Vitamin D and n-3 Polyunsaturated Fatty Acids (PUFAs) to Prevent Chronic Pain Following Major Thermal Burn Injury

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List of Abbreviations

n-3 PUFA: n-3 Polyunsaturated fatty acid
EPA: eicosapentaenoic acid
DHA: Docosahexaenoic acid
g: grams
IU: international units
FDA: Food and Drug Administration
MThBI: Major Thermal Burn Injury
RCT: Randomized Controlled Trial
SF-12: short form 12 item questionnaire
TBSA: total body surface area
UNC: University of North Carolina
GFR: glomerular filtration rate
IDS: investigational drug services
MAR: medication administration record
NRS: numeric rating scale
MCS: mental component score
PCS: physical component score
PTSD: post-traumatic stress disorder
DNA: deoxyribonucleic acid
RNA: ribonucleic acid
Cc: cubic centimeters
PT: physical therapy
OT: occupational therapy
RPS: regional pain scale
DASS: Depression, Anxiety, and Stress Scale
MOS: medical outcomes survey
UPLC/ESI/MS/MS: Ultraperformance Liquid Chromatography/Electrospray Ionization Mass Spectrometry
ELISA: enzyme linked immunosorbent assay
SAE: serious adverse events
IRB: institutional review board
DCC: data coordinating center
CRF: case report form
EC: ethics committee

Study Summary

Title	Pilot, double-blind, randomized controlled, multi-center study of the effects of fi**s**h oil **a**nd vitamin D in the prevention of chronic pain fo**l**lowing **m**aj**o**r thermal bur**n** injury.
Short Title	Vitamin D and n-3 PUFAs to prevent chronic pain
IRB Protocol Number	17-1971
Phase	2
Methodology	Double Blind, Randomized Controlled Trial using a 2x2 factorial design
Study Duration	3 years
Study Center(s)	University of North Carolina-Data Coordinating Center University of South Florida Washington Hospital Center
Objectives	Determine the safety and feasibility of administering n-3 PUFAs and Vitamin D to survivors of major thermal burn injury.
Number of Subjects	60
Diagnosis and Main Inclusion Criteria	Major Thermal Burn injury involving less than 30 percent TBSA that are severe enough to require surgery.
Study Product, Dose, Route, Regimen	n-3 PUFA (DHA:EPA 2:3), 2 grams (4 softgels) orally administered once per day for 6 weeks, Vitamin D 2000 IU (1 capsule) once per day for 6 weeks.
Duration of administration	6 weeks
Reference therapy	Matched placebo to fish oil and Vitamin D preparations
Statistical Methodology	Safety will be determined by qualitative and quantitative analysis of the adverse events. Feasibility will be determined by protocol adherence. Estimates of potential efficacy will be determined via repeated measures analysis using mixed effects models.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

The main objective of this study is to determine whether administration of n-3 polyunsaturated fatty acids and Vitamin D for 6 weeks following Major Thermal Burn Injury (MThBI) is feasible and safe. This phase II, multicenter, randomized controlled trial is a pilot study to determine feasibility, safety and potential efficacy (effect size, sample variation, response to study drug) that can be used to adequately power a larger randomized controlled trial to fully assess efficacy.

1.1 Background

Nearly 500,000 burn injuries occur annually in the US, and ~40,000 individuals experience major thermal injuries (MThBI) requiring hospitalization[1-3]. Approximately 3/4 of these individuals require skin grafting[4], a procedure in which skin is transplanted from a donor site to the site of burn injury (graft site). Up to 60% of MThBI patients receiving skin grafting experience severe ongoing pain despite treatment[5]. New therapeutics that prevent chronic pain, promote recovery, and reduce suffering in MThBI survivors are urgently needed.

Long chain n-3 polyunsaturated fatty acids (PUFAs) represent one of the most promising, untried treatment options to prevent pain in MThBI survivors because they have been demonstrated to be powerful, non-addictive analgesics[6] and also are readily available and easy to administer[7]. A wealth of preclinical and clinical evidence has shown that two of the most well-studied n-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are powerful analgesics, with a potency of effect many times that of oral opioids[8], and are able to prevent pain[6,9-11]. They also have anti-inflammatory, antidepressant, and neuroprotective effects and have been shown to improve general mental and physical health. Oral formulations of DHA and EPA are widely available[7], and burgeoning evidence from RCTs testing the efficacy of DHA and EPA in other neuro/inflammatory disorders (e.g., rheumatoid arthritis[12], migraine headache,[13] depression[14]) demonstrate that they are well-tolerated and highly effective.

Vitamin D represents a promising, untried treatment to prevent pain following MThBI because it is associated with worsened chronic widespread pain severity [15,16], increased central pain sensitivity [17], and other chronic pain conditions such as low back pain[18], and spinal stenosis[19]. Second, available evidence suggests that Vitamin D insufficiency, defined as serum 25-hydroxyvitamin D (25[OH]D) concentrations <74 nmol/l, or less than 20 ng/mL, is a common condition affecting approximately 76% of the U.S. population and disproportionately affects African Americans (97%)[20,21]. Third, in the CNS, vitamin D is known to upregulate two anti-inflammatory cytokines TGF-β1 and IL-4 [22,23] which have been shown to inhibit chronic pain [24]. Given this data, Vitamin D may be an important modifiable risk factor for chronic pain development following MThBI.

1.2 Investigational Agents:

DHA and EPA are n-3 Polyunsaturated fatty acids and are active components of fish oil which is known to have anti-hyperlipidemic, anti-inflammatory, and anticoagulation properties. n-3 fatty acids are essential fats that are not synthesized in vivo, and therefore must be consumed through dietary souces[25]. These dietary fats are metabolized into anti-inflammatory, pro-resolving lipid mediators such as resolvins and protectins. DHA is the major component in human tissues, especially neural tissue. EPA is another fatty acid found in human tissues and is known to have anti-inflammatory effects. It is known that DHA does ~ 2 g per day result in a near maximal plasma response[25]. Supplementation of DHA causes a modest increase in EPA levels, but EPA supplementation does not cause an increase in DHA owing to poor enzymatic conversion. Therefore, to increase both DHA and EPA levels in the plasma, the goal of this study, one effective approach is to supplement with both fatty acids[25].

Vitamin D is a fat soluble nutrient that has been previously associated with chronic pain outcomes[15,16,18,19]. Vitamin D deficiency is common in the population with a US prevalence of 76%[20,21]. Previous RCTs have used a supplement of 2000 IU of Vitamin D_3 targeting an optimal plasma level of 90 nmol/L[26,27].

Co-administration of Vitamin D and n-3 PUFAs (Fish Oil) has been safety performed in previous RCTs[26,28,29] and are potentially synergistic in improving pain outcomes.

1.3 Preclinical Data

Pre-clinical studies have shown that n-3 PUFAs and their downstream metabolites are effective anti-inflammatory agents that reduce neuroinflammation (e.g. microglial activation)[6], provide analgesia[6,30], and prevent the development of chronic pain following injury[6,31]. This data supports the use of n-3 PUFAs to prevent chronic pain in survivors of MThBI.

