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Validation of the Treatment Outcomes of a Fixed-Dose Combination Therapeutic in a High-Fidelity Porcine Model of Traumatic Brain Injury

PRINCIPAL INVESTIGATOR: Bruce May

CONTRACTING ORGANIZATION: IRR, Inc.

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Fort Detrick, Maryland 21702-5012

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**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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U.S. Army Medical Research and Development Command
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**14. ABSTRACT**

**Background:** Traumatic Brain Injury (TBI) is a multi-system disorder involving complex interactions between the brain and immune system. As such, mitigation of inflammation is a cornerstone of TBI treatment. Normally, microglia in the injured brain can produce anti-inflammatory mediators by scavenging cellular debris and orchestrating neurorestorative processes to promote post-TBI neurological recovery. However, microglia can become dysregulated and produce excessive proinflammatory mediators, which exacerbate brain damage by impeding brain repair and neurological functional recovery. Persistent neuroinflammation in TBI patients may contribute to progressive and long-lasting impairment to their physical, cognitive, behavioral, and social performance. Anti-inflammatory cytokines or interventions can counteract and downregulate these inflammatory and cytotoxic reactions. As one example, acute or delayed treatment with the partial anti-inflammatory medicine anatabine in a mouse TBI model improved long-term spatial memory and reduced pathology at later time points. A recent clinical case study (Dr. Chandler May, MD, JD, MS, FCLM) demonstrated the potential efficacy of levocetirizine (Xyzal ®), a well-established FDA-approved anti-inflammatory drug – a highly selective H1 receptor antagonist (antihistamine), in synergy with the leukotriene receptor antagonist montelukast (Singulair ®) in improving TBI outcomes. Thus, early treatment to control inflammation may reduce secondary injury cascades and improve recovery/prognosis. The development of TBI therapies has been challenged by the heterogeneity of TBI and inadequate modeling. To date, single-target anti-inflammatory therapeutics have failed to improve TBI outcomes in numerous clinical trials.

**Overall Objective:** Inflammatory Response Research, Inc. (IRR) is developing an anti-inflammatory formulation as a rapid time-of-injury TBI intervention. IRR's approach focuses on combining the unique synergistic properties of levocetirizine and montelukast into a fixed-dose combination (FDC). Both levocetirizine and montelukast are FDA-approved, low-risk, low-cost, and widely utilized. Completion of this project will advance IRR's product into Phase II/III clinical trials and support future 505(b)(2) new drug application (NDA).

**Study Design**: In collaboration with the University of Iowa Pharmaceuticals (UIP) and Dr. Todd Kilbaugh at the Children's Hospital of Philadelphia (CHOP), IRR will develop and validate an injectable montelukast and levocetirizine fixed-dose combination (FDC). Preclinical proof-of-concept testing will be performed in a translationally relevant porcine controlled-cortical impact (CCI) model of TBI. FDC development, manufacturing, validation, and preclinical testing will be accomplished over the following two objectives.

**Objective 1:** Develop and manufacture 1,500 ICH quality-compliant levocetirizine and montelukast FDC vials Preliminary combinations (soluble or lyophilized) of both drugs at target concentrations will be screened for stability up to 3 months across storage conditions and/or solvent composition (e.g. humidity, pH, buffer, cosolvents). Compatibility of suitable formulations with product contact materials will be verified. Lead combination products will be validated for sterility (endotoxins & bioburden), purity (HPLC), and active pharmaceutical ingredient (API).

**Objective 2:** Determine an optimally effective FDC dosing range by measuring the impact on translationally relevant outcomes in a porcine CCI model of TBI. CCI in pigs has become a newly accepted standard model for TBI research based on translational relevance, in part, due to similarities in brain size, shape, gyral pattern, vasculature, and gray/white matter distribution patterns between pigs and humans. (7-13th Annual Brain Injury Conferences (2017- 2023), Arlington VA). The FDC formulation developed in Objective 1 - outcome metrics will be measured via inflammatory biomarkers (IL-1B, IL-6, IL-8, TNF-α, and others), neuroimaging (MRI/MRS/DTI), actigraphy, and neuropathology. Efficacy will be tested across two FDC dose ranges following CCI using 50 mixed-sex young pigs distributed across experimental groups (including CCI-sham, FDC-vehicle, and dexamethasone controls).

