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14. ABSTRACT

Traumatic brain injury is divided into multiple phases. The primary injury phase of TBI is caused by mechanical stress applied to the tissue and causes irreversible damage. In contrast, secondary injury progresses over the following hours, providing an opportunity to save damaged tissue and reduce brain injury. We propose to apply a novel near infrared light (NIR) technology developed in the Sanderson and Hüttemann laboratories, to target the cellular injury processes that occur during the secondary phase and reduce overall brain damage.

This proposal addresses the focus areas of the FY22 CDMRP Traumatic Brain Injury and Psychological Health Research Program, Translational Research Award. The goal of this proposal is to reduce the brain injury following TBI with NIR therapy. We have previously shown that this technology limits toxic oxidative damage in the brain following ischemic stroke and following cardiac arrest. This therapy also provides a reduction in brain injury in both brain injury pathologies. Here, we discovered that the application of the infrared light technology also limits the progression of brain injury following trauma. Importantly, this treatment is noninvasive and without side-effects, therefore it offers significant advantages to other potential therapeutic approaches for TBI.

In this proposal we will accomplish several important steps that are necessary for this therapy to be used on humans in the future. We will use our large animal model of brain trauma to test the ability of NIR to reduce brain injury, a critical step before a new therapy can be transitioned into the clinic and tested on humans. We will also use our mouse traumatic brain injury model to conduct a detailed investigation of how NIR reduced brain injury. Finally, we will develop a human clinical device composed of a dual-laser system, a connector, and a durable helmet that deliver the NIR in the battlefield.

If successful, as suggested by our preliminary experiments, this new therapeutic approach could become a safe and non-invasive therapy for reducing brain damage after trauma. The therapy can be easily and flexibly delivered in the military and civilian hospital setting, vessels including aircraft carriers, and in the field. We expect that our therapy will reduce hospital and intensive care time, expedite rehabilitation, with a profound beneficial effect on the patients, their families, and society as a whole.

15. SUBJECT TERMS

Traumatic brain injury, neuroprotection, mitochondria, near infrared light, reactive oxygen species, medical device

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1. Introduction

This Progress Report details the progress for year 1 of our study titled “Robust Neuroprotection with Noninvasive Near Infrared Light: Translational Development of a Treatment for TBI” funded by the Translational Research Award through the Traumatic Brain Injury and Psychological Health Research Program to address the focus area related to developing a therapeutic device to treat traumatic brain injury (TBI). According to the Defense Health Agency, TBI has become a leading cause of death and lasting disability among active-duty military personnel and veterans. TBIs are heterogenous injuries that vary in severity, location and subtype. The molecular mechanisms that contribute to progressive cell death are highly complex, redundant and overlapping. Importantly, TBI is a chronic disease, characterized by progressive development of injury and associated with lasting symptoms. While this can result in a debilitating and serious condition, it provides a window-of-opportunity to limit cell death after the initial TBI. Nonetheless, there are no interventions that provide protection against cellular injury. Accordingly, there is a critical unmet need to advance our understanding the molecular mechanisms of injury and develop new treatment strategies. We have previously demonstrated robust neuroprotection with infrared light (NIR) as a treatment for post-ischemic brain injury. Accordingly, we hypothesized that, due to molecular overlap between the injury mechanisms with ischemic cell death, our NIR device – called LUCID (Light Utilizing Cytochrome c oxidase Inhibitory Device) – could provide protection against TBI.

Note: Our project was funded by exercising the Early-Career Investigator Partnering Option. Accordingly, this progress update will include a combined report describing accomplishments on the two parallel and related studies, TP210529 and TP210529P1.

2. Keywords

Traumatic brain injury

Neurotrauma

Controlled cortical impact

Cytochrome c oxidase

Near infrared light

3. Accomplishments

Major goals for TP210529 includes Specific Aims 1 and 2, and TP210529P1 includes specific Aims 3 and 4.

