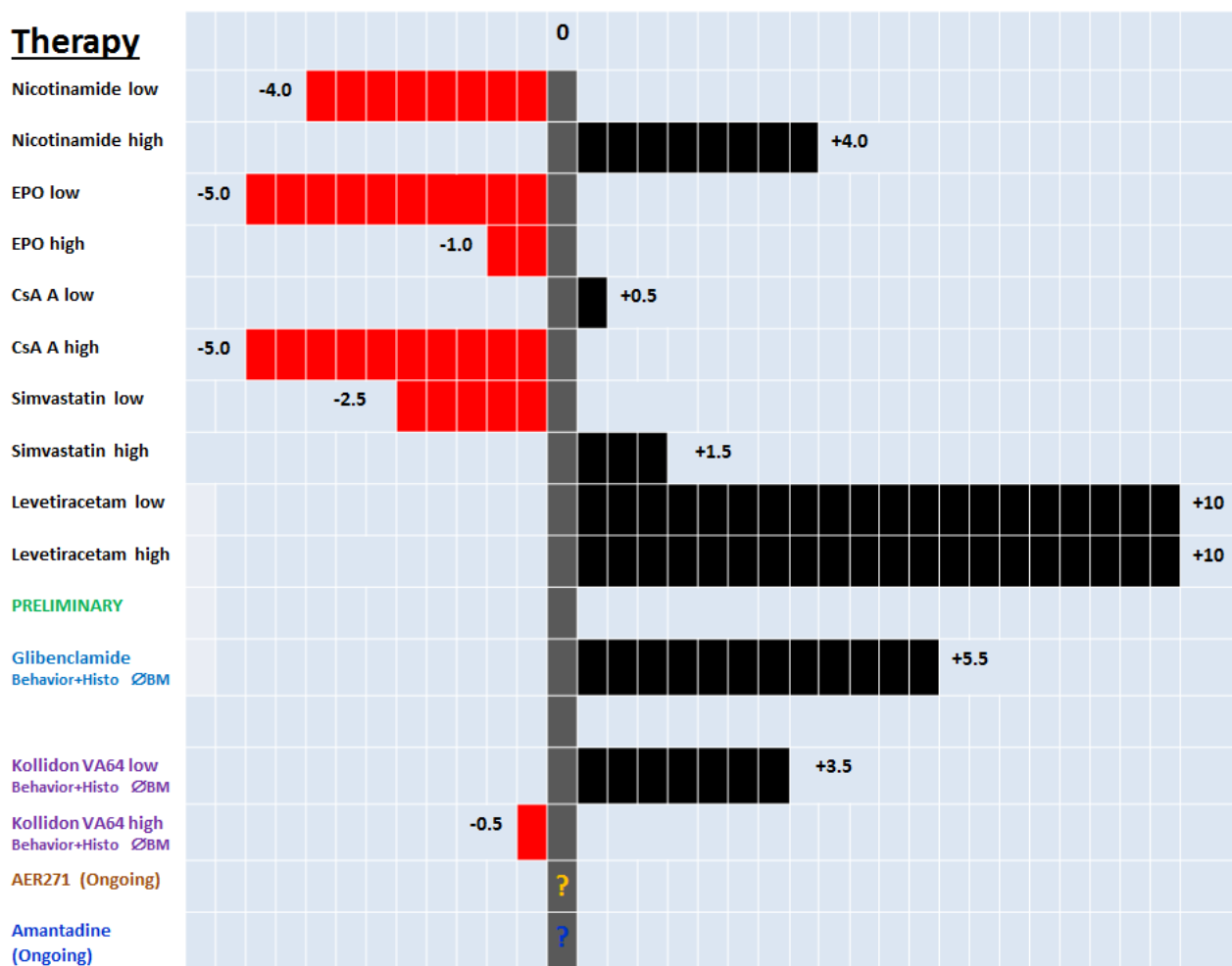


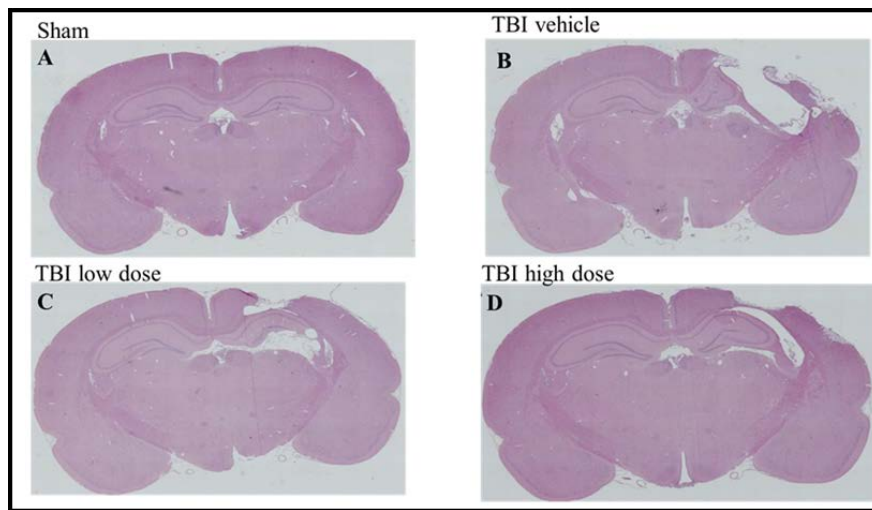
Figure 4. Results and status of the first 8 therapies tested by OBTT and OBTT-ES. All of the data have been analyzed and assembled (and either published or submitted for publication) for the initial 5 therapies (see Reportable Outcomes 4-11)—only the biomarker data are pending on therapies 6 and 7 so it is possible that the final scoring for those two therapies could change slightly. **The 2 most promising therapies that have been evaluated by OBTT/OBTT-ES to date are levetiracetam and glibenclamide.** Levetiracetam generated +10 overall positive points across models, showed efficacy in 2 of 3 models (FPI and CCI), was the only therapy that improved cognitive outcome in any model (FPI), was similarly effective at 2 different doses, and is the only therapy that did not generate any negative points in any of the models. It has been advanced to testing in micropigs; a mild insult with unique outcomes (axonal injury and vascular dysfunction). Glibenclamide showed considerable benefit in model (CCI) in OBTT suggesting that it may have specific benefit in clinical contusion. That is logical given that it targets the development of cerebral edema. Insults and behavioral testing of therapy 8, AER-271 have been completed and data are being analyzed. Therapy 9, amantadine is currently in the midst of testing across the OBTT consortium. See text for details.

improved cognitive outcome in any of the models (FPI), was similarly effective at both doses, and is the only therapy that did not generate any negative points in any model. As outlined later, it has been advanced to testing in the micropig model. As also shown in **Figure 4**, more recently, the anti-edema SUR-1 antagonist glibenclamide, showed benefit in one of the 3 models (CCI) in OBTT suggesting that it may have specific merit for testing in the clinical setting of cerebral contusion. In CCI, glibenclamide robustly improved motor function and also is the only agent tested thus far by OBTT to reduce contusion volume in CCI. These two agents thus support the hypotheses in OBTT namely that clues

regarding potentially useful drugs for testing across injury phenotypes and in specific injury phenotypes, respectively may be able to be identified by our consortium approach.

New findings on individual therapies evaluated by OBTT/OBTT-ES during this funding period

So that a cogent and manageable report could be presented, in general, only those findings that



generated points (positive or negative) in the OBTT scoring matrix are shown in this report.

Drug #5:
Levetiracetam (benefit on ultimate hemispheric tissue loss is predicted theranostically by 24h plasma levels of glial fibrillary acidic protein [GFAP])

Figure 5. Coronal section through the brain at 21d after CCI in rats from the levetiracetam study by OBTT. Sections shown are from the median rat in each group. High dose levetiracetam (panel D) significantly reduced hemispheric tissue loss vs. vehicle after CCI (see reference 6). This finding was predicted by the 24 h plasma GFAP levels (**Figure 6**).

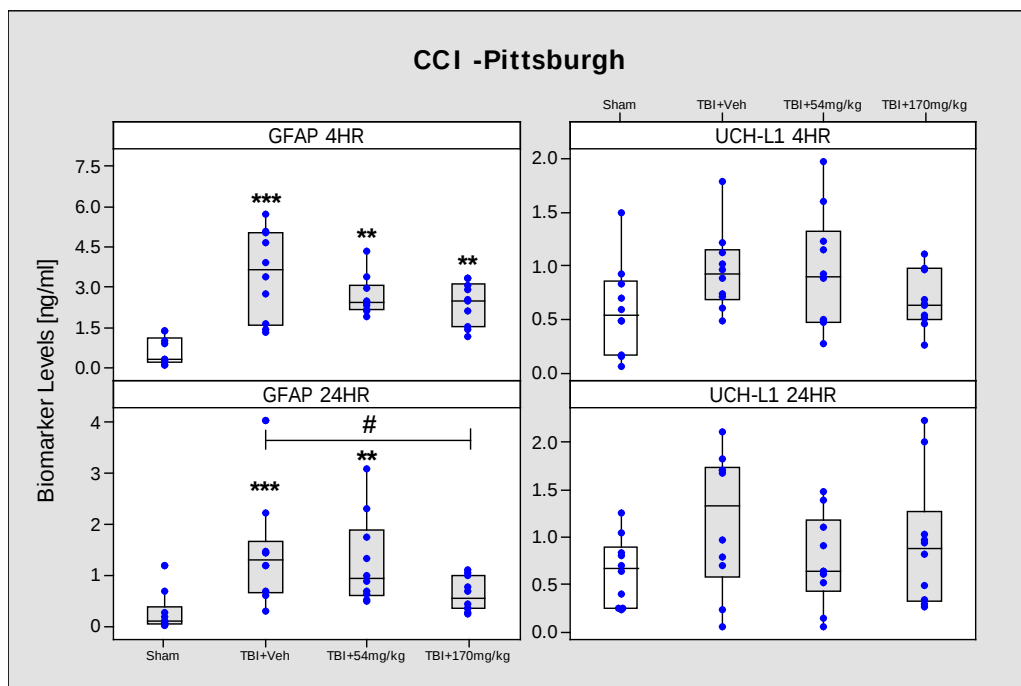


Figure 6. Box plots of GFAP and UCH-L levels in blood at 4 and 24 h post-insult in sham, vehicle, low dose and high dose levetiracetam treated rats (FPI [A], CCI [B], and PBBI [C]). The horizontal line in each box is the median. Boxes show interquartile range. Whiskers indicate the 90th and 10th percentiles. Each value is plotted as a dot superimposed on the graph. There were significant increases in GFAP in the TBI vehicle groups at 4 and 24 h vs. sham in all 3 models. **In CCI, high dose levetiracetam significantly reduced GFAP levels at 24 h after injury (# $p < 0.05$ vs vehicle) predicting the ultimate tissue sparing of this therapy. See text for details.**

