Pilot Study of Omega-3 Polyunsaturated Fatty Acid Treatment in Mild Acute TBI (OPTIMA-TBI pilot).

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Protocol Number: HUM00129045

Principal Investigator: Frederick Korley, M.D., Ph.D.

IND Sponsor: NA

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

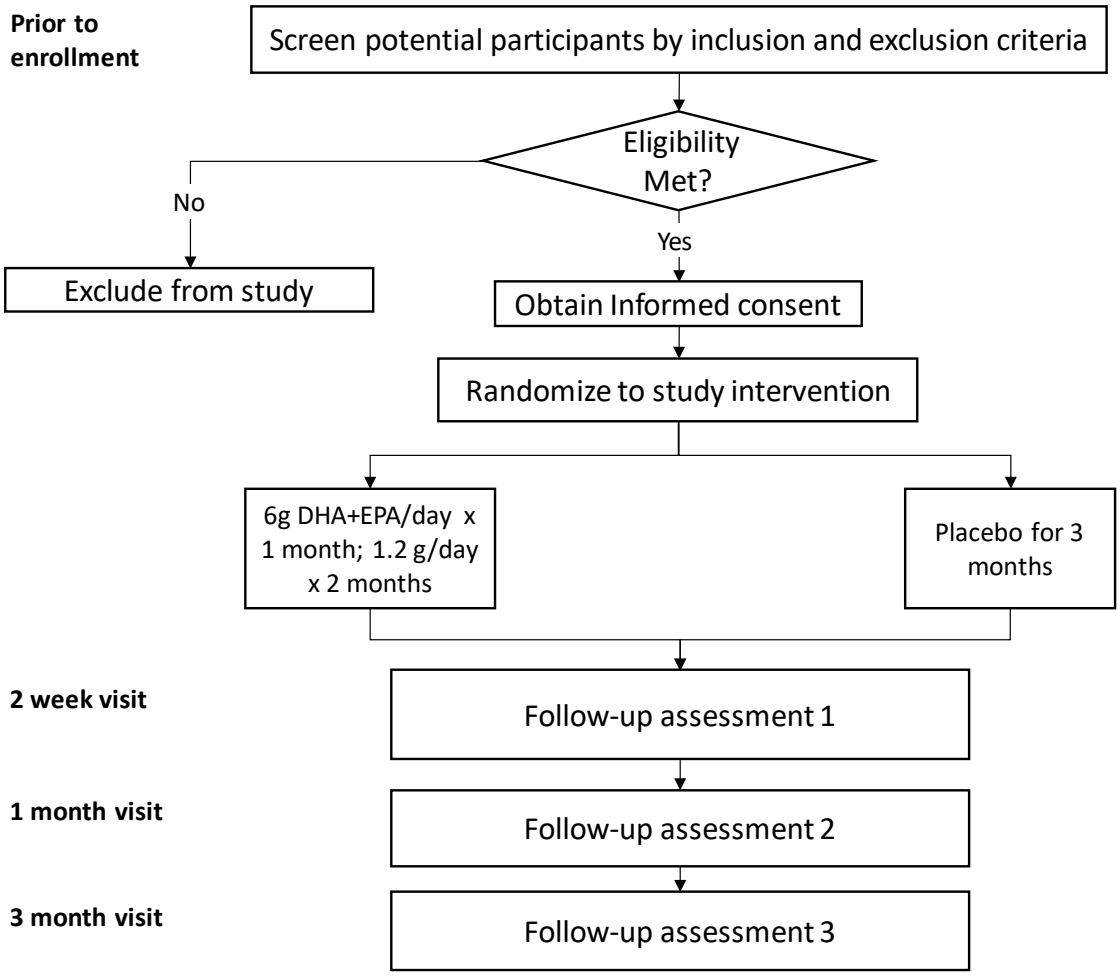
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Pilot Study of **O**mega-3 **P**olyunsaturated Fatty Acid **T**reatment **i**n **M**ild **A**cute **TBI** (OPTIMA-TBI pilot).
Study Description:	This is a double-blinded randomized control trials comparing the effect of omega-3 fatty acid versus placebo on blood biomarkers of brain injury, inflammation and neurogenesis.
Objectives:	Primary Objective: Biomaker endpoints include: neuronal injury measured by Neurofilament light chain (NFL), Neurogranin (NRGN) and Intercellular adhesion molecule 5 [ICAM5]; inflammation measured by high sensitivity C-reactive protein (CRP), IL-6 and IL-10 and neurogenesis measured by brain derived neurotrophic factor (BDNF) Secondary Objectives: 1. Delayed functional recovery defined as Glasgow Outcome Scale Extended (GOSE)<8 at 3 months 2. Moderate/severe post-concussive symptoms at 3 months defined as having at symptoms in at least three following ICD-10 symptom categories obtained from the Rivermead Post-Concussion Questionnaire (RPQ): (1) headaches, dizziness, general malaise, excessive fatigue, or noise intolerance; (2) irritability, emotional labiality, depression, or anxiety; (3) subjective complaints of concentration or memory difficulty; (4) insomnia; (5) reduced tolerance to alcohol; (6) preoccupation with these symptoms and fear of permanent brain damage. 3. Cognitive impairment at 3 months is defined based on a battery of 13 neurocognitive tests. Subjects are deemed cognitively impaired if their scores on at least 2 neurocognitive tests are more than 2 standard deviations below their IQ T-Score ascertained by the Wechsler Test of Adult Reading (WTAR). 4. Serious adverse events (SAEs): Life-threatening bleed including upper GI bleed, intracranial hemorrhage; hypotension; severe allergic reaction, blood glucose and LDL cholesterol levels
Endpoints:	Primary Endpoint: Serum levels of neurofilament light chain, C-reactive protein, IL-6, IL-10, Brain derived neurotrophic factor over a 3-month period Secondary Endpoints: Functional recovery (measured by Glasgow Coma Scale Extended), symptomatic recovery (measured by the Rivermead Post-concussive Symptoms Questionnaire) and cognitive impairment (measured by a cognitive battery) at 3 months post injury

