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Product Name:	Amytal® Sodium (amobarbital)
Title of Study:	A Single Dose Level Study to Assess the Safety and Tolerability, of Intra-Articular Injection of Amobarbital in a Formulation Containing Cross-linked Hyaluronate in Patients with Intra-articular Distal Tibial Fractures
Development Phase	Phase I/IIa
Study Centers:	University of Iowa (UI) and Indiana University (IU)
Estimated number of subjects:	A total of 66 subjects will be enrolled in this study. Ten alternate subjects will be recruited to replace those that screen fail, drop out, or are removed from the study. Except in cases of pregnancy or breast-feeding, subjects will be recruited without regard to sex.
Study Period:	August 2021 until completion
Estimated date of first enrollment:	June 2022 will be the earliest day of enrollment, pending approval from UI and IU IRB and FDA IND approval.
Estimated date of last enrollment:	We estimate that all subjects will be enrolled by October 2022.
Route of Administration:	Intra-articular injection
Dosing Regimen:	1.3 mL containing 0.8 mg amobarbital (Amytal® Sodium) and 7.5 mg cross-linked hyaluronate (Gel-One®) given once or twice.
Sponsor:	J. Lawrence Marsh
IND Number:	140778

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1. INTRODUCTION

Background and Rationale for Conducting this Study

Intra-articular fractures (IAFs) can be devastating. Despite optimal current treatment, many of these injuries result in crippling arthritis that compromises quality of life and, in some instances, longevity. Once post-traumatic osteoarthritis (PTOA) begins, it is progressive and irreversible. PTOA from all causes affects over six million civilians at a cost of nearly 15 billion dollars annually to the United States (Brown *et al.*, 2006). It is largely a disease of young active people, who often fare poorly with reconstructive operations. In civilians, 76% of IAFs occur in patients less than 45 years old. Clearly, there is a medical need to find new IAF treatments (Buckwalter and Felson, 2015).

Among all joints, IAFs of the distal tibia (the ankle joint), have especially high rates of PTOA; a recent meta-analysis reported rates from 22% to 93% (Zhang *et al.*, 2017). The vast majority of ankle OA is post-traumatic, with injuries often leading to disabling PTOA within two to five years. The impairment associated with ankle PTOA is comparable to that caused by end-stage kidney disease or congestive heart failure (Saltzman *et al.*, 2006). For these reasons, patients with IAFs of the tibial pylon provide an ideal population for translation and optimization of new treatments to prevent PTOA.

Unlike many diseases in which the time of onset is unknown, PTOA caused by IAFs originates from a single catastrophic event. Our investigations have defined two primary mechanical IAF components that lead to PTOA: acute mechanical damage caused by IAFs and harmful persistent elevated contact stress caused by residual joint surface incongruity (Anderson *et al.*, 2011). Current treatment of severe IAFs involves surgical articular surface reduction, which aims to minimize elevated joint contact stresses arising from residual incongruity. The efficacy of this approach appears to have reached a limit in practice, as a substantial number (up to 50% or more) of patients with IAFs have poor results, and outcomes have not improved in the past 50 years (McKinley *et al.*, 2010). Even well-reduced joints result in PTOA after severe IAFs. This has led us to investigate how IAF causes PTOA and to seek biologic interventions to mitigate this process.

Our approach to mitigating PTOA after IAF focuses on inhibiting acute harmful cell responses to articular cartilage impact and to residual elevated mechanical stresses (Buckwalter *et al.*, 2013). Impacts and residual elevated contact stresses trigger aberrant chondrocyte mitochondrial activity that results in excess reactive oxygen species (ROS) production, chondrocyte death, persistent mitochondrial dysfunction in surviving cell populations, and ultimately PTOA (Martin *et al.*, 2017). Targeting the mitochondrial electron transport chain member complex I, which is responsible for the initial one-electron reductions of oxygen producing these damaging ROS, has provided profound benefits in multiple experiments including *in vitro* bovine, *in vivo* rabbit PTOA models, and recently in our minipig IAF model (Coleman *et al.*, 2018) demonstrate that acute administration of the complex I inhibitor amobarbital after IAF preserves articular cartilage structure and composition at 6 months after IAF, a time point when control animals have already developed PTOA lesions (Figure 1A). We quantified these changes using semi-automated Mankin scoring algorithms applied over the length of the articular surface of the talus (Figure 1B). In addition, amobarbital treated animals have decreased synovial thickness (Figure 1C) and decreased pain (Figure 1D) at this time point. Lastly, we have determined ideal dosing of amobarbital for inhibition of electron transport activity (Figure 1E) and confirmed that the compound at the dose proposed has no

toxicity to human cartilage (fractured radial head retrievals) taken 1 week after fracture (Figure 1F).