Although much of the data on levetiracetam was presented and discussed in last year's report, after filing the report, we broke the biomarker code on this therapy. We were pleasantly surprised with the findings. Recall that levetiracetam significantly reduced hemispheric tissue loss vs. vehicle assessed at 21d after injury in CCI at the high dose—indicating a tissue sparing effect (**Figure 5**). Remarkably, at high dose, levetiracetam vs. vehicle also significantly reduced plasma GFAP levels

assessed at 24h after injury in CCI (**Figure 6**). This suggests theranostic potential for the use of blood levels of GFAP at 24h after injury in predicting ultimate tissue loss. Looking back on our data, we noted

that with simvastatin, high dose therapy actually increased lesion volume in FPI, again assessed at 21d after injury. Remarkably, once again 24h GFAP blood levels predicted that deleterious effect. The only other drug effect on tissue loss across OBTT was in CCI, where nicotinamide showed a significant dose dependent reduction in hemispheric tissue loss. In that case, there was a strong trend toward reduced 24h plasma GFAP by treatment vs vehicle. Taken together, our data suggest theranostic potential for the use of 24h blood levels of GFAP as a screening tool for tissue sparing effects in pre-clinical studies. It will be interesting to track the efficacy of GFAP in predicting ultimate tissue loss by CT or MRI in clinical studies given the fact that GFAP is a biomarker that is gaining traction in clinical trials. Unlike GFAP, ubiquitin C-terminal hydrolase-L1 (UCH-L1) did not predict tissue sparing (see **Figure 6**). **The potential theranostic use of circulating biomarkers as identified by OBTT is an exciting finding that was discussed in a platform presentation at the 2016 MHSRS, and is the topic of a manuscript in preparation for the *Journal of Trauma* special issue on the MHSRS.**

Drug 6. Glibenclamide

Glibenclamide is a sulfonylurea receptor (SUR1) regulated NC_{Ca-ATP} channel antagonist that has shown promise in pre-clinical stroke models. A review of its use in CNS insults has been published (Simard et al, *J Cereb Blood Flow Metab* 32:1699-1717, 2012). The SUR1 channel is a nonselective cation channel (ABC binding cassette transporter) regulated by intracellular calcium and ATP. The ABC proteins couple ATP hydrolysis to translocation of solutes, xenobiotics or drugs across membranes. SUR1 activation leads to Na^+ accumulation, cellular depolarization and ATP depletion. $SUR1NC_{Ca-ATP}$ channels are present in brain microvascular endothelium, neurons, and astrocytes, are induced by injury and $TNF\alpha$. Channel activation is associated with cell necrosis and cytotoxic edema. This channel can also be blocked by Riluzole which is also neuroprotective in pre-clinical studies. However, glibenclamide is much more potent than Riluzole (EC_{50} of 48 nM vs. 31 μ M, respectively). SUR1 can be up-regulated by TBI. Upregulation was seen by 6h after CCI in rat hippocampus, peaked at 12h and only partially resolved by 24h. Glibenclamide has shown promise in models of brain ischemia including transient and permanent MCAO, thromboembolic models, and malignant brain edema. Reductions in infarct volume and mortality were seen with a 10h treatment window. It has also shown benefit in experimental SAH. There have been 7 reports in spinal cord injury models. Most have been positive depending on injury severity—greater benefit in milder insults.

At the time of selection, two preclinical TBI studies in rat models were reported, both positive. Patel et al (*J Neuropath Exp Neurol* 69:1177-1199, 2010) studied Glyburide (10 μ g/kg IP at 10 min post TBI) followed by a SQ infusion of 200 ng/h for 7d by Alzet pump. This produced plasma levels of ~5 ng/mL with minimal effect on blood glucose. Treatment reduced cleaved Caspase-3 in hippocampus, Fluoro-Jade positive hilar neurons, and improved probe trial but not MWM latency. No motor data were presented. Hackenberg et al, <http://www.egms.de/static/en/meetings/dgnc2013/13dgnc397.shtml>) studied glibenclamide in rat CCI using a SQ bolus 15 min after CCI and a 7d infusion via Alzet pump. The exact dose was not described. Brain edema at 24h, and contusion volume at 8h, 24h, and 7d as assessed by MRI were reduced.

There is an ongoing phase 2 clinical trial of IV Glyburide vs. placebo in TBI using MRI outcomes funded by the US Army in the INTRuST consortium. It includes TBI patients from mild to severe (<http://clinicaltrials.gov/ct2/show/NCT01454154?term=A+randomized+Clinical+Trial+of+glyburide+for+TBI&rank=1>).

Finally, Glyburide can reduce blood glucose. The doses that reduce blood glucose (producing hypoglycemia) in rats are 30-400 times greater than those showing neuroprotection. However, blunting of hyperglycemia that is seen in CNS insults could potentially play some role in the observed benefit.

The best characterized dosing regimen and the one recommended in discussions with Dr. Simard from the University of Maryland come from his report. In a model of MCAO stroke in rats, a 10h therapeutic window was seen. We consulted his group on dosing and drug preparation for our studies. A loading

bolus (10µg/kg SQ) was given at 10 min post injury, followed by a 7d continuous SQ infusion (0.2µg/h, Alzet mini-pump).

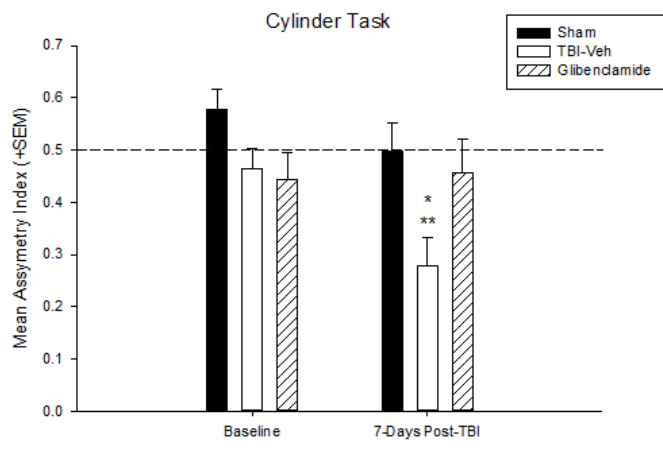


Figure 7. Effect of Glibenclamide on cylinder task in FPI. Rats performed equally on this task at baseline. After TBI, the vehicle group was impaired vs. sham (* $p<0.05$) and glibenclamide (** $p<0.05$) treated rats 7d post-injury. Glibenclamide treated rats showed marked improvement on forelimb use. ANOVA for group ($p=0.015$). **This yielded full points for this task.**

Glibenclamide: Effects on behavioral outcomes

As indicated in the introduction, thus far, in OBTT glibenclamide represents the agent with the second highest score. It has shown the most promise in the CCI model (as outlined below) and thus this agent may have the most clinical potential in the contusional phenotype.

We noted a significant benefit on motor function—full points for vehicle vs glyburide in both the FPI and the CCI models. This is shown in **Figures 7 and 8**. In FPI, significant benefit for glibenclamide treated rats

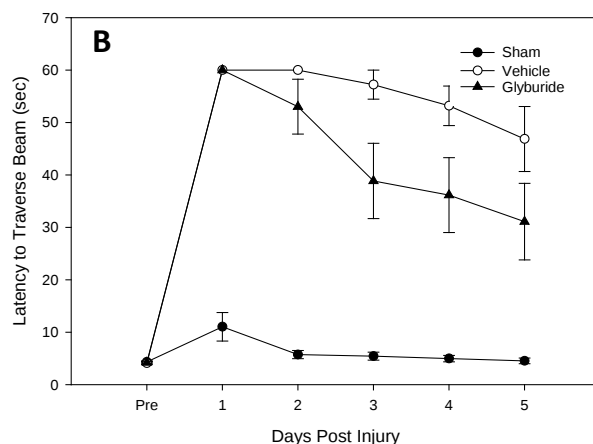
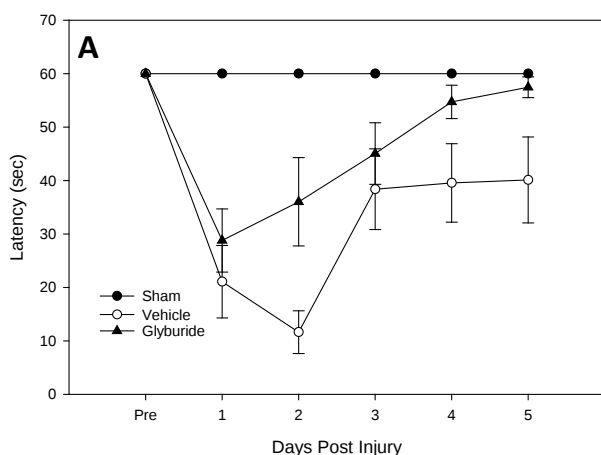


Figure 8A-B. Example showing the effect of glibenclamide on (A) beam balance and (B) beam walking motor tasks vs. vehicle treatment of rats after CCI. **Glibenclamide treatment yielded beneficial ($p<0.05$) effects vs. vehicle resulting in full points for treatment for both motor tasks.**

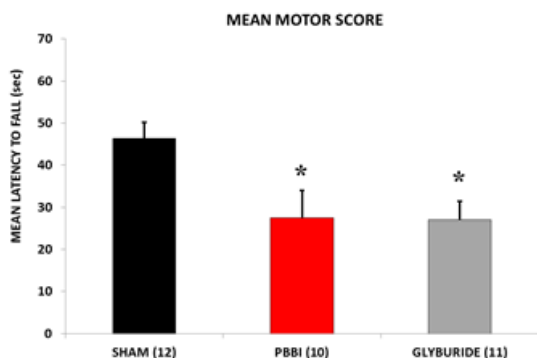


Figure 9. Effect of glibenclamide on motor function (Rotarod) after PBBI. Unlike FPI and CCI, benefit of Glibenclamide on motor function was not seen in the PBBI model.

was seen vs. vehicle after TBI on the cylinder task (**Figure 7**). In CCI highly significant benefit was seen for glibenclamide treatment on both the beam balance and beam walking (**Figure 8A-B**). In contrast, no motor benefit was seen in PBBI—an example (Rotarod) is shown in **Figure 9**.

Surprisingly, benefit from treatment with glibenclamide was not seen across models on cognitive outcome including latency testing for MWM hidden platform paradigm, working memory, or probe trial (an example is shown in **Figure 10** of the effect of glibenclamide on MWM latency to find the hidden platform in the CCI model).