MAJOR GOALS – SPECIFIC AIM 1, SPECIFIC AIM 2 – WIDER LABORATORY

The major goal of Specific Aim 1 and 2, conducted by the Wider Laboratory, are to evaluate the efficacy and safety of LUCID device in a translational model of focal TBI in swine and optimize the treatment paradigm. Device evaluation in Aim 1 includes identifying the most effective dose (i.e. light intensity) in the context of swine neurotrauma. Aim 2 focuses on identifying the effect of alternative treatment paradigms on the efficacy of NIR treatment. To test LUCID, we will induce TBI using the controlled cortical impact model followed by treatment with or without NIR. During recovery from surgery, cognitive outcomes will be analyzed with motor and memory tasks, and blood will be collected for biomarker analysis. After ten days, the brain is imaged with MRI and harvested for histological processing and analysis.

Specific Aim 1: Identify Light Utilizing COX Inhibitory Device (LUCID) treatment parameters that maximize neuroprotection in a translational model of TBI. Specifically, Aim 1 will identify the optimal NIR dose by evaluating neuroprotective efficacy of multiple power densities, including 0.5 Watts, 1.0 Watts and 2.0 Watts.

Subtask 1.1 Obtain IACUC approval through the University of Michigan IACUC to conduct experimental study in swine – to be completed by December 2022. We completed subtask 1.1 and obtained IACUC approval on June 14 2022 – IACUC approval ID#: PRO00010876. *Subtask 1.2 Obtain ACURO approval for the work being performed under this award – as described in the to-be approved institutional IACUC protocol* – to be completed by March 2023. We completed subtask 1.2 and obtained ACURO approval in August 2022 - USAMRDC Protocol ID#. TP210529.e001. *Subtask 1.3 Successful (blinded and randomized) enrollment to achieve required group sizes determined by power analysis.* According to Subtask 1.3, 50 animals are scheduled to be enrolled by May 2024. Currently, we have enrolled 11 (22% of the estimated total) subjects into the study and we are on track to complete enrollment by the deadline outlined in the SOW. *Subtask 1.4*

Identification of the optimal LUCID dosage. This will include but is not limited to (i) histopathological analysis of neurological injury (which may include but is not limited to) using Cresyl Violet Nissl stain, Fluoro-Jade C, and Luxol Fast and MRI (shown in figure 1), and 4-HNE (ii) quantification of biomarkers of neurotrauma, including GFAP, UCH-L1, NF-L and tau and (iii) neurocognitive function, analyzed by tasks such as gait analysis, open field/novel object test, and treat retrieval – to be completed by September 15, 2024. Neurocognitive evaluation shown in Fig 2. We have initiated subtask 1.4 and are completing the tasks in parallel with Subtask 1.3, and estimate that it will be completed by the deadline outlined in the SOW.