1.4 Clinical Data to Date

Previous studies demonstrate that Vitamin D and O3FAs are two safe, and effective treatment options that have been used to treat a range of painful musculoskeletal disorders. Both Vitamin D and O3FA have been shown to reduce chronic pain severity. Numerous studies, both randomized clinical trials and systematic reviews, have found O3FA to reduce pain related to rheumatoid arthritis[88-95], which is an inflammatory pain condition like burn injury. O3FAs have also shown efficacy in randomized controlled trials to treat fibromyalgia, a difficult to manage chronic pain condition. O3FAs have demonstrated improvement in post-operative pain following gastric bypass[96]. O3FAs have shown efficacy in treating osteoarthritis, and there does not seem to be an affect of high or low dose of O3FA[97]. Recent RCT data from a population with coronary artery disease indicates that O3FAs increases physical function, reduced risk of joint replacement and improves pain and stiffness[98]. Clinical evidence from a case series demonstrated that O3FA is effective in reducing neuropathic pain[99]. Strong clinical trial evidence indicates that O3FA is effective to reduce pain and improve quality of life in patients who suffer from migraine headaches migraine headache[25]. Vitamin D has similarly been shown to reduce chronic pain across a range of painful conditions. Clinical trial results demonstrate that Vitamin D supplementation improves symptoms experienced by patients suffering from fibromyalgia[35-39], a difficult to treat widespread pain condition. Vitamin D supplementation in a recent clinical trials also improved disability related to low back pain[40], osteoarthritis[41] and chronic pain related to migraine headache[42]. Not all studies are positive, for example a recent study demonstrated that Vitamin D supplementation did not change analgesic consumption, or improve pain outcomes among the general population[100]; however this study included the general population not those at high risk of chronic pain development as are the individuals we plan to recruit into this study. Taken together, the results of clinical trials assessing the effectiveness of O3FA and Vitamin D to address painful conditions are promising, and given the lack of available treatment options for burn survivors, safety profile of O3FA and Vitamin D, and therapeutic promise we propose O3FA and Vitamin D as a potentially safe and effective preventative treatment option to reduce chronic pain severity and promote opioid cessation following burn injury.

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1.5 Dose Rationale and Risk/Benefits

The study will follow a 2x2 factorial design. Two supplements will be used: one will consist of 2 grams of a 3:2 ratio of EPA/DHA consumed orally (4 softgels=4g fish oil) once daily for 6 weeks and another will be 2000 IU daily of Vitamin D (1 capsule). The n-3 PUFA dose has been previously used for studies of other painful conditions[11] and has been associated with a significant increase in the n-3 PUFA level and a reduction in the n-6:n-3 PUFA ratio over a 6 week period[34]. The Vitamin D dose of 2000 IU per day has been previously used in other clinical trials, has been shown to correct Vitamin D to optimal levels, and has been shown to be safe. Participants in the comparator arm for n-3 PUFAs will receive 4 grams of soybean/corn oil, which has been safely and effectively used as a comparator in a number of studies.

Chronic pain is associated with reduced mental and physical health and interferes with essential activities of daily life. Currently there are limited treatment options to address chronic pain once it has become established and the overarching aim of this clinical trial is to prevent chronic pain development, therefore there is a critical unmet need of safe, non-addictive, non-invasive preventative treatment options that can

be administered to burn survivors in the aftermath of injury. This study has the potential to benefit participants and future burn survivors and improve pain and general health outcomes. The risks of taking n-3 PUFAs and/or Vitamin D are small. Although n-3 PUFA preparations are well tolerated, potential side effects include fish like taste, belching, indigestion and GI upset, increased bleeding. Acceptance of this limited risk is reasonable based on the anticipated benefits to burn survivors at risk for chronic pain development. Side effects from Vitamin D are rare, however, it is possible that over supplementation of Vitamin D may be associated with headache, loss of appetite, dry mouth, metallic taste, and nausea/vomiting. **The risks to the subjects in experiencing these side effects is balanced against the potential for a new, safe, non-opioid preventative treatment option for chronic pain following MThBI.**

2 Study Objectives

Primary Objective: To assess the feasibility and safety of administering n-3 PUFAs and/or Vitamin D to MThBI survivors for 6 weeks following MThBI.

Secondary Objectives:
1. To demonstrate potential efficacy of n-3 PUFAs/Vitamin D on decreasing graft site pain severity in survivors of MThBI requiring surgery. This will provide data on the confidence interval around our model estimates, which will inform a larger clinical trial.

2. Demonstrate the potential efficacy of n-3 PUFAs/Vitamin D on reducing pain interference on important domains of life function measured by the Brief Pain Inventory and SF-12 Mental and Physical Component Scores.

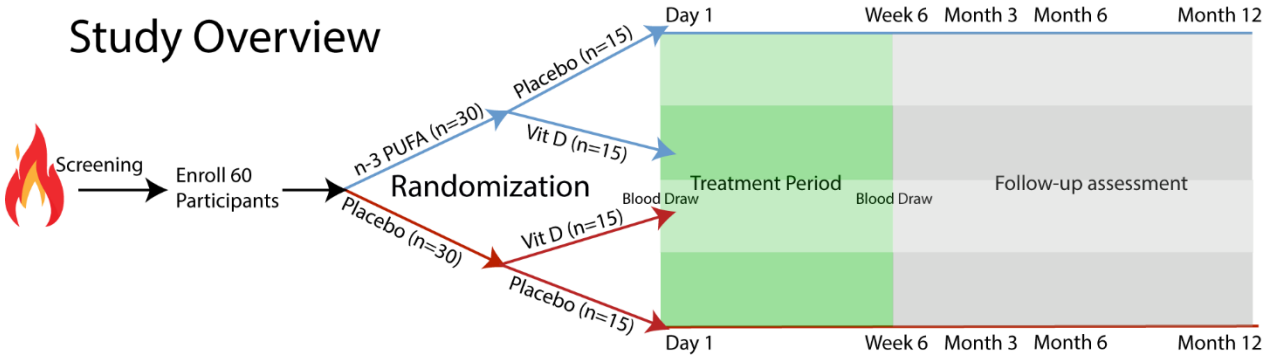
3. To demonstrate the utility of a clinical prediction tool based on a logistic function derived from a previous observational cohort study to enhance enrollment of a population of MThBI survivors at greater risk of chronic pain development.

4. To explore sex as a biological variable to determine whether there are differences in safety and efficacy among women and men.

Long-Term Aims Beyond the Present Proposal
To use data collected to design and adequately power a large-scale RCT assessing the efficacy of n-3 PUFAs and/or Vitamin D treatment in decreasing chronic pain in high-risk patients presenting for treatment in the aftermath of MThBI.

3 Study Design

3.1 General Design



Study Overview. Participants will be screened using a logistic risk stratification tool and will be enrolled if their probability of chronic pain is estimated to be ≥0.3. Comprehensive phenotyping will be performed on enrolled patients and a plasma sample will be obtained. Randomization will occur and 15 patients will be treated with n-3 PUFAs, Vitamin D, n-3 PUFA+Vitamin D or placebo for 6). Each patient will be assessed at Week 6, Month 3, 6, and 12 following MThBI. Follow-up

telephone assessments will be completed by staff at the UNC Institute for Trauma Recovery. Safety will be assessed with weekly phone calls during the 6 week treatment period.

In this pilot randomized, double-blind study with 2x2 factorial design, eligible patients will be randomized across three sites to receive either n-3 PUFA supplementation, Vitamin D, n-3 PUFA + Vitamin D, or comparator lipid (soybean/corn oil) for 6 weeks following MThBI. Randomization will occur after the enrollment questionnaire within 72 hours of burn injury. Plasma, DNA, and RNA samples will be collected on study enrollment and at 6 weeks following MThBI. Follow-up assessments will be completed at 6 weeks, 3, 6 months and 1 year following MThBI.