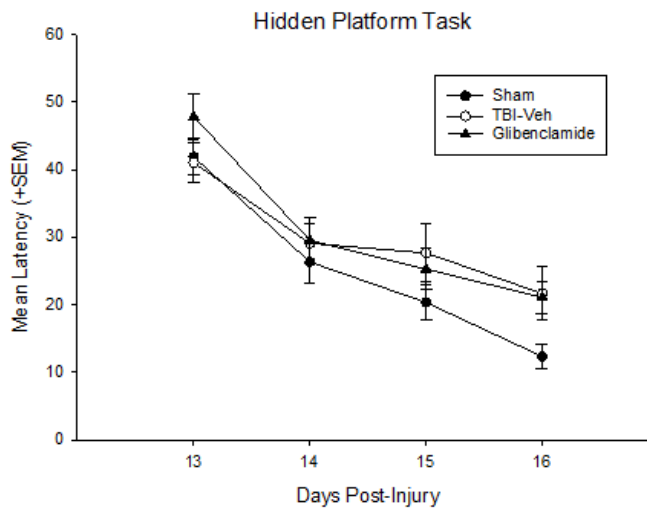


Figure 10. Lack of effect of Glibenclamide on cognitive outcome (MWM latency) after TBI induced by FPI. Contrasting benefit on motor function, no benefit was seen on MWM performance with Glibenclamide treatment. This same pattern was seen in the CCI model.

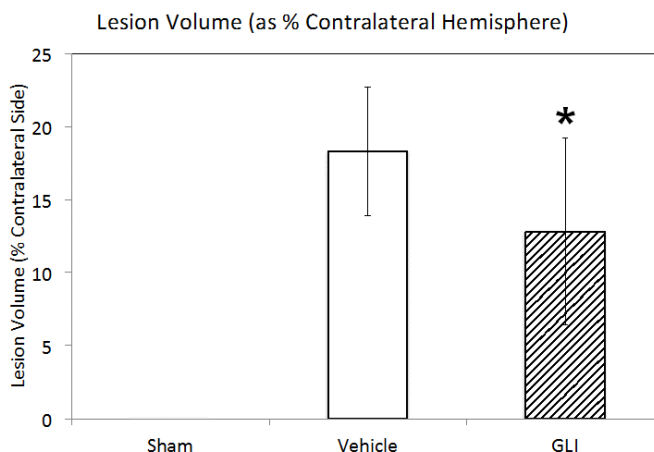


Figure 11a. Glibenclamide (GLI) reduced lesion volume ($12.8 \pm 6.4\%$) vs. vehicle ($18.3 \pm 4.4\%$) in the CCI model, $p < 0.05$. Data in this example are mean $\pm$ SD. GLI is the first therapy to reduce contusion volume in CCI as evaluated across OBTT and OBTT-ES.

Glibenclamide: Effects on neuropathology

Complementing the benefit of glibenclamide on motor function, assessment of histology (contusion volume and hemispheric tissue loss) revealed a beneficial effect of glibenclamide in the CCI model. Specifically, **glibenclamide significantly reduced contusion volume vs. vehicle (full points) in CCI (Figures 11a and 11b)**. Of note, glibenclamide is the first drug tested in either OBTT or OBTT-ES that has significantly reduced contusion volume in CCI (recall that two therapies have attenuated hemispheric tissue loss in CCI—nicotinamide and levetiracetam). However, glibenclamide is the only drug to reduce tissue loss in the contusion proper. Only a trend toward reduced hemispheric tissue loss was seen with glibenclamide treatment (19.1 ± 9.5 vs. $25 \pm 5.4\%$ of uninjured hemisphere in treated vs. vehicle groups) indicating that the effect in the contusion proper was greatest. That would make physiological sense given that the CCI model highlights contusional edema. We did not, however, see beneficial effects on lesion volume or hemispheric tissue loss in the FPI or PBBI models.

All of the biomarker samples have been processed and the results are being analyzed for both GFAP and UCH-L1. It will be extremely interesting to determine if 24h GFAP predicts theranostic effects on contusion volume for this therapy; the data are being currently analyzed.

Given that this drug targets brain edema, one possible explanation for the overall results is that motor performance is improved because it is tested in the acute phase (during the initial week after injury) when brain edema peaks in the TBI models. **It is also possible that the use of a craniotomy in all 3 models results in an under-estimation of the potential benefit of this drug given that some degree of decompression in each model**

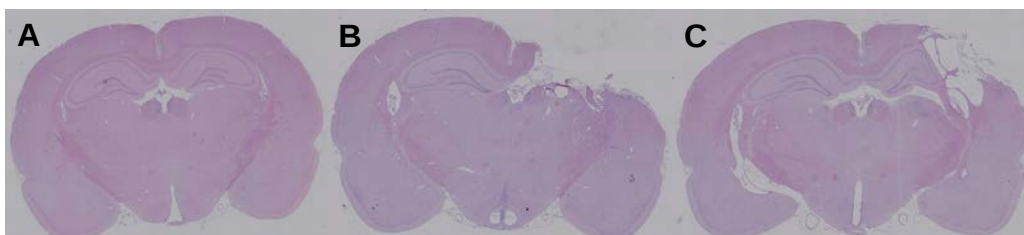


Figure 11b. H&E-stained coronal sections from the 3 median rats in sham (A) vs. TBI-vehicle (B) vs. TBI-glibenclamide (C) illustrates reduced contusion volume by glibenclamide treatment that is shown quantitatively in **Figure 11a**. This benefit was only seen in CCI.

would already reduce the impact of brain swelling on secondary injury. Glibenclamide is a therapy, based on our findings in OBTT, that is worthy of additional study and may have clinical potential specifically in contusional phenotypes. Given this finding, as outlined in the original OBTT grant proposal, we are currently evaluating the effect of glibenclamide in our model of combined CCI plus hemorrhage in mice—which because of the necessary fluid resuscitation—highlights the development of cerebral edema and intracranial hypertension (Blasiolo et al, *Anesthesiology*, 118:649-663, 2013). Those studies are ongoing and will be carried out in the no-cost extension and remainder of the OBTT-ES funding period.

Drug 7. Kollidon-VA64:

Kollidon VA 64 is an agent categorized into the higher risk higher reward classification and thus represented an OBTT-ES prototype drug. It is also known by its chemical name vinylpyrrolidone-vinyl acetate copolymer (see **Figure 3**). Kollidon VA 64 is used extensively as a vinylpyrrolidone excipient in the pharmaceutical industry. Although as a potential therapeutic agent in TBI, however, it is much more exploratory than the other agents tested thus far by our consortium. It was reported to have biological effects in TBI suggesting that it operates by a unique mechanism of action—via membrane resealing effects (Mbye et al, *J Cereb Blood Flow Metab*, 32:515-524, 2012). It is a large polymeric molecule with MWs ranging between 45,000 and 75,000. Thus, it is anticipated to only enter the injured brain in sites where there is substantial BBB permeability. In that seminal pre-clinical study on this agent in TBI, IV administration of a single dose (500 microliters of a 1 mmol/L solution) at 1h after CCI in mice significantly reduced acute cellular degeneration, BBB damage, brain edema, and motor deficits. It also re-sealed injured cell membranes in brain tissue, but it did not appear that the ultimate benefit on secondary damage was a result of that mechanism—given that the cells exhibiting propidium iodide uptake ultimately went on to die whether or not they were in the treatment group. Kollidon VA 64 also attenuated caspase 3/7 activation. The purported effect of this agent of BBB permeability was large—almost completely ameliorating Evans Blue extravasation, and consistent with that finding, brain edema was reduced by >50% in treated vs. control groups. Thus other mechanisms conferring beneficial effects of Kollidon VA 64 appear to be operating after TBI. Finally, there is ongoing unpublished investigation on the mechanism of action of this agent and it may have effects as a Pannexin channel inhibitor—which may explain in part its “membrane resealing” effects—however that is still speculative and remains to be clarified. In the published study, a lower dose of 250 μ L of a 1 mmol/L solution was also effective (i.e., half of the aforementioned dose), and thus, these two doses were pursued by our consortium.

Regarding drug preparation, dosing and information beyond what is published, in personal discussion with the author of the seminal paper on this agent (Dr. Whalen, at Harvard Medical School), IP administration was not effective. Also, the optimal way to prepare the agent is to dissolve 0.2 grams in 5 mL of sterile PBS and inject either 10 mL/kg or 20 mL/kg in the mouse ~3 or 6 mL, respectively, in a 300 gram rat. The concentration can also be doubled and to avoid administering 20 mL/kg of fluid which could alter our models, and it dissolves well in PBS, thus we took that approach—since 6 mL is a

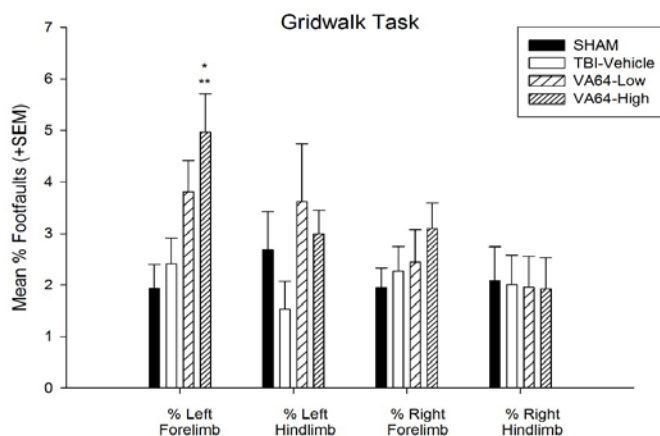


Figure 12. Effect of Kollidon VA64 on motor testing in FPI. Surprisingly, at high dose it showed a detrimental effect on gridwalk (** $p < 0.05$ vs sham and vehicle). This yielded negative 2 points in the scoring matrix.

fairly large volume. To ensure the capability of blinding treatment for this agent, we prepared two different stock solutions of drug, 0.4 g in 5 mL of PBS or 0.2 g in 5 mL of PBS and always administered 10 mL/kg. Appropriately, the vehicle was 10 mL/kg of sterile PBS. As in other studies in OBTT, the sham group did not receive treatment or vehicle.