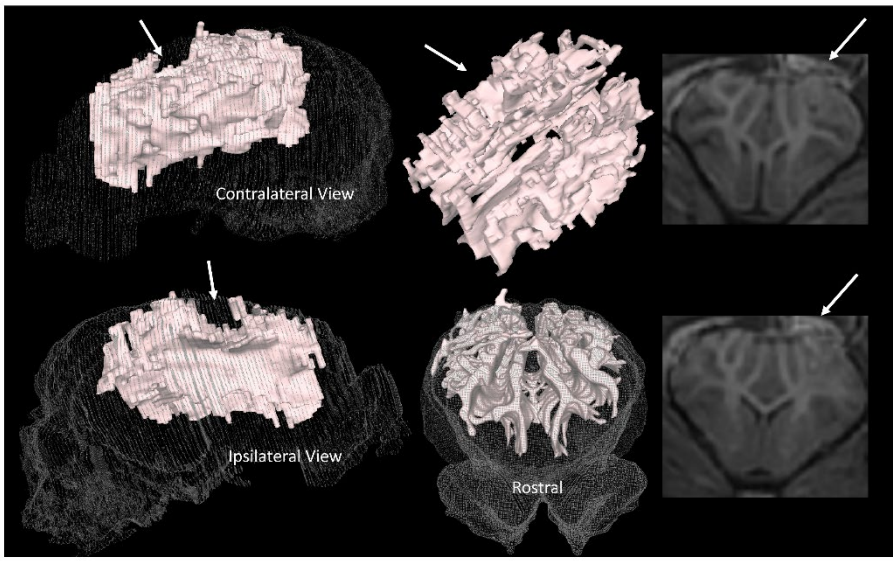

Fig 1. MR Imaging and analysis. MRIs are processed using machine learning to segment whole brain and white/gray matter to determine structural changes.

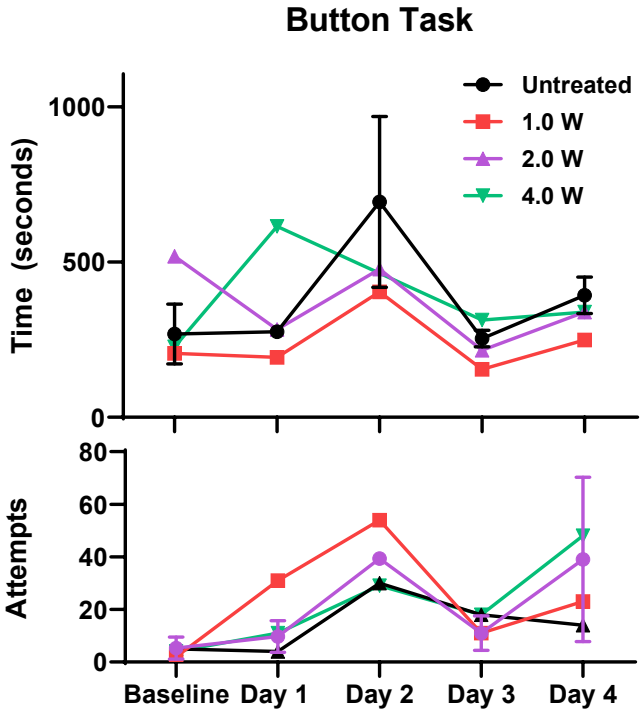

Fig 2. Ongoing Memory Task Results. Using a button test, memory is evaluated by the ability to learn the association between pressing a button with reward. After baseline training, numbers are the time in seconds it takes animals to empty the button device of food rewards. n=1-4/group

Specific Aim 2. Determine the efficacy of alternative treatment paradigms on neuroprotection to simulate conditions for clinic use and forward deployment. The treatment paradigm has not been optimized for the application of LUCID in swine TBI. Environmental variables may have positive or negative effects on treatment. Accordingly, Specific Aim 2 focuses on testing the impact of increased treatment duration and delayed treatment administration. This study will include a group with a 4-hour delay in treatment and a group with an extended treatment of 8-hours.

Subtask 2.1 Successful (blinded and randomized) enrollment that achieves required group sizes determined by power analysis. Subtask 2.1 is projected to be initiated September 2024. *Subtask 2.2 Explore the effect of alternative treatment paradigms on the neuroprotective effect of LUCID treatment. Alternative treatment paradigm groups will include delayed application of treatment and extended duration of treatment.* Subtask 2.2 is projected to be started September 2024. *Subtask 2.3 Complete evaluation of brain injury through histological analysis (Cresyl Violet nissl stain, Fluoro-JadeC, Luxol Fast Blude stain, MRI and 4-HNE), neurotrauma biomarker analysis (GFAP, UCH-L1, NF-L and tau), and neurocognitive function (gait analysis, open field/novel object test and treat retrieval.* Subtask 2.3 will be started in February 2025.