3.2 Posting on clinicaltrials.gov

The study protocol will be published to clinicaltrials.gov prior to commencement of the trial. Results will be posted on clinicaltrials.gov when available.

3.3 Primary Study Endpoints

The primary endpoint of this study will examine the safety, feasibility and efficacy of n-3 PUFAs/Vitamin D to improve chronic pain outcomes following major thermal burn injury. **Outcome to assess safety:** Safety outcomes will be met after descriptive review of adverse outcomes, and comparison of adverse events is made across treatment groups by investigative team at the conclusion of the study. **Outcome to assess feasibility:** 60 patients will be enrolled, randomized into one of 4 treatment arms in a 1:1:1:1 ratio, and treated with study drug for 6 weeks following Major Thermal Burn injury severe enough to warrant surgery within a 24 month period. Proportion of participants completing following up at 6 weeks will be reported. The concentration of Vitamin D and n-3 PUFAs will be calculated before and after the 6 week treatment in each treatment arm to assess the response (expect an increase in both Vitamin D and n-3 PUFA in treatment arms). **Outcome to assess efficacy:** Efficacy of Vitamin D/PUFA will be measured by difference in pain severity in treatment arms versus control arms. We will 1) calculate a model estimated mean of chronic pain severity and corresponding 95% Confidence interval in each study group and 2) determine the slope of pain recovery among individuals treated with Vitamin D and n-3 PUFAs relative to control participants using a linear mixed model adjusting for sex, ethnicity, and study site.

3.4 Secondary Study Endpoints

Several secondary endpoints will be included in this study. The efficacy of n-3 PUFA/Vitamin D to improve overall mental and physical health (SF-12 mental and physical component scores), pain interference, depression, anxiety, and stress, and opioid consumption will be assessed. Therapeutic response to Vitamin D and n-3 PUFA will be assessed in both women and men.

3.5 Primary Safety Endpoints

To assess safety, adverse events will be tracked using RedCap through emailed/text-messaged surveys during the drug treatment to assess side effects and safety profile of n-3 PUFAs and Vitamin D. Qualitative analysis of adverse events will be performed by investigative team.

Subject Selection and Withdrawal

3.6 Inclusion Criteria

1. ≥ 18 years and ≤ 65 years of age
2. Enrollment procedures can feasibly be completed within 96 hours of thermal burn injury
3. Estimated TBSA ≤ 30%
4. Surgical team has plans for surgical management of the burn wound (e.g. xenograft and/or autograft).
5. Patients experience a thermal burn injury, not an electrical or chemical burn.

6. Has a telephone to receive follow-up calls.
7. Able to speak and read English
8. Alert and oriented
9. Willing to take study medication for 6 weeks following enrollment
10. Subjects are capable of giving informed consent.
11. Predicted probability of chronic pain ≥ 0.3 when demographic parameters are entered into a logistic regression model developed from a previous cohort. (Initial pain score entered into this model will be based on the highest pain severity over the initial 24 hours after burn injury).
12. European American or African American

3.7 Exclusion Criteria

1. Unwilling to take study drug
2. Allergy to fish oil or corn/soybean oil.
3. Patient taking clopidogrel (Plavix)
4. Patient taking warfarin or dabigatran.
5. Substantial comorbid injury (e.g. long bone fracture)
6. Pregnancy/Breastfeeding
7. Prisoner status
8. Chronic daily opioid use prior to burn (>20 mg oral daily morphine equivalents).
9. Active psychosis, suicidal ideation, or homicidal ideation
10. Requires an escharotomy or fasciotomy for the treatment of burn injury.
11. Has a disorder of pain processing or diminished capacity to perceive pain (congenital insensitivity to pain)
12. Known Child-Pugh liver disease severity classification B or C.
13. Known chronic kidney disease stage 4 or higher (GFR≤29).
14. Known Hemophilia A/B
15. Known bleeding dyscrasia
16. History of an inability to tolerate fish oil or corn/soybean oil.
17. Severe gastroesophageal reflux disease
18. No other history or condition that would, in the investigator's judgment, indicate that the patient would very likely be non-compliant with the study or unsuitable for the study (e.g. might interfere with the study, confound interpretation, or endanger patient).
19. Intubated and sedated at time of enrollment.
20. Hypersensitivity to Vitamin D3, ergocalciferol, calcitriol, alfacalcidol, calcipotriol
21. Hypercalcemia (if not already completed, this will be assessed by clinical labs with albumin correction prior to enrollment).
22. Hypervitaminosis
23. Sarcoidosis
24. Hyperphosphatemia
25. Arteriosclerosis
26. Active myocardial ischemia
27. Frequent antacid use (calcium carbonate, cimetidine)
28. Cholestyramine or Colestipol use
29. Taking Vitamin D supplements in excess of 800 IU daily.
30. Taking >1g of fish oil per day.

3.8 Subject Recruitment and Screening

Prior to approaching the patient, medical history data will be reviewed for information pertaining to inclusion/exclusion criteria in section 4.2. If the patient is determined to be ineligible, the review will stop. The purpose of assessing this information is to approach only those patients who are potentially eligible. Because of the disproportionate burden of chronic pain in African Americans and women following MThBI,

we will over sample these populations if needed to achieve 20 African Americans and 20 women. A logistic prediction tool will be used to predict the probability of chronic pain and is designed to sample individuals most likely to develop persistent/chronic pain. Record will be kept of patients who are screened, but excluded and for what reason. A HIPAA waiver will be requested to permit the review of medical record data for evident exclusion criteria prior to approaching a patient (this will reduce study burden on the study population who are recovering from a burn injury). In patients who otherwise appear to be eligible for the study, the attending burn surgeon/medical record will be consulted to determine if surgery is planned (most of the time this can be determined through chart review). Reasons for exclusion will be recorded. To be eligible for the study, the burn must be severe enough for the burn surgery team to plan surgical management. Patients will be approached by study personnel and will be offered participation. Participants will undergo informed consent. If patients refuse consent, data collected to that point will be retained to describe the proportion of burn patients who were screened but refused to participate (because this study is regulated, we will maintain demographic information for audit purposes). Reason to refuse participation will be also documented and retained. If the patient is after 10 days following their last menstrual period, and is not on birth control, a urine pregnancy test will be performed prior to randomization. If the patient is found to be pregnant they will be excluded from the study. If not already collected, an albumin corrected calcium level will be drawn and patients will be excluded if >12 mg/dL. Once patients are determined to meet full inclusion criteria and have no reason for exclusion, consented patients will be randomized and study drug will commence on enrollment in the study. Patients who are initially intubated may be eligible for the study, as long as they are extubated, alert, and oriented at the time of enrollment and other eligibility criteria are met. Advertising for this study will occur through flyers placed on the burn unit that will be approved by the institutional IRB for each study site.

3.9 Early Withdrawal of Subjects

3.9.1 When and How to Withdraw Subjects

Participants may withdraw from the study at any point in time. If patients experience side effects such as gastroesophageal reflux/"heartburn", unpleasant taste that cannot be tolerated or any other unwanted effect and elect not to continue the study drug, they will be followed and included in the intention to treat analysis. Abrupt discontinuation of the study medication or comparator is not expected to affect/compromise patient safety. In other words, no wean would be needed. If patients are determined to be pregnant during the 6 weeks of drug administration (through home urine pregnancy test, or through an office based urine/serum pregnancy test) then the study drug will be discontinued, but the patients will be followed in an intention to treat fashion.