Kollidon VA 64 was provided free of charge from BASF (Catalog # CAS-No 25086-89-9, Florham Park, NJ) as a powder to Dr. Kochanek who distributed it

to the centers. Dr. Kochanek communicated with technical support and the distribution teams at BASF and they were pleased to supply the agent to us. Based on the aforementioned publication and discussion with Dr. Whalen, it can be dissolved in sterile PBS (AMRESCO Biochemicals and Life Science Research Products, Catalog E504), which will also serve as the solution of the vehicle. The treatment groups included: 1) sham (surgery and catheters but no treatment), 2) vehicle (PBS) 10 mL/kg IV over 5 min, 3) low dose 10 mL/kg of a 0.2g/5 mL PBS solution IV over 5 min, and 4) high dose 10 mL/kg of a 0.4g/5mL PBS solution IV over 5 min. The therapeutic window for this agent is suggested by the publication to be 1h but to be consistent with our other acute therapies, and maximize its potential efficacy in a post treatment paradigm, we again gave the treatment at 15 min after the insult. We also piloted administering the drug at the high dose to mice with arterial catheters in place to ensure that there was no adverse effect on blood pressure and it was well tolerated.

As shown earlier in this report in **Figure 4**, all of the final results for Kollidon VA64 have been tabulated with exception of the biomarker data, which have been run but the results are being analyzed.

Kollidon VA64 produced only limited benefit across the TBI models in OBTT. **Figure 4** shows the points generated for low (+3.5 overall) and high dose (-0.5 overall) using the literature based single dose regimens with treatment at 15 min after TBI.

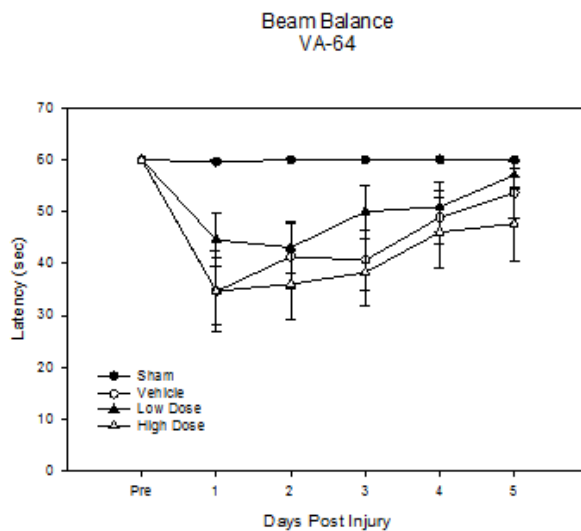


Figure 13. Effect of Kollidon VA64 on beam balance in CCI. Partial positive points were generated by low dose ($p=0.19$) which—unlike vehicle ($p=0.02$) or high dose ($p=0.004$) groups did not differ from sham.

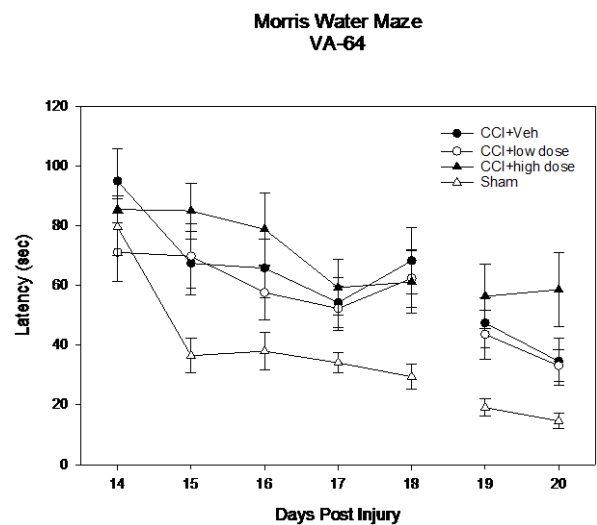


Figure 14. Effect of Kollidon VA64 on MWM task in CCI. Low dose scored partial points on this task (see text for details).

Kollidon VA64: Effects on behavioral outcomes

Once again, in general, only results of those tasks that generated points (positive or negative) in the OBTT scoring matrix are shown in this report. A complete description of findings will be provided in the full manuscript on this therapy that will be published at a later date.

In FPI, low dose VA64 did not yield any points for any behavioral task, while high dose actually produce a detrimental effect on one aspect of motor function—the gridwalk task (**Figure 12**).

In CCI, low dose Kollidon VA64 showed intermediate benefit on beam balance testing. Although TBI vehicle and TBI-high dose groups showed significant deficits vs sham, the low dose group did not (**Figure 13**). Similarly, the low dose group demonstrated an intermediate benefit on latency to find the hidden platform on the MWM task. Once again, although TBI vehicle and TBI-high dose groups showed significant deficits vs sham, the low dose Kollidon VA64 group did not (**Figure 14**). Both of these intermediate benefits generated partial points for the respective tasks.

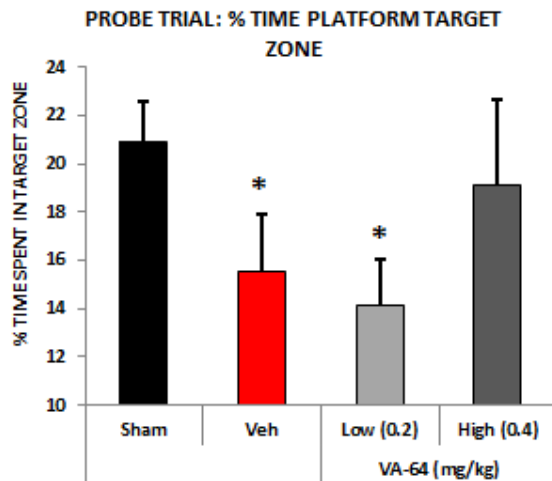


Figure 15. Effect of Kollidon VA64 on probe trial in the MWM in the PBBI model. Partial positive points were produced by high dose which—unlike either vehicle (* $p < 0.05$) or high dose (* $p < 0.05$) groups did not differ from sham indicating a beneficial effect on recovery of performance on this task.

In the PBBI model there was an effect of Kollidon VA64 on only a single behavioral task at either dose. Specifically, an intermediate benefit was shown for the high dose group in the probe trial which generated partial points for high dose therapy for this task in PBBI (**Figure 15**).

The histology code was recently broken on this therapy and no effect on either lesion volume or hemispheric tissue loss was seen at either dose in any of the 3 models across OBTT.

Thus, although a few beneficial effects of this therapy were seen on behavioral outcomes, in general Kollidon VA64 did not demonstrate a robust beneficial effect on any of the outcomes assessed in OBTT. As indicated above, analysis and interpretation of the results of serum biomarker data are pending.

Drug 8. AER 271 (a more speculative therapy tested on OBTT-ES)

Treatment of cerebral edema after TBI has largely been accomplished via five traditional strategies, namely, osmolar therapy (mannitol or hypertonic saline administration), CSF drainage, craniectomy, limitation of fluid administration, and diuretics. All of these in essence remove accumulated edema or deal with its consequences. In contrast, since the discovery of aquaporin (AQP) channels in the 1990s, there is hope that drugs targeting them might be developed to prevent the development of brain edema rather than deal with its consequences. Also given the fact that glibenclamide represented the second most positive therapy tested by OBTT to date, study of an additional therapy targeting edema was logical.

AQP4 at the BBB is a potential key target in this regard. AQP4 null mice have reduced edema and improved outcomes in a variety of CNS injury models such as water intoxication, stroke, and meningitis. In TBI, Shi et al (*Neurosci Bull* 28:61-68, 2012) studied that AQP4 KO mouse and showed that at 24h after cryo-injury the KO exhibited reduced edema and neuronal death, but at 7 and 14d, these outcomes were actually worse in the KO vs WT. This study exemplifies the current perspective on the role of AQP4 in CNS injury, namely, it plays a dichotomous role, with an important role in the early accumulation of cytotoxic edema, but a role in the clearance of edema in the delayed phase. This may be due to the bidirectional movement of water through this passive pore. There is also a suggestion that its effects can be further partitioned as AQP4 being involved in the formation of cytotoxic edema but in clearing of vasogenic edema. Several reports discuss this dichotomous role for AQP4, including its role in the recently discovered glymphatic pathway of protein and fluid clearance in models of brain injury and in humans (Verkman et al, *Biochim Biophys Acta* 1758:1085-1093, 2006; Liff et al, *J Neurosci* 34:16180-16193, 2014). There is thus from the standpoint of TBI therapy the theoretical need to consider AQP4 inhibition early but not late. There are two other issues that may reduce potential efficacy of AQP4 blockade after TBI. Binder et al (*Glia* 53:631-636, 2006) reported slowed potassium recovery and longer seizure duration in AQP4 KO mice (vs WT) subjected to stimulation-evoked seizures. Shi et al (*Neurosci Bull* 28:61-68, 2012) showed reduced astrocyte proliferation and glia scarring in AQP4 KO mice, but increase in the magnitude of the delayed microglial response after injury vs WT, suggesting a possible protective role for AQP4 in delayed neuro-inflammation. There also is a link between the danger signal HMGB1 and AQP4 up-regulation, and thus, this strategy might mitigate HMGB1 mediated detrimental effects after TBI which were dramatic in work by Laird et al (*Glia* 62:26-

38, 2014) and inhibited in the report of Xiong et al (*Neurochem Res* 39:216-224, 2014) by the transporter blocker probenecid.

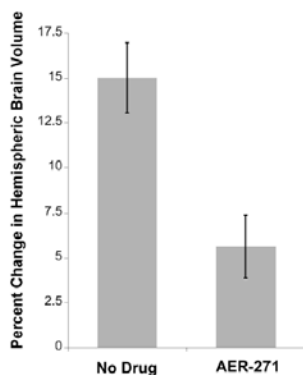


Figure 16. Effect of AER-271 administration on brain swelling after MCAO in mice.

Aeromics has been developing AER-270 and showed that it reduced ICP and brain edema in a murine model of water intoxication. They have an SBIR funded (NINDS) program to develop this and related agents. They also approached the DoD about development of this agent, and were referred to us by programs to consider testing of AER-271 in OBTT. The materials presented by Aeromics were reviewed and it was selected for testing by the OBTT administrative committee as a logical albeit more high risk high reward therapy in the OBTT-ES framework (which has a stated goal of adding study of more novel less well developed [albeit promising] therapies to the low hanging fruit agents currently investigated by the standard OBTT pathway).