Study Population:	Individuals presenting to the emergency department (ED) within 7 days of injury, who meet the American Congress of Rehabilitation Medicine (ACRM)'s definition of having mild traumatic brain injury (mTBI) will be eligible. The ACRM defines mTBI as a traumatically-induced physiological disruption of brain function as a consequence of the head being struck, striking an object, or undergoing an acceleration/deceleration movement without direct external head trauma and resulting in at least one of the following: any period of loss of consciousness (LOC); any loss of memory for events immediately before or after the injury; any alteration in mental state at the time of the injury (eg, feeling dazed, disoriented, or confused); and focal neurological deficit(s) that may or may not be transient. Subjects will be excluded if they have any of the following findings: 1. GCS<13 at any time during ED stay 2. Significant polytrauma including: bony fracture or solid organ injury 3. Study medication cannot be administered within 7 days of injury 4. Patient cannot be relied on to complete follow-up (i.e. no reliable telephone number, substance dependence, homeless) 5. Cannot communicate in English 6. Take an anticoagulant (coumadin or a novel oral anticoagulant) daily 7. Age less than 18 years or greater than 65 years 8. Patients already taking fish oil supplements daily 9. History of cognitive impairment 10. Allergic to fish/fish oil 11. Pregnant women (self-reported)
Phase:	II
Description of Sites/Facilities Enrolling Participants:	University of Michigan Emergency Department
Description of Study Intervention:	The intervention group will receive 6g/day of omega-3 fatty acids (fish oil) for 1 month followed by 1.2g/day for two additional months. The control group will receive placebo (olive oil) treatment for 3 months.
Study Duration:	1 year
Participant Duration:	3 months

1.2 SCHEMA


Prior to enrollment
Screen potential participants by inclusion and exclusion criteria
Eligibility Met?
No
Yes
Exclude from study
Obtain Informed consent
Randomize to study intervention
6g DHA+EPA/day x 1 month; 1.2 g/day x 2 months
Placebo for 3 months
2 week visit
Follow-up assessment 1
1 month visit
Follow-up assessment 2
3 month visit
Follow-up assessment 3