3.9.2 Data Collection and Follow-up for Withdrawn Subjects

If participants choose to withdraw because of side effects, they will be followed until their symptoms resolve or until it is determined that their side effects were not related to the study drug. If the patient voluntarily withdraws from the study, outcome measures from completed timepoints will be collected and included in intention to treat analysis. If the patient is lost to follow-up and is unresponsive to more than 4 telephone calls and 4 secure e-mails, the next of kin listed in the medical record will be contacted once to attempt to reach the participant. If this is not successful, then 1 certified letter will be sent to attempt to contact the consented participant. This is highlighted in detail in the manual of procedures.

4 Study Drug

4.1 Description

The study drugs are 1) 2g of DHA/EPA administered orally as 4 capsules comprising approximately 2 grams of EPA/DHA in a 3:2 ratio (this will require a total dose of 4 grams of fish oil) and 2) Vitamin D_3 (cholecalciferol) 2000 IU orally.

4.2 Treatment Regimen

For 6 weeks following enrollment in this study, participants will be provided with study drug or placebo. Both the n-3 PUFA and Vitamin D will be taken together once per day.

4.3 Method for Assigning Subjects to Treatment Groups

Once a potential subject has been successfully screened, does not meet any exclusion criteria, is willing to participate in the study, and signs informed consent, the patient will be randomized by the study site using a block randomization scheme with permuted block sizes stratified on ethnicity and sex. A randomization table will be generated by the study statistician and placed in the randomization module in RedCap. Only the investigational pharmacy and study statistician will be unblinded to the drug the patient is receiving. They will not reveal this to the patient care team, investigative team, or to the patient. Identical dosing schedule, route of administration, number of pills in each dose, and the appearance of the capsules will be identical among each group. At the time of patient discharge from the study site, the study site (nursing staff) will dispense a supply of study medication for the patient to complete a 6 week course (this medication was initially dispensed to the patient from IDS at the time of enrollment). Only the IDS will know the identity of each group's treatment, as well as the study statistician. This will only be provided to the study statistician at the end of the study.

4.4 Preparation and Administration of Study Drug

Study medication, including n-3 PUFA, Vitamin D_3 and comparator, will be ordered directly from the supplier and delivered to the investigational drug pharmacy (IDS) at each site. All medication will be stored in a climate controlled storage area. The containers will be labeled by the manufacturer. Each participant will receive two bottles of study medication one containing n-3 PUFA or control and anther containing Vitamin D or placebo to take once daily. The fish oil/placebo will contain 168 capsules, and will have instructions to "take 4 capsules by mouth each morning for 6 weeks." The Vitamin D3/placebo will contain 42 capsules, and will have instructions that read "take 1 capsule by mouth each evening for 6 weeks." Upon discharge, the patients will be given a quantity to complete 6 week course of study medication. Lost medication will be replaced upon request and this will be dispensed by the IDS pharmacy and shipped to participant by the site coordinator. At Washington Hospital Center, an IDS pharmacy will not be used, and procedures will be developed that will enable dispensing study drug and accounting for dispensed study drug per their protocol approved by the site IRB.

4.5 Subject Compliance Monitoring

Administration of the study drug will be completed by burn center nursing staff and will be documented in the medication administration record (MAR). Reasons for refusal will be documented in the MAR. Following the treatment period, patients will receive weekly surveys delivered by text message/e-mail via RedCap or by phone call that will assess the number of missed doses as well as a graft-site pain rating (0-10 NRS). In addition, a biological test for medication compliance will be performed by comparing starting concentration of n-3 PUFAs/Vitamin D to ending concentration (e.g. an increase in PUFA level is an additional marker of compliance).

4.6 Prior and Concomitant Therapy

Prior and concomitant analgesic, anti-inflammatory, anti-itching, and anticoagulants will be collected on enrollment, discharge from the hospital through data extraction, and at each telephone/e-mail follow-up assessment. The need for concomitant therapy with a therapeutic dose of anticoagulation for any indication will trigger a conversation with the prescribing physician about whether co-administration of fish oil is appropriate given the specific indication for anticoagulation. If it is not acceptable to take fish oil and anticoagulant in the prescribing physician's best judgement, then the study drug will be stopped without unblinding. Analgesic medications as would be normally prescribed are permitted throughout this study including anti-inflammatory agents which are part of routine care of burn survivors. Consumption of medication will be tracked and addressed in secondary analyses.

4.7 Packaging

Study drug will be shipped from supplier in the case of vitamin D an n-3 PUFAs in bulk containers and stored in the investigational drug pharmacy. Study drug will be administered to inpatient burn survivors by nursing staff or other appointed staff. On discharge from the burn unit, a quantity sufficient to complete a 6 week supply will be calculated by study staff and dispensed by the site at the time of patient discharge.

4.8 Blinding of Study Drug (if applicable)

Only the investigational drug services and unaffiliated staff will know the contents of the capsules. The study drugs and comparator will be packaged in identical capsules and will be indistinguishable. Each patient will be assigned to a treatment arm using the randomization table using a block randomization technique. Randomization table to be generated by Data Coordinating Center statistical support. A Randomization ID will be generated that corresponds to the treatment. Only the IDS or, in the case of Washington Hospital Center, an appointed staff with no affiliation with the study will dispense the study drug maintaining strict blinding.

4.9 Receiving, Storage, Dispensing and Return

4.9.1 Receipt of Drug Supplies

Our drug supplier will ship the study drug and comparator to the UNC Investigation Drug Pharmacy. Once the study drug arrives, receipt of the study drugs will be performed and a drug receipt log filled out and signed by the person accepting the shipment. Each site will develop their own process for maintaining a log of drug administration. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory supplied at the time of shipment. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files.

4.9.2 Storage

Drug will be stored at 77 degrees Fahrenheit with excursions permitted from 59-86 degrees Fahrenheit at the UNC investigational drug pharmacy. At each site, it will be kept in a climate controlled room. The drug will not be frozen. There is no requirement for the drug to be shielded from light or any other special requirements.

4.9.3 Dispensing of Study Drug

The study drug will be dispensed by burn unit nursing staff during the inpatient phase of treatment along with other daily medications. Upon discharge from the burn unit, the remaining number of tablets to complete a 6 week course will be calculated by study staff and dispensed to the patient in standard containers with a label outlining instructions. Study drug reconciliation will be performed at the conclusion of the study. This reconciliation will be logged on the drug accountability form and signed and dated by the study team. This log will be kept by the UNC site investigational drug pharmacy at UNC via an electronic system.

4.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

5 Study Procedures

5.1 Enrollment Procedures

This first visit will take place upon admission to the hospital following a major thermal burn injury within 72 hours of MThBI. Patients enrolled in the study will be randomized (1. once consents are signed and 2. baseline data is collected) into a 2x2 factorial design with 1:1:1:1 allocation as described in the study

overview. Burn survivors qualifying for this study are generally hospitalized for a period of time (typically 7-14 days) and therefore will start the study drug immediately after enrollment questionnaire has been completed and continue daily dosing during their stay. Upon discharge of the patient a supply sufficient to complete a 6 week course of study drugs will be dispensed by study staff.

Medical record information abstracted at time of admission will include information related to patient past medical history and initial burn care, and medication use prior to admission and prior to enrollment. Laboratory values relevant to patient renal and hepatic function will also be obtained, along with any toxicology labs obtained by the primary treating team.