AER-271 (Aeromics) is a proprietary AER-270 prodrug developed to increase solubility of this therapy, and was thus evaluated by our consortium in OBTT-ES. AER-271 is

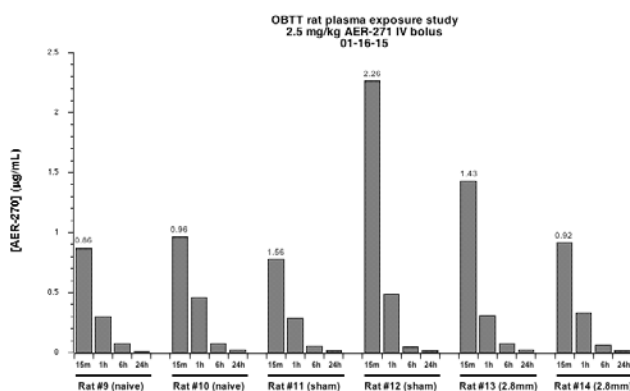


Figure 17. Pilot studies of AER-270 plasma levels in rats after a 2.5 mg/kg dose of the AER-271 pro-drug given over 30 min IV.

rapidly converted to AER-270 and after IP administration—indeed only AER-270 can be detected in blood. AER-271 has also been shown to reduce brain edema in water intoxication in mice and both mortality rate and brain swelling in a mouse model of MCAO (proprietary data). In the MCAO model, mice were treated with a 2 mg/kg IP bolus of AER-271 followed by an 8 µl/h maintenance infusion of a 1mg/mL solution. That regimen produced a 29% reduction in mortality and marked improvements in both brain swelling in the ischemic hemispheric (Figure 16) and neuro-outcome.

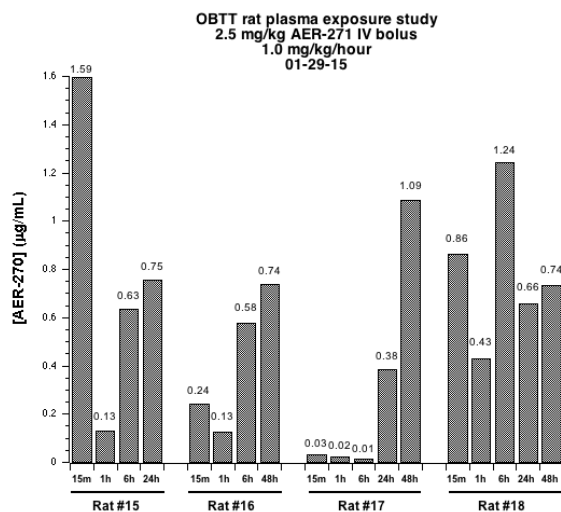


Figure 18. Blood levels of AER-270 in rats after IV administration of the pro-drug AER-271 as a 2.5 mg/kg (over 30 min) bolus followed by a continuous infusion of 1 mg/kg/h IV for 48h. Rat 17 did not receive a bolus. This regimen was used for our studies based on the desire for a target level >0.4µg/mL during the 48 h

We carried out a number of pilot studies of dosing in rats in the CCI model (by Dr. Dixon at the Safar Center). Data from the final 10 rats are shown in **Figures 17 and 18**. The effect of an AER-271 bolus alone on AER-270 plasma levels is shown in **Figure 17**. A 2.5 mg/kg IV bolus of AER-271 produced AER-270 levels between 0.86 and 2.26 µg/mL. The effect of bolus followed by continuous infusion of the pro-drug AER-271 on blood levels of AER-270 is shown in **Figure 18**. The bolus was again 2.5 mg/kg IV over 30 min and followed by an infusion of 1 mg/kg/h for 48h. **A therapeutic target of >0.4 ug/mL is desired and this regimen in general achieved that goal.** AER-270 plasma exposure seen in pilot rat #18 for example would be considered to be ideal.

+ AER-271 (2.5 mg/kg bolus IV beginning 15 min after injury and administered over 30 min, followed

immediately by a continuous IV infusion of 1 mg/kg/h for 48 h). The vehicle (0.07% Tris Base and ~0.84% NaCl [balanced to ~300 mOsm using water or NaCl as needed) was also be provided.

This protocol was used for studies at all sites and no complications were encountered. All the injuries have been completed and all of the behavioral outcome testing has been completed. Data analysis is being carried out currently on the behavioral outcome data and tissue processing is underway for contusion volume and hemispheric tissue loss. Biomarker samples are also being processed. Results for the behavioral outcomes should be available soon, and will be sent to Aeromics prior to release. We anticipating inclusion of all of these data in the upcoming presentation to the INTS conference in early 2016. We are also currently examining the effect of this agent in our murine model of TBI plus hemorrhage and resuscitation (as described previously for glibenclamide at the Safar Center site) to define its effects on brain edema and intracranial hypertension, which could also aid in defining dose optimization.

Drug 9. Amantadine

Amantadine (**Figure 19**) is another low hanging fruit candidate for TBI acute therapy that also has shown benefit in a high quality clinical trial when given in the sub-acute period after injury (Giacino et al, *N Engl J Med* 366:819-826, 2012). That trial was based on a prior publication by Dixon et al (*Restor Neurol Neurosci* 14:285-294, 1999) in CCI in rats. The rationale for amantadine as a therapeutic is based on its impact on three potential effects. First it augments dopaminergic neurotransmission after TBI and dopamine deficits are well known after TBI. Second it acts as a partial NMDA antagonist, and finally it inhibits microglial activation. **A positive trial using amantadine in both the acute and sub-acute periods in OBTT could readily set the stage for more expanded clinical trials of its use in TBI. We also believe that this agent has considerable chance to demonstrate a beneficial effect on cognitive outcome across models, and if so it could represent an excellent drug to include in future trial design that targets combination therapy—which along with cellular therapies, will be one of the areas of focus of our renewal application of OBTT.**

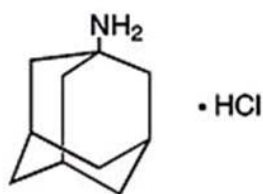


Figure 19. Amantadine HCl chemical structure

There are 5 published papers strongly supporting the use of amantadine in TBI rat models—2 in CCI and 3 in FPI. In the aforementioned seminal paper, amantadine (10 mg/kg IP) or saline vehicle were injected once daily for 18d after injury. The first dose was given one day after TBI. On days where behavioral testing was performed, treatment was given 15 min before testing. Treatment did not improve motor function or CA1/CA3 neuronal survival but significantly improved MWM performance—with a marked effect (a ~50% improvement in latency). In that study, amantadine did not improve MWM performance in shams suggesting a specific effect in TBI. That study was followed by a mechanistic study (Bales et al, *Exp Neurol* 229:300-307, 2011) in rats again treated with amantadine (10 mg/kg IP daily) vs saline which showed that treatment reversed the CCI-induced decrease in DARPP-32 phosphorylation at threonine-34 in striatum. Also, consistent with an NMDA antagonist effect, amantadine decreased threonine-75 phosphorylation. Recently, Wang et al (*J Neurotrauma* 31:370-377, 2014) in the lab of Bruce Lyeth, used a much more aggressive 16d treatment regimen—of either 15 mg/kg IP 3X daily (45 mg/kg/d), or 45 mg/kg IP 3X daily (135 mg/kg/d) and showed improved MWM performance and hippocampal neuronal (CA2-3) survival in the 45 mg/kg group with an almost identical MWM profile (albeit not quite significant) in the 15 mg/kg group. PK data showed that both groups achieved blood levels similar to those seen in humans treated with 100 mg BID. Recently, two studies from China also support efficacy of amantadine after FPI in rats. Huang et al (*PLoS One* 9:e86354, 2014) used a SQ infusion (3.6 mg/kg/h for 8wks) and reported improved rotarod and novel object recognition with treatment. Amantadine also reversed dopamine release deficits after FPI. Finally, Tan et al (*Behav Brain Res* 279:274-282, 2015) again in rat FPI used the regimen developed by Lyeth (45 or 135 mg/kg/d with 3 daily IP injections) and reported benefits on two outcomes related to depression (sucrose preference & immobility). They also reported a reduction in FluoroJade+ and

TUNEL+ neurons in substantia nigra with treatment. Both doses were effective but the higher dose performed better on each outcome.

Based on these studies, and with the focus of this therapy as a TBI rehabilitation agent, we are currently using the approach reported by Dixon et al with a single 10 mg/kg dose of amantadine 15 min prior to behavioral testing. But to evaluate dose response, with a treatment range and PK closer to the human levels, we are also using 45 mg/kg IP as our high dose—mimicking the Lyeth protocol—but once again given 15 min prior to behavioral testing. Based on all of this information, we have taken following approach with amantadine hydrochloride, 10 mg/kg; Sigma A1260:

Groups

- Sham
- TBI + vehicle
- TBI + 10 mg/kg
- TBI + 45 mg/kg

First treatment is on d1 after injury and treatment is administered 15 min before behavioral testing and at a similar time during the day on days when there is no behavioral testing. The vehicle is sterile saline.

This protocol (both doses) was piloted in the CCI model and demonstrated no concerns. Studies are currently ongoing at all sites. We also hope that these data will be able to be included in the upcoming presentation to the INTS conference in early 2016.

Drug 10. Minocycline

Minocycline (**Figure 20**) is a low hanging fruit candidate for TBI. At the time of selection, there were 17

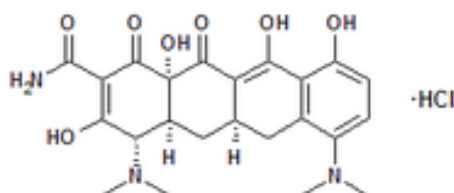


Figure 20: Minocycline chemical structure

manuscripts published on effects in TBI models in rodents (please see OBTT manual of operations for a complete list of references). This also includes a positive report in a blast TBI model (Kovesdi et al, *Front Neurol* 3:111.2012), a beneficial report in combined TBI plus sepsis, and two reports on combination therapy of minocycline plus NAC (Abdel Baki et al, *PLoS One* 5:e1249016, 2010; Haber et al *Exp Neurol* 249:169-77, 2013). There is also an excellent phase II human trial in SCI (Casha et al, *Brain* 135:1224-1236, 2012). There is a feasibility trial (NCT01058395) that is listed on

Clinical Trials.GOV that is purportedly ongoing at Wayne State that is proposed to be completed in 2016. Most of the initial TBI work came from two labs (Morganti-Kossmann, and Plotkine) using closed head injury in mice—with a relatively mild lesion—certainly compared to either CCI or PBBI. There are two negative reports carried out in unusual models from the perspective of TBI, the metallothionein KO mouse, and facial nucleus crush injury. There is a recent study by the Hoane lab (Vonder Haar et al, *J Neurotrauma* 31:961-975, 2014) using oral dosing of 60 mg/kg for 72h that showed no behavioral benefit on MWM but a reduction in lesion volume in CCI. There is a compelling report in CCI that showed that minocycline outperformed progesterone, simvastatin, cyclosporine, and NAC on active place avoidance after CCI—minocycline was then carried forward. In that study, the combination of Minocycline and NAC was highly effective and a dose of 45 mg/kg IP was given at 1, 24 and 48h after CCI. Benefit of that combination was confirmed in a follow up study in a rat CCI model. Highly relevant to OBTT, Kovesdi et al (*Front Neurol* 3:111.2012) from the group of Agoston showed that 4d of treatment with 50 mg/kg improved multiple behavioral outcomes and had beneficial effects on a number of other biomarkers. Also relevant to OBTT, as indicated above, minocycline just completed a Phase II clinical trial in SCI and the results 52 patients randomized to 7 d of IV Minocycline vs. placebo showed that the Minocycline regimen was safe, feasible, and associated with a tendency towards improvement in several outcomes.