1.3 SCHEDULE OF ACTIVITIES (SOA)

Activity	Enrollment	2 week visit	1 month visit	3 month visit
Screening	X			
Demographics	X			
Medical History	X			
Pre-hospital Events	X			
Informed consent	X			
Clinical data elements	X			
Rivermead Post-Concussion Questionnaire	X	X	X	X
Blood draw	X	X	X	X
Study medication	X	X	X	X
Glasgow Outcome Scale Extended		X	X	X
Montreal Cognitive Assessment			X	X
Brief Test of Attention (BTA)			X	X
Trail Making Test, Part A, Part B			X	X
Stroop Test			X	X
Controlled Oral Word Association Test (COWAT)			X	X
Wisconsin Card Sorting Test (WCST)			X	X
Hopkins Verbal Learning Test (HVLT)			X	X
Brief Visual Memory Test (BVMT)			X	X
Wechsler Test of Adult Reading (WTAR)			X	X
Patient Health Questionnaire 9 (PHQ9)			X	X
Generalized Anxiety Scale GAD 7			X	X
Davidson Trauma Scale to assess PTSD			X	X
Pittsburg Sleep Questionnaire			X	X
Satisfaction with life scale			X	X
Social Ties Check List			X	X
McGill pain scale			X	X

2 INTRODUCTION

2.1 STUDY RATIONALE

The objective of this study is to determine whether the treatment of traumatic brain injury patients with omega-3 fatty acids results in a decrease in blood biomarkers of neuronal injury and inflammation and an increase in biomarkers promoting neurogenesis.

2.2 BACKGROUND

Primary brain injury, the initial physical injury to brain tissue post-trauma, responds only to measures that prevent TBI from occurring in the first place. However, secondary brain injury, a complex cascade of events causing additional brain injury following primary brain injury, is more amenable to pharmacologic treatment. Neuroinflammation is one of the recognized mechanisms of secondary brain injury. In response to primary brain injury, activated microglia and injured neurons both release signaling proteins including cytokines and chemokines. Ω-3 and ω-6 fatty acids are major components of immune cells and neuronal cell membranes. They are also precursors to neuromodulatory lipds such as eicodanoids, endovanilloids and endocannabinoids that have antinociceptive and anxiolytic properties. Docosahexaenoic acid (DHA) is one of the most abundant fatty acid components of brain cell membrane phospholipids.[1] In rodent model studies, dietary supplementation with omega-3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) decreased secondary axonal injury,[2,3] attenuated endoplasmic reticulum stress response,[4] decreased neuroinflammation post-TBI,[5] and improved short and long-term neurologic outcomes.[3] Additionally, DHA supplementation post-TBI enhances neurogenesis by counteracting reductions in neuroplasticity biomarkers such as brain-derived neurotrophic factor.[6,7] Furthermore, DHA deficient rodents are more likely to have a greater amount of axonal injury and slower recovery neurologic recovery post-TBI.[8] To our knowledge there are no human studies examining the effect of omega-3 fatty acid supplementation post-TBI on functional, symptomatic and neurologic outcomes. However, a study of collegiate football players who were randomized to 2, 4 or 6g/day of DHA or placebo for a total of 189 days (including 80 pre-season days). Irrespective of the dose of DHA supplementation, those receiving DHA had lower values of serum neurofilament light chain, a biomarker of axonal injury, than those receiving placebo.[9]

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Gastrointestinal distress (nausea, vomiting, diarrhea), low risk of bleeding

2.3.2 KNOWN POTENTIAL BENEFITS

Improvement in functional, cognitive and symptomatic recovery from traumatic brain injury; cardiovascular benefits