MAJOR GOALS – SPECIFIC AIM 3, SPECIFIC AIM 4 – SANDERSON LABORATORY

The major goals of Specific Aim 3 and 4, conducted by the Sanderson Lab, are to interrogate the molecular mechanisms of LUCID treatment in the context of TBI and enhancement of the LUCID device design for specific use by the military. Aim 3 focuses on utilizing robust biochemical analysis of the effects of TBI on mitochondrial function and how LUCID therapy can modulate mitochondrial function. Transgenic mice expressing a mitochondrial-targeted oxidation marker will be used to monitor the effect of TBI on oxidative damage and the antioxidative effect of NIR treatment. Aim 3 also is focused on quantifying the change in mitochondrial function after TBI and how NIR mitigates these changes. In Aim 4, the LUCID device is to be modified in collaboration with our engineering team.

Specific Aim 3. Interrogate the mechanism of mitochondrial dysfunction in TBI and LUCID-induced protection. The molecular mechanisms of TBI are complex and heterogenous, however mitochondria and oxidative damage are considered common and primary contributors. Emerging evidence has indicated that mitochondrial hyperpolarization drives generation of oxidative radicals responsible for acute neurological injury, however this mechanism is less clear in TBI. LUCID technology harnesses the biological properties of NIR to modulate mitochondrial function to reduce hyperpolarization. Molecular markers and mitochondrial function assays are used to explore the role of mitochondria in TBI and the protective mechanism of LUCID.

Subtask 3.1 Obtain IACUC approval through the University of Michigan IACUC for experimental study in mice. Subtask 3.1, which was scheduled to be completed by December 2022, was completed in June 2022

– IACUC Protocol ID#: PRO00010879. *Subtask 3.2 Obtain ACURO approval for the work being performed under this award – as described in the to-be approved institutional IACUC protocol.* Subtask 3.2 was completed in August 2022 – ACURO Protocol ID#: TP210529P1.e001. *Subtask 3.3 Successful (blinded and randomized) enrollment that achieves required group sizes determined by power analysis and initiate TBI in mice using the controlled cortical impact model.* Subtask 3.3 has been initiated and is underway and is scheduled for completion in November 2023. Groups include shams, TBI with brain collected at 2, 6 and 24 hours after injury and TBI + LUCID (A) collected at 2, 6, and 24 hours after injury. *Subtask 3.4 Measure cellular respiration using oxygen electrode in mitochondria and cytochrome c oxidase isolated from TBI brains to evaluate the effect of LUCID treatment on TBI-induced deficits in mitochondrial respiration.* Subtask 3.4 is underway, utilizing tissue from TBI experiments and include respirometry and cytochrome *c* oxidase in-gel activity assays. Subtask 3.4 is scheduled to be completed by November 2023.
Subtask 3.5 Characterize supercomplex formation in TBI brains and how LUCID reverses supercomplex breakdown. Subtask 3.5 is underway and is scheduled to be completed by November 2023.

Subtask 4.1 Histological assessment of mitochondrial oxidation at multiple time points after caused TBI using MitoTimer, a genetic marker for oxidative stress in mitochondria. Subtask 4.2 Immunohistochemical analysis of tissue oxidative stress by detecting 4-HNE marker of lipid peroxidation. Subtasks 4.1 and 4.2 are scheduled to be completed by September 2025. We are on schedule, with mouse colonies being expanded to have adequate numbers to complete this aspect of the study.

Specific Aim 4. Human Medical Device Development for Specific Use by the Military for TBI. LUCID technology is in advanced stages of preclinical and translational development for neonatal, pediatric and adult cerebral ischemia-reperfusion injury. The primary objective of Aim 4 is to collaborate with a medical device manufacturer to modify the current LUCID system for specific use in adult TBI and military use.