The drug is suggested to have multiple effects including inhibition of microglial activation, prevention of oligodendrocyte and/or neuronal apoptosis, and inhibition of other aspects of inflammation such as MMP activation. Beneficial effects on oligodendrocytes has been suggested as a key mechanism of protection in SCI.

Regarding dosing, route of administration and PK, as discussed above, in TBI, IP doses of either 45 mg/kg, 50 mg/kg or 90 mg/kg are given acutely and then either daily or q12 h in most studies for either one to 4d. In experimental TBI, an exception to this approach is by the group of Plotkine who have used a more acute regimen of 90, 45 and 45 mg/kg given a 5 min, 3h, and 9h after TBI, with the 9h dose variably given depending on the study. They reported acute and long term benefit with this regimen. **Given the many studies in TBI, the varying therapeutic targets (neuronal death, neuroinflammation, etc.) and varying dosing regimens, selection of dosing and duration of therapy for minocycline for OBTT are challenging, but we believe extremely important. We thus outlined the plan described below and believe that we have developed a strong protocol for pre-clinical testing.**

The dosing regimen in the human SCI study discussed above was complex; an 800 mg IV load, tapered by 100 mg every 12 h until a plateau of 400 mg was achieved. Target blood level was 7-10 micrograms per mL.

The most comprehensive study on Minocycline PK in rats was published by Fagan et al (*Exp Neurol* 186:248-51, 2004) who reported that peak levels were similar between 20 mg/kg IV vs 90 mg/kg IP but the IP dose produced more sustained increases. However, the IP route produced great variability in drug levels vs the IV approach. The IV dosing route showed low levels by 8 h after a 20 mg/kg dose. Info on physiology post minocycline in TBI is scant, although the PI of OBTT has used it after in several un-published studies in mice without hemodynamic effects, even in mice with hemorrhagic shock.

There are two major problems with IP use of minocycline, namely 1) very erratic blood levels and 2) sclerosing of the peritoneal membrane. **Our collaborators in the University of Pittsburgh School of Pharmacy indicated that the most logical approach is that dosing be done via the IV route and with an approach geared to achieve a concentration of ~7-10 ug/ml (mg/L) because that is what Casha et al (*Brain* 135:1224-1236, 2012) achieved in the clinical spinal cord study.** Matsukawa, et al. (*BMC Neurosci* 10:126, 2009) reported neurotoxicity at high doses *in vitro* and *in vivo*, specifically at 100µM (49.4 mg/L) and 100 mg/kg respectively. Levels in CSF are between 11 and 56% of blood concentrations as reported by Saivin and Hovin (*Clin Pharmacokinet* 15:355-366, 1988).

Our team at the University of Pittsburgh School of Pharmacy also carried out a one-compartment

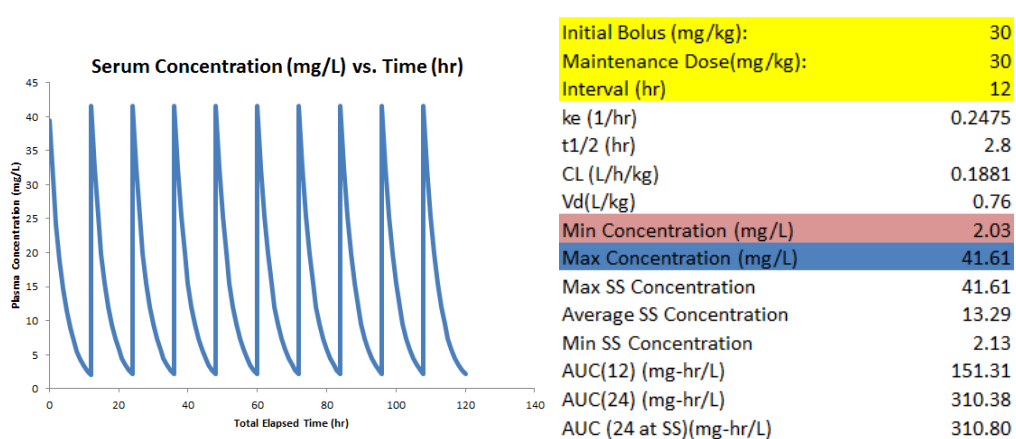


Figure 21. One-compartment predicted PK analysis using a 30 mg/kg dose in rats and based on the reported data of Fagan et al (see text for details).

predicted PK analysis using a 30 mg/kg dose in rats and based on the reported data of Fagan et al discussed previously (**Figure 21**). Based on all of this information, they suggested evaluating in pilots a 30 mg/kg bolus followed by a continuous infusion targeting steady

state levels of ~7 mg/L as suggested in the human spinal cord injury trial.

Duration of therapy is somewhat empiric, but based on all of the stated literature while also recognizing

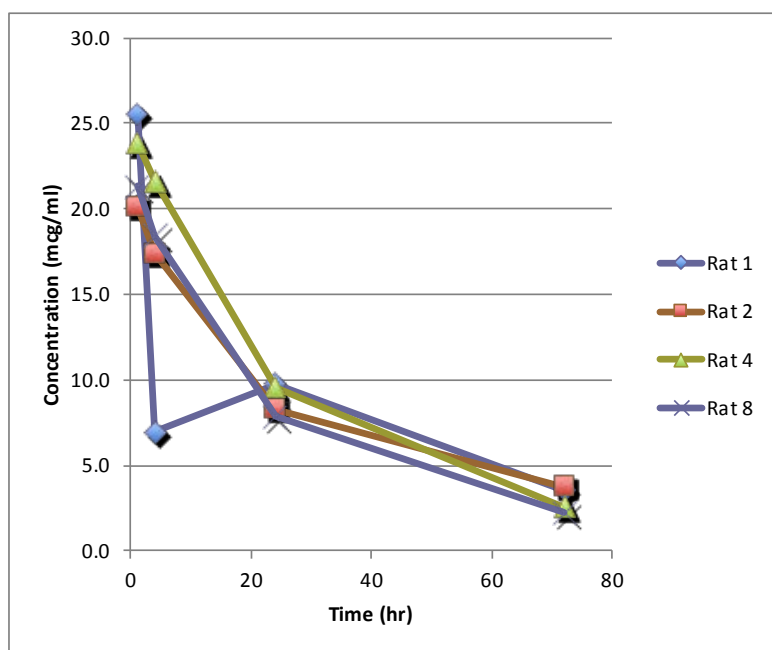


Figure 22. Blood levels of minocycline assessed in pilot rats administered an IV bolus of 30 mg/kg followed by a continuous IV infusion of 2 mg/kg/h for 72h.

the potential limitations of IV drug administration, we proposed treating for 72h. To determine what type of blood levels and PK would be seen with such an approach, within OBTT, Dr. Shear at WRAIR piloted testing of the following protocol for minocycline with serial drug levels measured by our pharmacy team at the University of Pittsburgh School of Pharmacy.

Minocycline hydrochloride (Sigma Catalog # M9511)

15 min after TBI Bolus of 30 mg/kg IV

Followed immediately by an IV infusion at 2 mg/kg/h—the duration of infusion was 72h.

Blood samples were collected at 1, 4, 24, and at sacrifice 72h (final) after injury.

Figure 22 shows the minocycline PK profile produced in rats using this protocol. Peak levels were well below the aforementioned reported toxic range of 100µM (49.4 mcg/mL). And levels in the target concentration of ~7 mcg/mL were seen for at least 48h with levels near or above 5 mcg/mL for the final 24h. **We thus propose using this treatment regimen across sites for testing of minocycline by OBTT. Studies with this therapy will commence as soon as testing of amantadine has been completed.**

Additional therapies and future directions for work in primary screening across the rat models

After minocycline, we will test another highly novel therapy supported by the OBTT-ES funding. We have identified the novel agent P7C3-A20 which has shown promise in several recent studies. It is in the P7C3 class of aminopropyl carbazole agents with potent neuroprotective properties for both newborn neural precursor cells in the adult hippocampus and mature neurons in other regions of the CNS. P7C3-A20 represents a putative therapeutic intervention to augment the survival of newly generated hippocampal neurons and enhance neurogenesis targeting improvement in hippocampal-dependent functional outcomes after TBI. Of note, it was identified using a target-agnostic *in vivo* screen of 1000 compounds to identify small drug-like molecules with neurogenic efficacy and has shown promise in early studies in the FPI model (Blaya et al, *J Neurotrauma* 31:476-486, 2014). We are currently working with Calico Life Sciences to obtain an MTA that will allow the studies to move forward, and we are very optimistic. A similar MTA was successfully obtained with Aeromics for AER 271. **We will discuss P7C3-A20 more in the next OBTT/OBTT-ES report but we believe that testing of this agent dovetails perfectly with our proposed plan to add investigation of cell-based therapies in the OBTT renewal application.**

We had hoped to be able to obtain the drug N-acetyl cysteine amide (NACA) a BBB permeable NAC analog for testing in OBTT-ES, however despite considerable effort and an MTA with Sentient Life Sciences, they were concerned that they would not be able to provide sufficient quantities of the drug for the entire consortium—which precluded our ability to move forward. Others agents with favorable voting at our prior drug selection meetings include edaravone and etanercept, although based on the

mission of OBTT-ES, another more novel agent may be considered depending on funding that remains; OBTT funding ends Sept 29, 2015 and a one-year NCE was requested. OBTT-ES ends Apr 30, 2016.