Demographic and phenotypic data will be collected at this time. Patients will be asked to report demographic information including age, sex, ethnicity, education level, income level, and marital status. Pre-burn physiological traits will be assessed using pain catastrophizing[38], SF12 MCS[39], depression anxiety stress scale (DASS21)[40], and Life events checklist. Characteristics of the patients' injuries will be reported through estimated percent total body surface area, mechanism their burn, and the donor/graft site location. Current pain at the graft site will be assessed using a 0-10 numeric rating scale[41-44], brief pain inventory, DN-4, S-LANSS[35] and Regional Pain Scale (RPS). The patient's physical function will be assessed with the SF12[39] and a work/disabilities questionnaire. Depressive symptoms will be recorded using the DASS21[40]. PTSD symptoms will be assessed with the PTSD Symptom Severity index-interview[45]. Patient somatic symptoms will be recorded with the somatic symptoms inventory. The patient's burn wound will be documented with digital photos taken of the injury site. Dissociative symptoms will be documented with the Michigan Critical Events Perception Scale[48]. Analgesic medication use will be recorded as daily milligram morphine equivalents. All patient information will be recorded by a trained research assistant or principal investigator. We additionally will assess treatment expectation using a modified survey that uses Likert scales prior randomization.

At the time of enrollment, blood for DNA will be collected using a PAXgene DNA storage tube (8.5cc). These samples will be stored at -70 ^{o}C or below for future testing. Blood for RNA will be collected using a PAXgene RNA storage tube (2.5cc). These samples will be stored at -70 ^{o}C or below for future testing. Blood for immune profiling will be collected in sodium heparin tubes (16cc). Whole blood collected from sodium heparin tubes will be aliquoted into SmartTubes which allow storage for later processing by mass cytometric methods. Additional aliquots from these tubes will used to assess adaptive and innate immune function *in vitro.* Blood for lipidomics, vitamin D level and protein expression analysis will be obtained via EDTA tubes containing K_2EDTA for sample anticoagulation (8cc). Plasma will be separated from the cells by centrifuging the collection tube for 10 minutes at 2000 rpm at 25 °C, within two to 48 hours of collection. Plasma will be stored in 100-250 µL aliquots in plastic microcentrifuge tubes at -70 °C or below for future testing. Total amount of blood collected via venipuncture will be 35-36cc. A drop of blood will be applied to two dried blood spot cards, one for Vitamin D testing and another for fatty acid profiling.

Primary study measures by timepoint.

Domain	Measure	D1	6W	3M	6M	1Y
Demographic information	Age, sex, ethnicity, education level, income level, marital status	●				
Pre-burn psychological traits	Pain catastrophizing, SF12 MCS, DASS21, Life events checklist	●				
Burn Injury Characteristics	Estimated % TBSA, mechanism of burn, donor/graft site location	●				
Graft Site Pain severity	0-10 numeric rating scale; Brief Pain Inventory, DN-4, S-LANSS †RPS	●	●	●	●	●
Physical function	SF12, work/disability questions	●	●	●	●	●
Depressive Symptoms	Depression anxiety stress scale (DASS21)	●	●	●	●	●
PTSD symptoms	PTSD Symptom Severity index-interview	●	●	●	●	●
Somatic Symptoms	Somatic symptoms inventory	●	●	●	●	●
Alcohol and drug use	TWEAK/SAOM		●	●		●
Burn wound evaluation	Digital photos of injury/wound site	●	●	●	●	●
Dissociative Symptoms	Michigan Critical Events Perception Scale	●				
Analgesic Medication Use	Daily milligram morphine equivalents, non-opioid analgesic use	●	●	●	●	●

†RPS = Regional pain sale, M=month, W=week, D= day, PTSD=post-traumatic stress disorder, TBSA: total body surface area, DN-4 Douleur Neuropathique 4, neuropathic pain questionnaire, S-LANSS: self report version of the Leeds Assessment of Neuropathic Pain scale SF-12: 12 item short form from the medical outcomes survey.

5.2 Inpatient Medication Administration

Daily the clinical nursing staff or study staff will administer the study drug orally along with the patient's morning medications or a separate time if this proves not feasible. This administration will be recorded in the patients electronic medical record in the medication administration record (MAR). Review of the MAR by study personnel will help calculate the supply of medication needed for each subject to complete a 6 week course of study medications: n-3 PUFA, Vitamin D, both or comparator (corn/soybean oil).

5.3 Data Extraction

Medical record information abstracted after discharge will include information on the patient's health status and care during hospitalization and at the time of discharge. This information will include burn characteristics (e.g. TBSA, depth, location, mechanism), medications taken during admission, and discharge medications. Data collected regarding the surgical procedure will include donor site location, size, mesh ratio, mechanism of grafting (e.g., staples or fibrin glue), location of grafting, and date of surgery.

5.4 Text messaged or e-mailed outpatient Weekly Pain and Compliance Assessments

Brief, weekly assessments of number of missed doses in the past week and graft site pain severity (0-10 NRS) over the past 24 hours will occur via automated text message/e-mailed surveys administered through our secure study database RedCap. If patients are not able to respond via text messaging or e-mail they will be called by investigative team. Patients will be called by study staff if they miss 3 or more doses in a 1 week period to provide counseling and determine any potential barriers to taking the study medication. Study staff will contact the participants at 3 weeks for a brief assessment of missed doses, to provide adherence counseling, and collect any reported information about adverse events. At this time a condensed bleeding assessment will be performed to assess bleeding events since the beginning of treatment[36].

5.5 6 week assessment

The goal of this assessment is to perform a comprehensive phenotypic follow-up and perform a blood draw to assess for lipid concentration, Vitamin D concentration and related gene expression (RNA), and epigenetic changes (DNA).

Outcome assessments will be performed via structured and scripted interview/survey either at the burn clinic or via telephone or via a web based survey emailed to their on file email address. Multiple methods of contacting participants may be used to best meet the needs of the participants. Average pain and itch (0-10 NRS), frequency of pain and itch during the past 24 hours at the patient's donor site, as well as average pain and itch (0-10 NRS) and frequency of pain and itch during the past 24 hours at the patient's graft site will be assessed. The condensed bleeding assessment tool will be repeated to assess for bleeding events between the 3 and 6 week timepoints. The presence of neuropathic pain symptoms (DN4[37], S-LANSS[35]) will also be assessed, as well as pain and itch related interference in daily activities (Brief Pain/Itch Inventory Interference Subscales).[38] Information regarding current medication use (both standing and PRN) and health services utilization (including burn care such as PT, OT) will also be obtained. At these time points the following assessments will also be administered: The frequency of scratching and its effect on itch related to the burn, at either the donor site or the graft site, will be evaluated. The location (Regional Pain Scale[39]) and intensity (0-10 NRS) of pain and itch symptoms (using a modified RPS) in any locations other than the donor and graft sites will be assessed, along with the presence and severity of other somatic symptoms (e.g. headache, dizziness, and itching). General health (SF-12[40]), depressive and anxiety symptoms (DASS[41]), and PTSD symptoms (PSS-I[42]) will be assessed. Sleep quality will be assessed using the MOS Sleep Scale.[43] Global recovery after burn injury will be evaluated using a 0-100 percent improvement scale (0%=no recovery, 100%=completely recovered). Alcohol abuse is common among injured patients,[44] and increased alcohol or drug use after trauma is likely to be related to chronic pain and itch outcomes. Quantity and frequency of alcohol and drug use will be assessed at the 24 week and 48 week follow-up time points, using questions from the TWEAK[45] and Substance Abuse Outcomes Module.[46]

During the 6 week visit to the burn clinic, patients will provide plasma, RNA and DNA samples as described above via routine venipuncture performed by trained personnel. The total amount of blood obtained will be approximately 14-15cc (less than 2 tablespoons). During this process 1 drop will be applied to two dried blood spot cards one for Vitamin D assessment and another for fatty acid profiling. If this is not possible or feasible, a mobile blood draw technician may be dispatched to a location convenient to the patient to obtain this sample. If this blood draw proves not to be feasible, we will provide (either by mail or, in the case of expected difficulties, at the time of enrollment) a patient a kit to perform a finger-prick to blot a drop of blood on two dried blood spot cards for 1. Assessment of Vitamin D level and for 2. Fatty acid profiling. This will be the same cards in which are used for initial blood collection. A dietary assessment will be performed as part of the 6 week assessment.