**Impact and Relevance to Military Health:** IRR's solution would directly address sub-area 3a within the "Treat" FY21 TBIPHRP TRA Focus Area. Active duty and reserve service members are at increased risk for sustaining a TBI. As such, TBI has become a major focus for the Department of Veterans Affairs (VA) healthcare system. Veterans may sustain TBIs throughout their lifespan, resulting in high levels of disability; those with severe TBI were four times more likely to have Alzheimer's Disease (AD) or twice as likely following moderate TBI diagnosis compared to healthy controls in a longitudinal study of WWII Veterans. Development of a low-cost and easily implemented TBI intervention usable between ROC-1 to ROC-4 at the time of injury has immediately realizable benefits to citizens at risk for TBI, active-duty military personnel, and veterans alike.

**15. SUBJECT TERMS**

Traumatic Brain Injury, Closed cortical concussion, Antihistamine, Leukotriene receptor antagonist

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## 1. Introduction

Despite numerous clinical trials worldwide, there remains a profound unmet need for a safe and effective therapeutic for the treatment of traumatic brain injury (TBI). The current approaches to the treatment are ameliorative and do not significantly alter the biochemical and complex pathophysiologic changes that follow an insult to the brain. To date, there is no FDA-approved pharmacotherapeutic agent to specifically treat the effects of TBI across the entire spectrum of patients and effectively mitigate inflammation without causing concurrent host toxicity. IRR has proposed the development and testing of a levocetirizine and montelukast injectable. The injectable will be assessed in a swine model of TBI. Compared to rodents, which have most commonly been used in studies of TBI, swine has evolved as an improved model whereas the size, shape, gyral pattern, vasculature, and gray/white matter distribution patterns of the brain are more similar between swine and humans than between rodents and humans. The results of this study will lead to testing of the combination therapy in human clinical trials in patients with TBI.

## 2. Keywords

Traumatic brain injury (TBI), Closed cortical concussion (CCI), inflammation, antihistamine, leukotriene receptor antagonist, Fixed dose combination (FDC), ACURO (Animal Care and Use Review Office), IACUC (Institutional Animal Care and Use Committee), ARIES (Animal Research Information Electronic Submissions system), ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use)

## 3. Accomplishments

**What were the major goals of the project?**

The major goals (Objectives) of the projects are as follows:

**Objective 1:** Develop and manufacture 1,500 ICH quality-compliant levocetirizine and montelukast FDC vials. Preliminary combinations (soluble or lyophilized) of both drugs at target concentrations will be screened for stability up to 3 months across storage conditions and/or solvent composition (e.g. humidity, pH, buffer, cosolvents). Compatibility of suitable formulations with product contact materials will be verified. Lead combination products will be validated for sterility (endotoxins & bioburden), purity (HPLC), and active pharmaceutical ingredient (API).

**Objective 2:** Determine an optimally effective FDC dosing range by measuring the impact on translationally relevant outcomes in a porcine CCI model of TBI. CCI in pigs has become a newly accepted standard model for TBI research based on translational relevance due, in part, to similarities in brain size, shape, gyral pattern, vasculature, and gray/white matter distribution patterns between pigs and humans. (7-13th Annual Brain Injury Conferences (2017- 2023), Arlington VA). The FDC formulation developed in Objective 1 - outcome metrics will be measured via inflammatory biomarkers (IL-1B, IL-6, IL-8, TNF-α, and others), neuroimaging (MRI/MRS/DTI), actigraphy, and neuropathology. Efficacy will be tested across two FDC dose ranges following CCI using 50 mixed sex young pigs distributed across experimental groups (including CCI-sham, FDC-vehicle, and dexamethasone controls).