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Omega-3 fatty acids have an excellent safety profile. It is FDA approved for treating hypertriglyceridemia. The potential risks from taking omega-3 fatty acids are minimal. Gastrointestinal symptoms resolve upon stopping the medication. The risk of bleeding is minimal and at times disputed. There are currently no treatments for traumatic brain injury. Animal studies have demonstrated that omega-3 fatty acid supplementation may hasten recovery from TBI. Therefore the potential benefits from taking this medication outweigh the risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the effect of omega-3 polyunsaturated fatty acids (PUFA) treatment on blood biomarkers of neuronal injury and blood biomarkers of inflammation and a blood biomarker of neurogenesis	Neurofilament light chain [NFL], Neurogranin [NRGN] and Intercellular adhesion molecule 5 [ICAM5], high sensitivity C-reactive protein [CRP], IL-6, IL-10, and brain derived neurotrophic factor [BDNF]	If omega-3 fatty acid supplementation decreases the levels of biomarkers of neuronal injury and inflammation and increases the levels of biomarkers of neurogenesis, then there is a strong likelihood it will improve clinical outcome
Secondary		
1. To demonstrate the feasibility of enrolling, treating and conducting follow-up assessments on mTBI patients presenting to the University of Michigan 2. To acquire preliminary data on the effect of omega-3 PUFA treatment on delayed functional recovery, moderate/severe post-concussive symptoms and cognitive impairment. 3. To acquire preliminary data on the types and incidence of serious adverse events (SAEs) in the Omega-3 PUFA treatment arm compared to the placebo arm	1. Successful enrollment of 50 subjects within 1 year 2. Measurement of functional recovery using the Glasgow Outcome Scale Extended (GOSE), measurement of symptomatic recovery using the Rivermead Post-concussive symptoms questionnaire (RPQ) and cognitive impairment using a neurocognitive battery of tests 3. Measurement of the incidents of gastrointestinal distress and clincally significant bleeding	These endpoints will help inform the design of a multi-center phase II trial

4 STUDY DESIGN

4.1 OVERALL DESIGN

We hypothesize that treatment of traumatic brain injury patients with omega-3 fatty acids will result in a decrease in blood concentrations of biomarkers of neuronal injury and inflammation and an increase in the blood concentration of biomarkers of neurogenesis. This is a randomized, placebo-controlled, double-blinded pilot phase II clinical trial.Participants will be randomized either a single dose of 6g of DHA+EPA for 1 month followed by 1.2g/day of DHA+EPA for two additional months or to placebo treatment for 3 months. Blood samples will be drawn at enrollment, 2 weeks, 1 month and 3 months post-injury. Biomarkers of neuronal injury, inflammation, and neurogenesis will be measured in batches. Functional and symptomatic recovery will be assessed at 2 weeks, 1 month and 3 months. Additionally, cognitive impairment will be assessed at 3 months.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

There are limited data on treatment of TBI with omega-3 fatty acids. The performance of a randomized control trial will allow an unbiased determination of the efficacy of omega-3 fatty acids in treating TBI

4.3 JUSTIFICATION FOR DOSE

Some animal studies have used a dose of 40mg/kg. Human studies of high dose omega-3 fatty acids for preventing cardiovascular disease have used our study dose of 4g. The maximum tolerable dose of omega-3 fatty acids is 21g/day. Case reports of severe TBI treatment with omega-3 fatty acids have reported using up to 19g/day. In our population of mild TBI patients we have selected a dose of 6g/day which is about 2 times the dose used in animal studies but significantly below the maximum tolerable dose or the dose used in other TBI studies.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.
The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Individuals presenting to the emergency department (ED) within 7 days of injury, who meet the American Congress of Rehabilitation Medicine (ACRM)'s definition of having mild traumatic brain injury (mTBI) will be eligible. The ACRM defines mTBI as a traumatically-induced physiological disruption of brain function as a consequence of the head being struck, striking an object, or undergoing an acceleration/deceleration movement without direct external head trauma and resulting in at least one of the following: any period of loss of consciousness (LOC); any loss of memory for events immediately before or after the injury; any alteration in mental state at the time of the injury (eg, feeling dazed, disoriented, or confused); and focal neurological deficit(s) that may or may not be transient.