5.6 3, 6 and 12 month assessments

Outcome assessments will be performed via structured and scripted telephone interviews. Average pain and itch (0-10 NRS), frequency of pain and itch during the past 24 hours at the patient's donor site, as well as average pain and itch (0-10 NRS) and frequency of pain and itch during the past 24 hours at the patient's graft site will be assessed. The presence of neuropathic pain symptoms (DN4[37], S-LANSS[35]) will also be assessed, as well as pain and itch related interference in daily activities (Brief Pain/Itch Inventory Interference Subscales).[38] Information regarding current medication use (both standing and PRN) and health services utilization (including burn care such as PT, OT) will also be obtained. At these time points the following assessments will also be administered: The frequency of scratching and its effect on itch related to the burn, at either the donor site or the graft site, will be evaluated. The location (Regional Pain Scale[39]) and intensity (0-10 NRS) of pain and itch symptoms (using a modified RPS) in any locations other than the donor and graft sites will be assessed, along with the presence and severity of other somatic symptoms (e.g. headache, dizziness, and itching). General health (SF-12[40]), depressive and anxiety symptoms (DASS[41]), and PTSD symptoms (PSS-I[42]) will be assessed. Sleep quality will be assessed using the MOS Sleep Scale.[43] Global recovery after burn injury will be evaluated using a 0-100 percent improvement scale (0%=no recovery, 100%=completely recovered). Pending litigation or workman's compensation claims related to the burn injury will also be evaluated (at month 12). Alcohol abuse is common among injured patients,[44] and increased alcohol or drug use after trauma is likely to be related to chronic pain and itch outcomes. Quantity and frequency of alcohol and drug use will be assessed at the 24 week and 48 week follow-up time points, using questions from the TWEAK[45] and Substance Abuse Outcomes Module.[46] Photos of both the graft site and donor site (if present) will be obtained using a standard algorithm using any available digital camera/smartphone and stored on the research database. If patients do not have access to a digital camera one will be mailed to their address with a return label, or they will have the photos taken during a follow-up clinic visit. We will also collect information about the number of servings of fish, olive oil, and any Vitamin D or fish oil supplements that have been consumed since the 6 week treatment period. Participants will be contacted at 9 months for a brief participant contact information update. This will ensure that the participant's address, phone number, and e-mail address are up-to-date for study contact purposes.

6 Statistical Plan

6.1 Sample Size Determination

The purpose of this study is to assess study feasibility, safety and to obtain initial effect size estimates and confidence intervals to use in sample size calculations for a larger study. Previous studies of Vitamin D and n-3 PUFAs for the treatment of chronic pain are poorly generalizable to this setting because 1) they examine treatment of established chronic pain states versus developing pain states, 2) do not involve trauma exposure/acute stress, and 3) are not preventive studies. Therefore, this pilot study will be instrumental in demonstrating feasibility and safety of the protocol, and estimates of efficacy along with confidence intervals will be helpful for planning a larger follow-up study.

Justification for the sample size. We will target 15 individuals in each group (60 total) so that feasibility and safety may be determined as well as provide potential evidence for efficacy. Below, calculations are provided to justify sample size for each objective.

Feasibility: Assuming that observed follow-up will be around 80%, this study will allow us to estimate follow-up rates to within ± 10% with a 95% confidence interval given we reach the enrollment target of 60. Allowing for up to 20% loss, with 30 participants treated in each n-3 PUFA arm of our study, our study is powered at 90% to detect a difference of 2.6% of DHA (an n-3 PUFA) in total fatty acid content considering a standard deviation of 1.3%[47]. This study (30 per Vitamin D arm is powered at 83% to detect a change of 9 ng/dl in Vitamin D level assuming a SD of 10.5[48].

Efficacy: Allowing for up to 20% loss and assuming a common standard deviation of 2.6 (approximated from our pilot dataset) and no interaction between treatments, randomizing 30 participants to each comparison group (Vitamin D/n-3 PUFA versus control) will allow us to estimate the difference in mean pain severity to within ±1.4 using a two-sided 95% confidence interval.

6.2 Statistical Methods

Primary outcome assessments and analysis:

Feasibility: The primary objective of this pilot study is to ensure that we are able to enroll participants and make follow-up assessments. The subsequent study will be considered feasible if we are able to collect follow-up assessments on >80% of enrolled participants at 6 weeks following MThBI, and >80% of participants at 1 year. Medication adherence will be assessed by pill counts at the end of the study and quantitative determination of plasma fatty acid/Vitamin D concentration. The subsequent study will be considered feasible if at least 75% of participants take at least 80% of their assigned medication (per pill count) and there is a statistically significant increase in Vitamin D and fatty acid concentration in treated individuals versus controls. T-tests will be used and a $p<0.05$ will be considered statistically significant.

Safety: Another main objective of this pilot study is to ensure safety of both treatments as well as combined. To determine safety, we will tabulate the number of serious adverse events in each treatment arm and perform a descriptive review of adverse events in the study, and make a determination about the degree of relatedness of each adverse event with the intervention using CTCAE criteria outlined in Appendix 2. The recorded adverse events in each arm will be tabulated descriptively, but no inferential statistics (p-values and confidence intervals) for comparing arms will be reported.

Efficacy: Estimates of efficacy will be obtained via repeated measures analysis using mixed effects models (PROC MIXED in SAS 9.4). Prior to unblinding the data, our study statistician will perform a blind review of the data in which statistical models will be developed and tested using dummy (computer generated) treatment assignments. The goal of this is to fit the most appropriate model given aggregate data. Graft site pain severity will be collected in four waves across time; therefore, we will use mixed effects modeling with random error using an unstructured covariance matrix. The model will include fixed effects for PUFA, Vitamin D, their interaction, time, and the interactions between groups and time, as well as fixed effects for site, gender, and race/ethnicity. During blind review, competing nested fixed effect models will be compared. We will consider modeling time using either a) time as continuous variable with a linear model (i.e., slopes only), b) time as a continuous variable with a higher order polynomial model (up to cubic, given 4 time points), or c) time as a class variable. Competing fixed effect models will be fitted using maximum likelihood and compared using the BIC criterion. Once the form for the fixed effect model is selected, the final model will be fit using restricted maximum likelihood. After unblinding the data, we will use appropriately specified linear contrasts of the model parameters to estimate mean differences across groups at each time point along with 95% confidence intervals.