**What were accomplished under the goals?**

**Objective 1:** University of Iowa Pharmaceuticals - Formulation of levocetirizine and montelukast was recently completed (September 2023) as a lyophilized powder, the details of which have been transferred to the manufacturing department. Initial attempts to create a stable (soluble) solution of the compounds were limited by the intrinsic properties of the molecules and the desired physiologic pH of the final solution. As such, efforts were redirected to the successful formulation of a lyophilized powder, which dissolves in ~ 20 sec with sterile water at room temperature. To date, the product is stable at room temperature, 40 ºC (104ºF) / 75 % humidity, and 55 ºC (131ºF) / 75% humidity for one month. Extended stability testing is ongoing; all laboratory activities are being conducted under cGMP requirements as described by 21 CFR Parts 210 and 211. Qualification of the written test protocol will be conducted according to the protocol. 1500 vials of the lyophilized powder will be manufactured during the next three months (October – December 2023) and shipped to Children's Hospital of Philadelphia Animal Laboratory (PENN) to initiate Objective 2.

The **Statement of Work** (Incorporating Objectives 1 and 2) was divided into three major Tasks:

Major Task 1: Develop a fixed-dose combination (FDC) of levocetirizine and montelukast injectable.
*Outcome: formulated – currently in manufacturing (Q3, Q4 2023)*

Major Task 2: Complete the IACUC protocol submission and obtain IACUC approval.
*Outcome: Reviewed and updated*
University of Pennsylvania's Office of Animal Welfare (OAW) of Institutional Animal Care and Use Committee (IACUC) Approval for the submission: AIRES protocol #806943; 3-Year Expiration Date: 07/17/2026.

Department of the Army, Headquarters, U.S. Army Medical Research and Development Command, 810 Schreider Street, Fort Dietrick, MD 21702-5000
Krinon Moccia, DVM, MPH, DACLAM, LTC, VC, USA, Director, Animal Care and Use Review Office (ACURO) Approval Letter – for collective Awards under the PENN AIRES protocol #806943: 09/11/2023.

Protocol #806943 includes animal work for three funded studies at PENN: MT22002.157.e001, TP210687.e001, and TP210651P1.e001, i.e., they all share the same IACUC protocol.

Major Task 3: Determine optimal dosing of the FDC in a porcine – Q1-Q2 2024
*Outcome: To be conducted in 2024*

Major Task 4: Data Analysis and Reporting – Q3 2024
*Outcome: To be conducted in 2024*

**What opportunities for training and professional development has the project provided?**

Nothing to report.

**How were the results disseminated to communities of interest?**

An overview of the science and planned development was presented at the Military Health System Research Symposium in Orlando, Florida, at the Gaylord Convention Center on August 15, 2023, Poster Session 2 - #67. The results of the lyophilized powder lead product dissolution test (video) were disseminated in August 2023 via email to the following individuals:

Sarah K. Gross, Scientific Officer, CDMPR Program

Dwayne Taliaferro, PhD, Program Area Lead, Program Manager, Science Officer and Contracting/Grants Officer Representative (COR/GOR)

Krista L. Caudle, Ph.D., Department of the Army Civilian, Product Manager, Traumatic Brain Injury Warfighter Brain Health Project Management Office (WBH PMO), US Army Medical Materiel Development Activity (USAMMDA)

Kenneth C. Curley, MD, Principal Subject Matter Expert, Contractor, Iatrikos Research and Development Strategies, Subcontractor to General Dynamics Information Technology (GDIT), Supporting the US Army Medical Materiel Development Activity (USAMMDA), Warfighter Brain Health Project Management Office (WBH-PMO)

**What do you plan to do during the next reporting period to accomplish the goals?**

Objective 2 involves the determination of the optimally effective FDC by measuring the impact on translationally relevant outcomes in a porcine CCI model of TBI. Data derived from the 50-pig, 10-day, double impact (time zero, day 3) study will include outcome metrics on 9 biomarkers of inflammation, advanced neuroimaging, actigraphy, and neuropathology. Results will be collated, ideally presented at the Military Health System Research Symposium during August 2024, and thereafter published**.**

## 4. Impact

**What was the impact on the development of the principal disciplines(s) of the project?**

Completion of Objectives 1 & 2 will facilitate ongoing product development through a proof-of-principle II (PoP2) study. Specifically, outcomes from this current (PoP1) study will provide the necessary support for initiating IND-enabling studies; these outcomes will also serve as the basis for preclinical safety testing in other nonhuman model species and a Phase II Clinical Trial in TBI patients.