Subtask 5.1 Collaborate with Lumitex, Inc. to initiate translational design plan: (a) Conduct voice of customer studies to document new adult military patient application user requirements. (b) Define use cases and updated system requirements. (c) Create verification plan including new TTM subsystem and new military use specification requirements. We have established a relationship with Lumitex, Inc. and have been working

to enhance the adult prototype design to increase the power capabilities for use in adult patients, including the TTM subsystem. Subtask 5.1 was scheduled to be completed by May 2023, and has been completed. Subtask 5.2 Collaborate with Lumitex, Inc. to design new LUCID cap design, is scheduled to be completed by September 15, 2024. Subtask 5.3 Fabricate β-prototype LDUs, light guide and cap for verification testing and integration into adult patient helmet, scheduled to be completed by September 15, 2024. Subtask 5.4 Perform subsystem and full-system verification testing, is scheduled for completion September 15, 2025.

4. Impact

What was the impact on the development of the principal discipline(s) of the project? Specific Aims 1 and 2 are utilizing the controlled cortical impact model of TBI in swine. To detect treatment effects, we are utilizing a variety of metrics, including histopathology, neuroimaging, biomarkers and neurocognitive assessment tasks. In addition to the potential of advancing neuroprotective treatment, integrating these methods will provide insight into the pathophysiology of this model, as well as TBI in general. Understanding the relationship between these metrics could lead to more efficient and robust injury evaluation in translational models.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems

Nothing to report.

6. Products

Nothing to report.

7. Participants & Other Collaborating Organizations

Name: Joseph Wider
Project Role: Principal Investigator
Researcher Identifier: 0000-0002-2859-4846
Effort: 4.8 months
Contribution to Project: Directly/indirectly managing every aspect of project. Conduct specific aims 1 & 2.

Name: Thomas Sanderson
Project Role: Principal Investigator

Researcher Identifier: 0000-0003-0112-8164
Effort: 1.8 months
Contribution to Project: Directly/indirectly managing projects. Conduct specific aims 3 & 4.

Name: Jennifer Mathieu
Project Role: Licensed Veterinary Technician
Effort: 6.0 months
Contribution to Project: Contributes to specific aim 1 as veterinary technician, providing surgical and large animal care.

Name: Erin Gruley
Project Role: Laboratory Technician
Effort: 2.4 months
Contribution to Project: Contributes to general laboratory matters, data acquisition and analysis.

Name: Maik Hüttemann
Project Role: Subcontract Principal Investigator
Researcher Identifier: 0000-0001-6310-7081
Effort: 0.5 months
Contribution to Project: Subcontract manager, contributing to Specific Aim 3.

Name: Tasnim Arroum
Project Role: Research Fellow
Effort: 3.0 months
Contribution to Project: Contributing to Specific Aim 3, conducting biochemical analyses of mitochondrial related endpoints.

Name: Robert Neumar
Project Role: Key personnel
Researcher Identifier: 0000-0001-7942-8496
Effort: 0.6 months
Contribution to Project: Contributes to study oversight and provides perspective.

Name: Fred Korley
Project Role: key personnel
Researcher Identifier: 0000-0003-4920-8278
Effort: 0.6 months
Contribution to Project: Contributes to study oversight and provides perspective.

Name: Hayley Zaroff, Keegan Kochanek, Caroline Carothers
Project Role: Undergraduate technicians
Effort: Hourly
Contribution to Project: Late night/overnight large animal postoperative care.

Subcontract Progress:

Organization Name: Wayne State University

Location of Organization: Detroit, Michigan USA

Contribution Type: Collaboration

This study includes a subcontract to be carried out by the Hüttemann Laboratory at Wayne State University in Detroit. This portion of the study is part of Major task 3 and will include biochemical analysis of mitochondrial function following TBI and LUCID treatment. Samples have been generated in the

Sanderson laboratory, using the controlled cortical impact model of TBI in mice. Tissue is frozen and sent to the Hüttemann Laboratory for measurement of mitochondrial respiration, cytochrome *c* oxidase activity and supercomplex formation of the respiratory chain complexes. This subcontract is on track and should be completed on schedule.

8. Special Reporting Requirements

Nothing to report.

9. Appendices