Interim analysis: Two interim analyses are planned after the enrollment of the 20th and 40th patients in the study. This will be conducted to ensure safety, perform a feasibility assessment, and assess potential efficacy. This optimizes study ethics, as it ensures safety of current and future study participants, allows

optimization of study protocol based on results of safety and feasibility assessment, and allows an efficacy assessment with appropriate statistical "penalty" for an interim look. Adverse events will be qualitatively reviewed at two interim analyses occurring after enrollment of the 20th patient and after enrollment of the 40th patient. While study investigators, staff, and participants remain blinded, the study statistician will prepare a qualitative summary of adverse events by type over the treatment groups. The results will be reported to investigators in aggregate such that blinding is maintained.

Significance thresholds for interim analyses for potential efficacy will be adjusted using the O'Brien-Fleming approach. In order for the trial to stop enrolling because efficacy (superiority) is established after the first interim analysis (after 20 patients enrolled), the boundary critical value of 3.428 and corresponding alpha of 0.0006 must be reached. In order for the trial to stop enrolling because efficacy is established after the second interim analysis (after 40 patients enrolled), the boundary critical value of 2.431 and corresponding alpha of 0.0151 must be crossed. After the final analysis (third analysis) a critical value of 1.985 and a p-value of 0.0471 will be used. This final, adjusted p-value threshold of 0.0471, allows for a statistical "penalty" to be assessed because of the interim look. Given that this is a pilot study, we believe it is critically important to assess our safety, feasibility and potential efficacy outcomes at interim analysis.

Given that the study was terminated early after enrollment of the 24th patient after one interim analysis, the final, adjusted p-value threshold of 0.0492, allows for a statistical "penalty" to be assessed because of the interim look.

Secondary Analyses:
Similar models as for the primary outcome will be fit for both the Brief Pain Inventory and the SF-12 Mental and Physical Component Scale Scores.

Sex as a biological variable (SABV) will be examined in treatment response including sex-by treatment interaction terms in the mixed effect model specified above and exploring sex differences in therapeutic response to Vitamin D and n-3 PUFAs.

General mental and physical health will be assessed using SF-12 physical and mental component scores (PCS and MCS) and the impact on physical and mental functioning will be determined in each of the treatment groups. A clinically important reduction in SF-12 PCS and MCS ranges between 2.5-4.5[49]; therefore, a reduction of ≥ 4.5 will be considered a clinically significant improvement MCS/PCS.

Brief Pain Inventory: We will also use multivariable analysis to determine the role of n-3 PUFA/Vitamin D supplementation in pain interference using composite and individual measures of the Brief Pain Inventory.

6.3 Subject Population(s) for Analysis

We will examine effect of n-3 PUFA, Vitamin D, and n-3 PUFA+Vitamin D treatment on pain outcomes using the following populations; however, the All-randomized population will be the primary analysis performed in this study following an intention to treat paradigm.
Primary Analysis:

- All-randomized population: Any subject randomized into the study, in the group to which they were randomized, regardless of extent of study drug received (intention-to-treat).

Secondary Analyses:

- Protocol-compliant population: Any subject who was randomized and maintained >80% study medication adherence, and no major protocol violations.

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.).
- Related or possibly related to participation in the research (i.e. 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).
- Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the

investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor will conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

7.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site.

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

7.3.2 Investigator reporting:

For reportable deaths, the initial submission to the UNC IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

7.3.3 Investigator reporting:

Investigators who are not UNC faculty or affiliated with a UNC research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

7.3.4 Sponsor reporting: Notifying the FDA

The study PI will report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening
    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3.

7.3.5 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

7.4 Unblinding Procedures

Unblinding the group in which the patient is randomized will only occur in exceptional circumstances when the knowledge of treatment is essential for the management of an individual patient. In emergency situations (e.g. anaphylaxis) the principal investigator may contact IDS to have the identity of the patient's treatment drug revealed to help manage the patient more effectively. If this occurs, the unblinding procedure will be documented in the patient's source documents. If emergency unblinding is needed, it will likely occur in the context of a serious adverse event and will be reported to the FDA along with the SAE. Unblinding will be reported similar to an SAE within 24 hours by phone or fax, followed by a written narrative of the event within 48 hours.

7.5 Stopping Rules

The entire trial will stop if any investigator judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, or good clinical practice.

7.6 Medical Monitoring

A. Safety Monitoring Plan: Overall safety oversight will be the responsibility of the data coordinating center PI (DCC PI). Monitoring the study (across one or more study sites) is considerably facilitated by the use of a single electronic database which can be reviewed by the network PI, study site PI(s), and other investigators. The DCC PI will monitor for adverse events that change study risk level and this will be communicated with the independent medical monitor if needed. Adverse events at individual study sites will also be monitored by the site PI. If an adverse event occurs which changes the study risk level, the DCC PI will immediately report this event to the DCC IRB (UNC IRB), inform the site PI(s), and oversee the process of modifying the study at the study site(s) as appropriate to address the change in risk. The DCC PI will also provide annual reports summarizing AE data to the data coordinating center IRB (UNC IRB) and any other requesting IRB. The site PI(s) will oversee patient safety at their site, with delegation of responsibilities to other investigators and designated study personnel as appropriate. The DCC PI, together with their study site teams, will ensure that all entry criteria are met prior to the initiation of the protocol, and that all study procedures and reporting of adverse events are performed according to the protocol. Serious adverse events (SAE's) will be monitored by the study site investigative teams in real time throughout the trial.

Serious AE	Death Life threatening reaction Inpatient hospitalization prolonged, or rehospitalization Persistent or significant disability/incapacity Congenital anomaly/birth defect Represents other significant hazards or potentially serious harm to research subjects (similar to "important medical event")
Expected AE	Known potential effect of the drug (based on drug description information from manufacturer) or disease process

Patient monitoring: An adverse event is any physical or clinical change experienced by the patient. This includes the onset of new symptoms and the exacerbation of pre-existing conditions. Adverse event assessments will be performed according to the following schedule:

Adverse event monitoring after discharge: Adverse events will be assessed at each patient contact (telephone or text-messaged/emailed survey), which will occur weekly while on the study drug.

Additional reporting: In addition to receiving these regular side effect assessments, patients will be given a toll free study number to reach the study team at the DCC, as well as telephone/pager numbers for individuals at the study site, and will be instructed to call these numbers if they experience any changes in their health status or if they have questions regarding the study medication.

Category definitions for adverse events are shown in Table 7. An electronic adverse event form will be completed for all unanticipated AEs. The severity of the AE will be assessed a CTCAE grade will be recorded for each. An investigator will also assess the relationship of any adverse event to study medication or procedures. Severity assessments will be performed using the Common Toxicity Criteria (http://ctep.cancer.gov/). The time table for reporting serious and other adverse events is shown in Table 7.

Unanticipated problems involving risks to participants or others, defined as any incident, experience or outcome that is both

Table 7. Time table for reporting unanticipated adverse events.

	Unanticipated
Serious AE	One week
Not Serious AE/other	Two weeks

unexpected and related or possibly related to the research, will be reported to the IRB. Unanticipated problems that are serious adverse events will be reported to the IRB within 1 week of the investigator becoming aware of the event. Any other unanticipated problem will be reported to the IRB within 2 weeks of the investigator becoming aware of the problem. The study team will report all serious unanticipated adverse events to the DCC PI immediately, and will review all non-serious unanticipated adverse events with the PI within 24 hours.

Patients with adverse events will be monitored with relevant clinical assessments (and laboratory tests if necessary) as determined by an investigator in collaboration with the patient's care team. All adverse events will be followed to satisfactory resolution or stabilization of the event(s). Any actions taken and follow-up results will be recorded on the appropriate AE form and given a CTCAE grade.