**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.**

Development of a low-cost & easily implemented TBI intervention usable between ROC-1 to ROC-4 at the time of injury has immediately realizable benefits to citizens at risk for TBI, active-duty military personnel, and veterans alike.

## 5. Changes/Problems

**Changes in approach and reasons for change**

Initial attempts to create a stable (soluble) solution of levocetirizine and montelukast were limited by the intrinsic properties of the molecules and the desired physiologic pH of the final solution. As such, efforts were redirected to the successful formulation of a lyophilized powder, which dissolves in ~ 20 sec with sterile water at room temperature. To date, the product is stable at room temperature, 40 ºC (104ºF) / 75 % humidity, and 55 ºC (131ºF) / 75% humidity for one month. Extended stability testing is ongoing; all laboratory activities are being conducted under cGMP requirements as described by 21 CFR Parts 210 and 211.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

**Changes that had a significant impact on expenditure**

Nothing to report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

**Significant changes in use or care of human subjects**

Not Applicable.

**Significant changes in use or care of vertebrate animals**

Nothing to report.

**Significant change in use of biohazards and/or select agents**

Not Applicable.

## 6. Products

**Publications, conference papers, and presentations**

Military Health System Research Symposium in Orlando, Florida, at the Gaylord Convention Center on August 15, 2023, Poster Session 2 - #67.

**Journal publications**

Nothing to report.

**Books or other non-periodical, one-time publications**

Nothing to report.

**Other publications, conference papers, and presentations**

Nothing to report.

**Website(s) or other internet sites(s)**

Nothing to report.

**Technologies or techniques**

Nothing to report.

**Invention, patent applications, and/or licenses**

Nothing to report.

**Other products**

Nothing to report.

## 7. Participants & Other Collaborating Organizations

| | |
|---|---|
| Name: | **Bruce May** |
| Project Role: | PI |
| Nearest person month worked: | 3 |
| Contribution to Project: | Dr. May coordinated the activities at Children's Hospital of Philadelphia (CHOP) with Dr. Todd Kilbaugh to ensure the IACUC protocol submission. He also worked with UIP to formulate / manufacture the drug product that will be used in Year 2. |
| Funding Support: | U.S. Army Medical Research and Development Command |

**What individuals have worked on the project?**

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

**What other organizations were involved as partners?**

**Organization Name:** University of Iowa Pharmaceuticals
**Location of organization:** 115 South Grand Avenue G-20

Iowa City, IA 52242
**Partner's contribution to the project:** Facilities

**Organization Name:** Children's Hospital of Philadelphia
**Location of organization:** Wood Center 6th Floor
3615 Civic Center Blvd
Philadelphia, PA 19104
**Partner's contribution to the project:** Collaboration on IACUC submission and approval

**Organization Name:** Children's Hospital of Philadelphia
**Location of organization:** Resuscitation Science Center
Anesthesiology and Critical Care Medicine
3401 Civic Center Blvd.
Philadelphia, PA 19104
**Partner's contribution to the project:** Collaboration on IACUC submission and approval

## 8. Special Reporting Requirements

### Collaborative awards

Nothing to report.

### Quad chart

Attached.

## 9. Appendices (pages 10-16)

Quad Chart – updated 10/09/2023 (1 page)

MHSRS poster presentation – August 15, 2023 #67 / Session 2 (1 page)

PowerPoint Slides - dissolution time of the lead product – pdf (2 pages)

University of Pennsylvania Office of Animal Welfare (OAW) of Institutional Animal Care and Use Committee (IACUC) Approval for the Submission Protocol #: 806943 (1 page)

Department of the Army, Headquarters, U.S. Army Medical Research and Development Command, 810 Schreider Street, Fort Dietrick, MD 21702-5000
Krinon Moccia, DVM, MPH, DACLAM, LTC, VC, USA, Director, Animal Care and Use Review Office (ACURO)
Approval Letter – for collective Awards under the PENN AIRES protocol #806943: 09/11/2023 (2 pages)