5.2 EXCLUSION CRITERIA

Subjects will be excluded if they have any of the following findings:

1. GCS<13 at any time during ED stay.
2. Significant polytrauma including: bony fracture or solid organ injury
3. Study medication cannot be administered within 7 days of injury
4. Patient cannot be relied on to complete follow-up (i.e. no reliable telephone number, substance dependence, homeless)
5. Cannot communicate in English
6. Take an anticoagulant (coumadin or a novel oral anticoagulant) daily
7. Age less than 18 years or greater than 65 years
8. Patients already taking fish oil supplements daily
9. History of cognitive impairment
10. Allergic to fish/fish oil
11. Pregnant women (self-reported)

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Potential subject for this trial will be recruited from all patients with mild TBI (GCS 13-15) presenting within 7 days of injury to the University of Michigan Emergency Department. Trained research personnel will perform enrollment of subjects.

Target sample size: 50 participants

Anticipated accrual rate: 5-10 patients per month

Anticipated number of sites: 1

Source of participants: Emergency Department and Inpatient admissions of the University Hospital, University of Michigan

How potential participants will be identified and approached: Trained research coordinators will screen the Emergency Department Dashboard of MiChart for eligible patients. They will then approach emergency physicians/physician assistants taking care of eligible patients and ask for permission to approach their patient to further discuss the study and obtain informed consent. This will occur in the emergency department. If the potential subject agrees to the referral, the research coordinator will approach the potential subject and begin the consent process. This process will help maintain patient privacy, by allowing potential subjects to have control over who has access to them. For patients lacking the capacity to consent, an authorized legal representative will be identified for consent purposes. Participation in the study will be voluntary.

Incentives for visit attendance: A cash incentive of $75 will be provided for attending the 2 week visit, $75 for attending the 1 month visit and $150 for attending the 3 month visit.

Inclusion of women and minorities: Traumatic brain injury occurs more frequently in men. However, efforts will be made to ensure that all eligible women are included. Additionally, efforts will be made to ensure an adequate representation of under-represented minorities.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The study intervention is an omega-3 fatty acid capsules manufactured by Wiley's Finest. We will utilize a formulation that contains 500 mg of docosahaxaenoic acid (DHA) and 100 mg of eicosapentaenoic acid (EPA) in one capsule

6.1.2 DOSING AND ADMINISTRATION

Every attempt will be made to start treatment as soon as possible after enrollment. The first dose will be administered within 7 days of injury. The intervention group will receive 6g per day of DHA+EPA for 1 month followed by 1.2 g/day of EPA+DHA for two additional months. The control group will receive placebo treatment for 3 months.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Study medications will be acquired from Wiley's Finest and stored and dispensed by the University of Michigan Research Pharmacy Group

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Study medication will be supplied as soft-gelatin capsules filled with light-yellow oil.

6.2.3 PRODUCT STORAGE AND STABILITY

- Store study medications at room temperature between 68°F to 77°F (20°C to 25°C).
- Do not freeze study medication
- Safely throw away medicine that is out of date or no longer needed

6.2.4 PREPARATION

The study drug will not need additional preparation

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

A web-based central randomization system will be developed and utilized. Study team members will be blinded to study group allotment.

6.4 STUDY INTERVENTION COMPLIANCE

Adherence to protocol will be assessed by measuring plasma levels of EPA and DHA and red blood cell index of omega-3 fatty acids at each follow-up visit.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from taking the study medication does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Blood samples, functional, symptomatic and cognitive outcomes data

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy (self-reported)
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive the study medication for 2 weeks or more

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for a scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Biomarker Assays: Neurofilament light chain, Intercellular adhesion molecule 5 and brain derived neurotrophic factor will be measured using a single molecule counting technology from Quanterix. Neurogranin will be measured using an ELISA assay made by ADx Neurosciences.
high-sensitivity C-reactive protein, IL-6 and IL-10 will be measured using standard ELISA assays.