Appendix 2 describes the adverse event grading scale and management guidelines for potential adverse events related to the study drugs. This chart covers a number of common adverse effects, but will not be comprehensive. As other situations are encountered and managed by the study team, this decision rule chart will be updated and expanded so that it remains current and inclusive.

Summary information regarding adverse events will be provided to the IRB at the time of annual IRB renewal. As described below, medical monitor reports will also be submitted to the UNC IRB on a quarterly basis. Criteria for stopping the study trial among all participants for review are shown in Appendix 2.

Patients experiencing adverse effects that in the opinion of the investigators merit drug dosage adjustment will have their dosage advanced more slowly, or the next dose reduced or held as appropriate. If necessary, patients will be maintained on a lower dose, or their dose will be re-advanced depending on the nature of the side effect(s). Patients who decline further participation and patients experiencing adverse effects that in the opinion of study investigators indicate that study drug should be stopped will be discontinued or tapered from study medication.

Independent Medical Monitor: To handle individual problems that may require communication directly with physician caring for the patient, or if decision making involves altering the study drug administration on a patient level, an independent Medical Monitor will become involved. This is to avoid any conflicts of interest that may arise. Data Handling and Record Keeping

7.7 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

7.8 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

7.9 Case Report Forms (as applicable)

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded in RedCap. All missing data will be noted. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be written. If the item is not applicable to the individual case, "N/A" will be written. All entries will be entered electronically in RedCap database. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. For clarification of illegible or uncertain entries, the clarification above the item will be printed initialed and dated.

7.10 Records Retention

Essential study documents (consents, paper questionnaires, screening forms) will be maintained for the duration of the IND. Data will not be de-identified/unlinked from original dataset to preserve the ability to contact patients should any unknown adverse events become known following the study. This data will be stored on a password encrypted server (RedCap at UNC).

8 Study Monitoring, Auditing, and Inspecting

8.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan described above. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

8.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Risk based monitoring plan will include study site visits from study sponsor (Mauck) after 2, 5 and 10 enrolled patients at sites once non University of North Carolina sites become active.

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

9 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Appendix 1 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent

form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

10 Study Finances

10.1 Funding Source

This study is financed by the NIH BIRCWH K12 program at UNC, the UNC Department of Anesthesiology, and the Institute for Trauma Recovery.

10.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All UNC investigators will follow the University conflict of interest policy.

10.3 Subject Stipends or Payments

To compensate participants for their time for completing follow-up interviews, we will offer patients $35 for the enrollment interview. Participants will be contacted weekly for the subsequent 5 week and will be compensated $10 for each week's survey completion. At 6 weeks, participants will be compensated $20 for survey completion, $45 for blood collection, and $10 for study drug/bottle return, for a total of up to $75 at the 6 week time point.. At the 3 and 6 month interviews, patients will be compensated $25 for each assessment completed. Participants will be compensated $10 at 9 months for a successful contact information update. Upon completion of the 12 month interview patients will receive $30. The maximum amount that an individual study participant may receive is $250.

11 Publication Plan

Principal investigator, in coordination with investigative team will determine the publication plan as appropriate on an ongoing basis. We will make every effort to publish study results within 1 year of database completion.

12 Data Management Plan

RedCap will be used as our research database and is a secure, widely-used system for recording study information. Access to enter data and view patient information will be password protected and accessed only by study personnel. At the time of enrollment or subsequent follow-up interview, data will be directly entered into RedCap. A codebook for the dataset will be compiled and saved along with the completed dataset. Our laboratory data manager will be responsible for assembling the final de-identified dataset and distribution to the study statistician for analysis. Completeness of the dataset will be determined as a percentage of non-missing data. When possible, reasons for missing values will be recorded (e.g. not applicable, refused to answer). Each research assistant that enters data in the system will be trained on mock patients and coding of patient responses will be checked. Data will be recorded by study sites directly into RedCap. Any discrepancies in coding will be handled by direct communication with study personnel at each study site.

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14 Appendices

14.1 Appendix 1: Adverse Event Monitoring Table

AE	Relatedness	CTCAE Grade				
		1	2	3	4	5
Back Pain		Mild Pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL		
	Possible	O	O	O		
	Probable	O	O	SI		
	Definite	O	SI	SI		
Gastrointestinal pain		Mild Pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL		
	Possible	O	O	O		
	Probable	O	O	SI		
	Definite	O	SI	SI		
Belching (or Flatulence)		Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae			
	Possible	O	O			
	Probable	O	O			
	Definite	O	SI			
Rash (maculo-papular?)		Macules/papules covering <10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	Macules/papules covering 10 - 30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL	Macules/papules covering >30% BSA with or without associated symptoms; limiting self care ADL		
	Possible	O	O	O		
	Probable	O	O	SI		
	Definite	O	SI	SI		
Dysgeusia		Altered taste but no change in diet	Altered taste with change in diet (e.g., oral supplements); noxious or unpleasant taste; loss of taste			
	Possible	O	O			
	Probable	O	SI			
	Definite	O	SI			
Bruising		Localized or in a dependent area	Generalized			
	Possible	O	O			
	Probable	O	O			
	Definite	O	SI			
Hematoma		Mild symptoms; intervention not indicated	Minimally invasive evacuation or aspiration indicated	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
	Possible	O	O	SI	SI	
	Probable	O	SI	SI	SI	
	Definite	O	SI	SI	SI	
Atrial fibrillation		Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
	Possible	O	SI	SI	SI	
	Probable	O	SI	SI	SI	
	Definite	O	SI	SI	SI	

Atrial flutter		Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker), or ablation	Life-threatening consequences; urgent intervention indicated	Death
	Possible	O	SI	SI	SI	
	Probable	O	SI	SI	SI	
	Definite	O	SI	SI	SI	
Alanine aminotransferase (ALT) increased		>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	
	Possible	O	SI	SI	SI	
	Probable	O	SI	SI	SI	
	Definite	O	SI	SI	SI	
Aspartate aminotransferase increased		>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	
	Possible	O	SI	SI	SI	
	Probable	O	SI	SI	SI	
	Definite	O	SI	SI	SI	
Cholesterol high		>ULN - 300 mg/dL; >ULN - 7.75 mmol/L	>300 - 400 mg/dL; >7.75 - 10.34 mmol/	>400 - 500 mg/dL; >10.34 - 12.92 mmol/L	>500 mg/dL; >12.92 mmol/L	
	Possible	O	O	O	SI	
	Probable	O	O	SI	SI	
	Definite	O	O	SI	SI	
Chills		Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics		
	Possible	O	RD	SI		
	Probable	O	SI	SI		
	Definite	O	SI	SI		
Fever		38-39ºC	>39-40ºC	>40ºC for ≤24 hours	>40ºC for >24 hours	Death
	Possible	O	O	SI	SI	
	Probable	SI	SI	SI	SI	
	Definite	SI	SI	SI	SI	

Criteria for stopping entire trial for review: The entire trial will stop if any investigator judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, or good clinical practice.

Possible = The AE **may be related** to the intervention
Probable = The AE **is likely related** to the intervention
Definite = The AE **is clearly related** to the intervention

*O = observe; SI = stop individual; ST = stop trial

*Grades defined by NCI CTCAEv4.0
*This chart covers many common adverse effects, but will not be comprehensive. This chart is intended to provide guidelines for handling adverse events but ultimate judgment will be the responsibility of the investigator. In addition, as other situations are encountered and managed by the study team, this decision guideline chart will be updated and expanded so that it remains current and fully inclusive.