Additional update of work on circulating biomarkers in OBTT

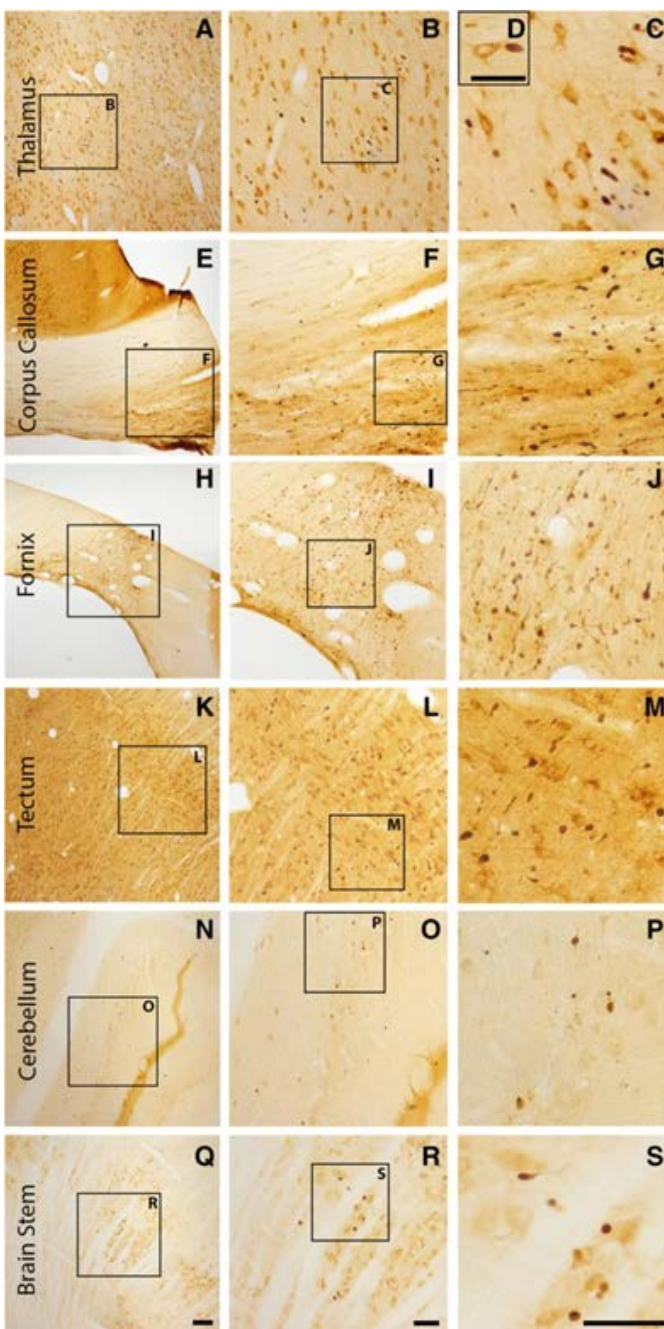


Figure 23: Axonal injury is seen in various regions throughout the micro pig brain after central FPI. Representative photomicrographs of APP immunohistochemistry in regions of the micro pig brain showing DAI in animals sustaining cFPI. Images in the middle panel (B, F, I, L, O, R) are magnified regions indicated in the images of the left panel (A, E, H, K, N, Q) and images in the right panel (C, G, J, M, P, S) are magnified regions indicated in the middle panel (B, F, I, L, O, R), respectively. Scale bar in Q: 200µm; R and S: 100µm; D: 50µm.

central FPI in micro pigs generates the full spectrum of diffuse axonal injury in multiple brain loci (**Figure 23**). These findings are consistent with human head injury, as evidenced by the routine

With regard to the biomarker work, as indicated over 5000 biomarker levels have been measured in rats thus far by Banyan Biomarkers, LLC for the rat studies within the OBTT consortium. In 2015 alone, 1614 samples from 392 rats had biomarker assessments. As outlined in the 8 manuscripts addressing the initial 5 therapies tested (please see manuscripts 4-11 in Reportable Outcomes), including the specific manuscript in that supplement devoted to the biomarker data from those studies (Reportable Outcomes 10), **GFAP has performed extremely well within OBTT and the incorporation of circulating biomarker assessments into the pre-clinical drug screening work by our consortium has been quite fruitful and highly informative.** UCH-L1 has not performed as well as GFAP in our rat and micropig models, although it has provided some insight into the models and cross model comparisons. **We plan to include Tau and/or phosphoTau (P-Tau) as additional serum biomarker(s) in the renewal application for OBTT and work on these assays along with development of GFAP and UCH-L1 assays for the micropig have also been ongoing for OBTT.** Tau is an axonally located microtubule-associated protein. Tau and P-Tau have been implicated in the post-TBI formation of chronic traumatic encephalopathy (CTE). We will also evaluate the feasibility of the axonally located neurofilament-H protein (NF-H) as an alternate biomarker.

Studies in the micropig at the Virginia Commonwealth University site (John Povlishock, PhD, Site PI and Audrey Lafrenaye, Co-I)

In year 5 of the OBTT and OBTT-ES studies the team at Virginia Commonwealth University has continued characterization of the micro pig model of mild diffuse TBI using the central FPI paradigm. These data generated a manuscript that was published recently in the *Journal of Neuroinflammation* (please see manuscript 12 in Reportable Outcomes). We found that

involvement of thalamic and colossal loci 6h post-injury. Importantly, this axonal injury occurred without micro-vascular damage and/or contusion, demonstrating the mild nature of the injury. Further, this axonal injury occurred without attendant systemic physiological abnormalities (please see **Table 1**);

Variable	Group	Pre-injury	Post-injury
Weight	Sham	19.13±4.75	
	TBI	20.12±3.37	
pH	Sham	7.47±0.03	7.48±0.03
	TBI	7.49±0.03	7.52±0.02 *
paCO ₂ mmHg	Sham	39.23±4.20	37.92±1.37
	TBI	40.83±2.81	37.61±1.11
paO ₂ mmHg	Sham	585.25±53.92	359.74±187.80
	TBI	558.50±41.42	492.18±116.09
MABP mmHg	Sham	94.29±14.94	86.13±20.19
	TBI	89.53±8.83	79.90±8.24
Hemoglobin O ₂ (%)	Sham	99.90±1.74x10 ⁻¹⁴	99.19±0.61
	TBI	99.83±0.167	99.50±0.58

Table 1: Systemic physiology was within normal ranges throughout the 6h post-injury monitoring period. TBI: traumatic brain injury; MABP: mean arterial blood pressure; *P<0.05 vs. sham values at same measurement point. Values are mean± SD.

therefore pathology observed can be attributed to the mTBI and not to additional systemic physiological changes. We also observed a robust neuroinflammatory microglial response following injury in the micro pig, the assessment of which will be continued.

In concert with these evaluations, serum was collected for ongoing biomarker assessments. This model positions us to directly correlate the biomarker analyses with the ongoing pathology that could ultimately guide our interpretation of biomarker efficacy as potential clinical markers of injury. In addition to the above,

we have also conducted functional studies in the micro pig's pial microcirculation, exploring the vasculature reactivity to a known vasodilator, acetylcholine. Despite the mild level of injury, all micro

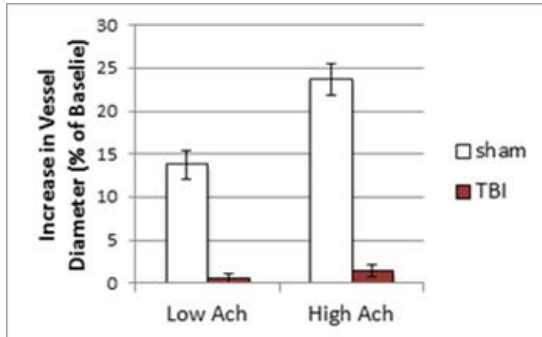


Figure 24: Pial vessel vasodilatory reactivity to acetylcholine (ACh) is lost with mTBI in the micro pig. Graph depicting the percent increase in vessel diameter in response to low or high dose Ach.

pigs to date demonstrate a loss of vasoreactivity to acetylcholine (**Figure 24**). Collectively, based on all the above, we feel well positioned to continue screening multiple therapies to determine their potential protective impact upon multiple clinically relevant endpoints.

Finally, as previously discussed, levetiracetam, the most promising agent tested thus far by OBTT was advanced to the micro pig model for testing. To date, fourteen micro pigs have been administered high dose levetiracetam (170mg/kg) or saline IV from 15min to 1hr following injury, maintaining the rigorous administration paradigm utilized in the rodent models. All animals were assessed for systemic physiological stability and stringently maintained within physiological normal ranges. Assessments of axonal injury,

neuroinflammatory response, vasoreactivity, and biomarker analysis are currently underway.

KEY RESEARCH ACCOMPLISHMENTS Since THE INCEPTION OF OBTT—Accomplishments for this funding year (ongoing or new) are bolded for convenience of the reviewer.

1. IACUC and ACURO Approval at all sites along with necessary updates
2. Creation and continual updating of an Operations Manual for the OBTT consortium by Dr. Kochanek
3. Monthly consortium investigator conference calls
4. TBI drug therapy literature review, investigators survey, and selection of therapies to be evaluated by the OBTT consortium (ongoing)
5. Comprehensive review of the TBI literature for the first nine drugs, nicotinamide, EPO, CsA, Simvastatin, Levetiracetam, Glibenclamide, Kollidon VA64, AER 271, Amantadine, Minocycline, P7C3-A20, NACA, NIM-811, Edaravone, and Etanercept, among many others by Dr. Kochanek, with updating of the manual through the most current agent (IACUC and ACUROs either submitted or approved at all sites).
6. Publication of 3 manuscripts on 1) the OBTT concept in the *Journal of Trauma*, and on therapy reviews germane to the US Army (see Reportable Outcomes 1-3)
7. **Biomarker assessments of >5000 rat samples, including 1614 in 2015.**
8. **Biomarker assay development for micropig assays of GFAP and UCH-L1**
9. **Presentation of 50 abstracts and/or National or International presentations since the inception of OBTT including 12 in year 5**
10. Report sent by Dr. Kochanek on the launching of OBTT to the Therapy and Oversight Committee and Consultants
11. Dr. Kochanek represented OBTT at the US Army Neurotrauma, Pharmacology Work Group. He was the second author of the comprehensive document generated by that group and recently published in the *Journal of Neurotrauma*.
12. Preparation of a full grant application titled Operation Brain Trauma Therapy-Extended Studies requested by CCCRP. Dr. Kochanek prepared the application.
13. **Completion of all experiments for drugs #1 (nicotinamide), #2(EPO), #3 (CsA), #4 (Simvastatin), #5 (Levetiracetam), #6 (Glibenclamide), #7 (Kollidon VA 64), #8 (AER 271), #9 (Amantadine—ongoing) and preliminary PK studies on #10 (Minocycline)—in primary screening across three rodent models with ~2000 rats studied. In addition, literature review and MTA preparatory work for #11 (P7C3-A20) and preparatory and MTA work for NACA.**
14. Investigators meeting held on at the 2011-15 National Neurotrauma Society Meeting
15. Presentation of a panel on OBTT by the PI and two site PIs (Shear and Mondello) at the 2015 meeting of the National Neurotrauma Society.
16. Presentation of a platform talk at the 2015 MHSRS by Dr. Mondello on the biomarker results in OBTT.
17. Re-establishment and continued refinement of the large animal micropig model of FPI TBI at Virginia Commonwealth University with publication of a manuscript on the neuroinflammatory response in that model in the *Journal of Neuroinflammation*.
18. Biomarker studies in the large animal micropig model to characterize parallel markers in that model in 2015.
19. Detailed characterization of axonal injury and the associated inflammatory response in the micropig model (ongoing).
20. Testing of Levetiracetam therapy in the micropig model (ongoing).
21. Submission of 8 manuscripts by the OBTT investigators for invited submission as a special issue of the *Journal of Neurotrauma* devoted to OBTT. All manuscripts have been accepted and are in press.
22. Submission of a manuscript on the theranostic applications of circulating biomarkers of brain injury in pre-clinical drug screening for TBI for the *Journal of Trauma* (in preparation by Dr. Mondello)
23. Invitation to present a plenary lecture at the 2016 conference of the International Neurotrauma Society, February 4, 2016, Cape Town South Africa (Dr. Kochanek).