Other outcome measures: Functional outcome will be assessed with the Glasgow Outcome Scale Extended (GOSE). Symptomatic outcome will be assessed with the Rivermead Post-concussion Symptom Questionnaire (RPQ). Depressive symptoms will be assessed with the Patient Health Questionnaire 9 (PHQ9). These outcomes will be measured at 2 weeks, 1 month, and 3 months.
Neurocognitive impairment will be assessed based on a battery of neurocognitive test. These include:

- the Wechsler Test of Adult Reading (WTAR)
- the Hopkins Verbal Learning Test (HVLT) (3 scores),
- the Brief Visuospatial Memory Test - Revised (BVMT-R) (3 scores),
- the Brief Test of Attention (BTA) (1 scores),
- the Trail making test A and B (1 scores),
- the stroop test (2 scores), and
- the Wisconsin Card Sorting Test (WCST) (2 scores),

Using the WTAR (word pronunciation) as an estimate of IQ, we will compare the 13 neurocognitive test T-scores of interest against the subject's IQ T-score. The standard deviation (SD) of T-score is 10. Each of the subject's 13 neurocognitive test T-scores is considered as aberrant if it is more than 2 SD below this subject's IQ T-score. A subject is considered cognitively impaired if at least 2 (based upon .05 rule; 5 out of every 100 test scores will be outside of expected range by chance alone) out the 13 T-scores are aberrant.

8.2 SAFETY AND OTHER ASSESSMENTS

At each study visit, we will evaluate patients for the following:

1. Gastrointestinal distress (nausea, vomiting, diarrhea)
2. Clinically significant bleeding such as intracranial hemorrhage and upper GI bleed

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

Study research coordinators will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the

training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study research coordinators will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.3.5 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 7 days of the investigator becoming aware of the problem.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):

Omega-3 fatty acid supplementation results in a decrease in the area under the concentration versus time curve for neurofilament light chain, CRP, IL-6 and IL-10 during the 3 months following TBI and an increase in the area under the concentration curve for brain derived neurotrophic factor.

- Secondary Efficacy Endpoint(s):

Omega-3 fatty acid supplementation improves functional outcome, post-concussive symptoms and cognitive decline post-TBI

9.2 SAMPLE SIZE DETERMINATION

This is a pilot study of 50 patients.

9.3 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

We will examine blood levels of biomarkers of omega-3 intake, axonal injury, and inflammation (red blood cell and plasma omega-3 index, NFL, ICAM5, CRP, IL-6 and IL-10) at baseline and at 3 months in both the intervention and the control groups and test the hypothesis that omega-3 PUFA treatment increases omega-3 PUFA blood levels and decreases levels of biomarkers of axonal injury and inflammation. To examine the effect of omega-3 PUFA treatment on biomarkers of neuronal injury and inflammation, we will construct multiple linear regression models with generalized estimating equations (GEE) and robust variance estimation to account for correlations within repeated measurements. This will allow us to determine whether variations in the levels of NFL, ICAM5, CRP, IL-6 and IL-10 over the 3 month study period differ among those receiving omega-3 PUFA supplementation versus those receiving placebo.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The written informed consent document is submitted with this protocol.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant or legally authorized representative will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data will be stored in a secure password protected and data-encrypted online database (REDCap). This will not include the participant's contact or identifying information. At the end of the study, all study databases will be de-identified and archived at the on a University of Michigan password protected computer.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the University of Michigan. Study data will be available for use by other researchers including those outside of the study. Permission to transmit data to the other collaborators will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the University of Michigan with the same goal as the sharing of data with other collaborators. These samples could be used for research the causes of traumatic brain injury, its complications and other conditions for which individuals with traumatic brain injury are at increased risk, and to improve treatment. Collaborators will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Provide the name and contact information of the Principal Investigator

Principal Investigator
Frederick Korley, M.D., Ph.D.
Department of Emergency Medicine
University of Michigan
24 Frank Lloyd Wright Drive, H3100
Ann Arbor, MI 48105

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical

Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or MOP requirements. The noncompliance may be

either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the principal investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices

GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale

11 REFERENCES

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