REPORTABLE OUTCOMES (All reportable outcomes since project inception are shown)

Manuscripts

1. Kochanek PM, Bramlett H, Dietrich WD, Dixon CE, Hayes R, Povlishock J, Tortella F, Wang K: A novel multi-center pre-clinical drug screening and biomarker consortium for experimental traumatic brain injury: Operation Brain Trauma Therapy. *J Trauma* 71(1 Suppl):S15-24, 2011.
2. Diaz-Arrastia R, Kochanek PM, Bergold P, Kenney K, Marx C, Grimes JB, Loh Y, Adam GE, Oskvig D, Curley KC, Salzer W: Pharmacotherapy of Traumatic Brain Injury: State of the Science and the Road Forward: Report of the Department of Defense Neurotrauma Pharmacology Workgroup. *J Neurotrauma* 31:35-58, 2014.
3. Kochanek PM, Jackson TC, Ferguson NM, Carlson SW, Simon DW, Brockman EC, Ji J, Bayir H, Poloyac SM, Wagner AK, Kline AE, Empey PE, Clark RSB, Jackson EK, Dixon CE: Emerging Therapies in Traumatic Brain Injury. *Seminars in Neurology* 35:83-100, 2015.
4. Kochanek PM, Bramlett HM, Dixon CE, Shear DA, Dietrich WD, Schmid KE, Mondello S, Wang KKW, Hayes RL, Povlishock JT, Tortella FC: Approach to Modelling, Therapy Evaluation, Drug Selection, and Biomarker Assessments, for a Multi-Center Pre-Clinical Drug Screening Consortium for Acute Therapies in Severe Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
5. Shear DA, Dixon CE, Bramlett HM, Mondello S, Dietrich WD, Deng-Bryant Y, Schmid KE, Wang KKW, Hayes RL, Povlishock JT, Kochanek PM, Tortella FC. Nicotinamide Treatment in Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
6. Bramlett HM, Dietrich WD, Dixon CE, Shear DA, Schmid KE, Mondello S, Wang KKW, Hayes RL, Povlishock JT, Tortella FC, Kochanek PM. Erythropoietin Treatment in Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
7. Dixon CE, Bramlett HM, Dietrich WD, Shear DA, Yan HQ, Deng-Bryant Y, Mondello S, Wang KKW, Hayes RL, Empey PE, Povlishock JT, Tortella FC, Kochanek PM: Cyclosporine Treatment in Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
8. Mountney A, Bramlett HM, Dixon CE, Mondello S, Dietrich WD, Wang KKW, Caudle K, Empey PE, Poloyac SM, Hayes RL, Povlishock JT, Tortella FC, Kochanek PM, Shear DA: Simvastatin Treatment in Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
9. Browning M, Shear DA, Bramlett HM, Dixon CE, Mondello S, Schmid KE, Poloyac SM, Dietrich WD, Hayes RL, Wang KKW, Povlishock JT, Tortella FC, Kochanek PM: Levetiracetam Treatment in Traumatic Brain Injury: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
10. Mondello S, Shear DA, Bramlett HM, Dixon CE, Schmid KE, Dietrich WD, Wang KKW, Hayes RL, Glushakova O, Catania M, Richieri SP, Povlishock JT, Tortella FC, Kochanek PM. Insight into Preclinical Models of Traumatic Brain Injury Using Circulating Brain Damage Biomarkers: Operation Brain Trauma Therapy. *J Neurotrauma*, in press.
11. Kochanek PM, Bramlett HM, Shear DA, Dixon CE, Mondello S, Dietrich WD, Hayes RL, Wang KKW, Poloyac SM, Empey PE, Povlishock JT, Mountney A, Browning M, Deng-Bryant Y, Yan HQ, Jackson TC, Catania M, Glushakova O, Richieri SP, Tortella FC: Synthesis of Findings,

Current Investigations, and Future Directions: Operation Brain Trauma Therapy. J Neurotrauma, in press.

12. Lafrenaye AD, Todani M, Walker SA, Povlishock JT: Microglia processes associate with diffusely injured axons following mild traumatic brain injury in the micro pig. J Neuroinflammation, in press.

Abstracts and presentations

1. Kochanek PM, Dixon CE: Operation Brain Trauma Therapy (OBTT) Consortium: Program Overview/University of Pittsburgh Program. Presented at the ATACCC Meeting, Ft. Lauderdale, FL, August 15-19, 2011.
2. Bramlett HM, Dietrich WD: Operation Brain Trauma Therapy (OBTT) Consortium: University of Miami Miller School of Medicine Program. Presented at the ATACCC Meeting, Ft. Lauderdale, FL, August 15-19, 2011.
3. Shear DA, Schmid KE, Tortella FC: Operation Brain Trauma Therapy (OBTT) Consortium: The WRAIR Program (Penetrating Ballistic-Like Brain Injury). Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference, Fort Lauderdale, FL, 2011.
4. Povlishock, JT: Operation Brain Trauma Therapy: The Virginia Commonwealth University Program. Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference in Fort Lauderdale, FL, 2011.
5. Wang KKW, Hayes RL: Operation Brain Trauma Therapy (OBTT) Consortium: Banyan Biomarkers Core. Presented at the Advanced Technology Applications to Combat Casualty Care (ATACCC) Conference in Fort Lauderdale, FL, 2011.
6. Kochanek PM: Operation Brain Trauma Therapy (OBTT). Oral plenary presentation, 2012 Congress of the National Neurotrauma Society, Phoenix, AZ, July, 2012.
7. Kochanek PM, Bramlett H, Dixon CE, Shear DA, Mondello S, Schmid K, Dietrich WD, Wang KK, Hayes RL, Tortella FC: Cross model comparison of behavior, neuropathology, and serum biomarkers after controlled cortical impact, parasagittal fluid percussion, and penetrating ballistic-like brain injury: results from Operation Brain Trauma Therapy. J Neurotrauma 29:10, A23-A23, 2012.
8. Mondello S, Bramlett HM, Dixon CE, Shear DA, Zhang Z, Zoltewicz S, Schmid K, Dietrich WD, Wang KKW, Hayes R, Tortella FC, Kochanek PM: Differential effect of nicotinamide on serum damage marker profiles following controlled cortical impact, parasagittal fluid percussion, and penetrating ballistic-like brain injury: results from Operation Brain Trauma Therapy. J Neurotrauma 29:10, A48-A48, 2012.
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10. Shear DA, Pedersen R, Sun J, Long M, Schmid K, Tortella FC: Operation Brain Trauma Therapy consortium: dose-response evaluation of nicotinamide in the WRAIR model of penetrating ballistic-like brain injury. J Neurotrauma 29:10, A72-A73, 2012.
11. Dietrich WD, Bramlett H, Furones-Alonso O, Sanchez-Molano J, Sequeira D, Moreno W: Assessment of nicotinamide on outcome after fluid percussion brain injury: an Operation Brain Trauma Therapy study. J Neurotrauma 29:10, A165-A165, 2012.

12. Kochanek PM, Bramlett H, Dixon CE, Shear DA, Mondello S, Schmid K, Dietrich WD, Wang KK, Hayes RL, Tortella FC: Cross model comparison of behavior, neuropathology, and serum biomarkers after controlled cortical impact, parasagittal fluid percussion, and penetrating ballistic-like brain injury: results from Operation Brain Trauma Therapy. Proceedings of the MHSRS, Ft. Lauderdale, FL, August, 2012.
13. Mondello S, Bramlett HM, Dixon CE, Shear DA, Zhang Z, Zoltewicz S, Schmid K, Dietrich WD, Wang KKW, Hayes R, Tortella FC, Kochanek PM: Differential effect of nicotinamide on serum damage marker profiles following controlled cortical impact, parasagittal fluid percussion, and penetrating ballistic-like brain injury: results from Operation Brain Trauma Therapy. Proceedings of the MHSRS, Ft. Lauderdale, FL, August, 2012.
14. Kochanek PM: Operation Brain Trauma Therapy (OBT). Oral plenary presentation, Proceedings of the MHSRS, Ft. Lauderdale, FL, August, 2012.
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Conclusion

The unique multicenter pre-clinical drug screening consortium OBTT supported by both the parent OBTT grant and the linked OBTT-ES grant continues to be highly productive. This year 8 manuscripts comprising a special issue of the *Journal of Neurotrauma*, and addressing the results of the first 5 therapies has been submitted, all of the manuscripts have been accepted by the journal. In addition, a manuscript on the micropig model is in press in the *Journal of Neuroinflammation*. The consortium has completed studies on 9 therapies and has performed the PK studies to launch the 10th therapy. Two drugs have shown promise as tested by OBTT. Levetiracetam has shown benefit on a number of outcomes in 2 of the 3 models while glibenclamide has shown benefit on a number of outcomes in the CCI model. This suggests that Levetiracetam may have utility and it deserves to be investigated across the injury spectrum in TBI. Levetiracetam is currently being tested in the micropig model. It also suggests that glibenclamide may have special utility in the setting of cerebral contusion and deserves to be studied further in that specific setting. Given the safety profile of both of these agents and prior clinical use both would be able to be used in clinical trials in TBI in a seamless fashion. It is OBTT's recommendation that they be considered for clinical investigation as described in this report. Finally, with regard to biomarkers, GFAP has performed extremely well in the rat models from both a diagnostic and theranostic perspective. Our data suggests that GFAP may be useful in preclinical studies to serve as a screening tool which is much less labor intensive than assessing lesion volume. Our findings also parallel what appears to be successful development of GFAP in clinical TBI that is emerging. As outlined in the future directions section above, additional therapies are being evaluated by the consortium and not all data on the aforementioned therapies is complete. We anticipate being able to assemble and publish a second special issue of the *Journal of Neurotrauma* based on these additional drug studies across the consortium.

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Please note that references were incorporated directly into the text.