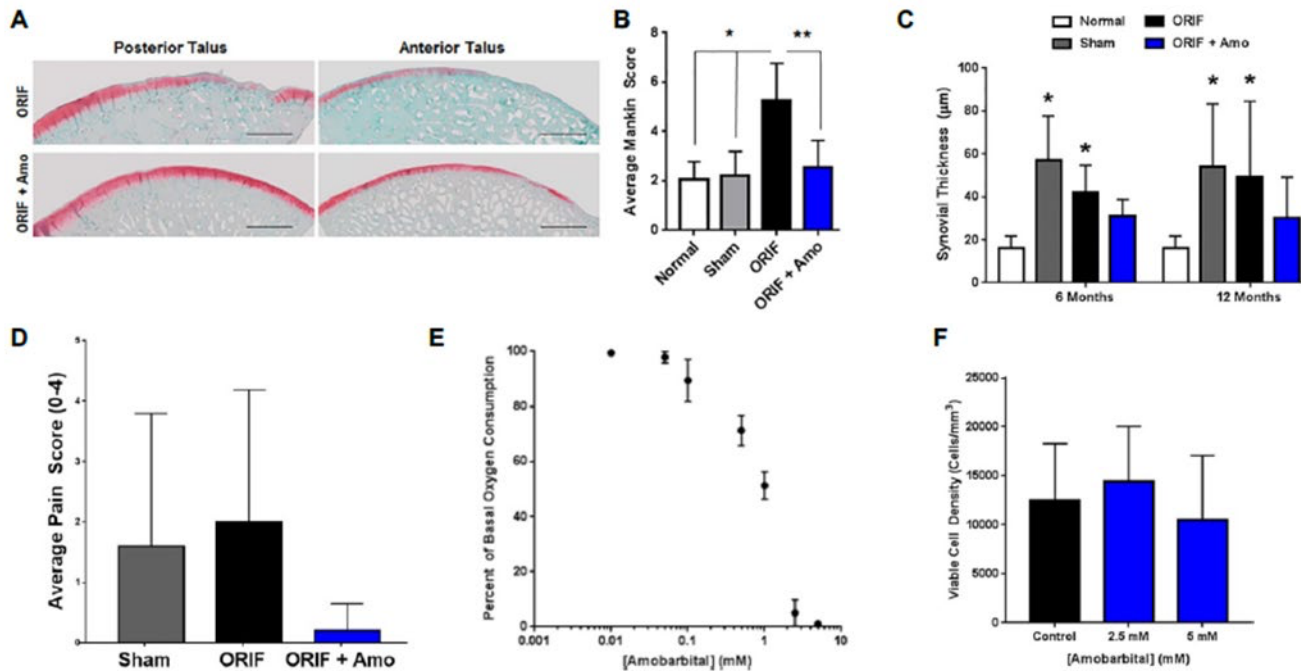


Figure 1. (A-D) Data support the benefits of amobarbital treatment following IAF in the minipig. (E) Ideal amobarbital dosing and (F) lack of toxicity were established in human radial head retrievals. [ORIF=open reduction and internal fixation, Amo=amobarbital.]

We have identified a series of events that describes how injury to cartilage from IAFs results in high levels of oxidant production. Further, we have found that blocking production of oxidants with amobarbital prevents chondrocyte death, and when fractured minipig hock joints are treated soon after injury, PTOA is significantly decreased. The doses of amobarbital showed no toxicity to local tissue in human cartilage retrievals or clinical manifestations in minipigs. This drug has a lengthy track record of use in humans. This combined with the evidence we have presented supports a small-scale Phase I and Phase IIa randomized controlled clinical trial that will assess safety and provide initial evidence for efficacy.

2. HYPOTHESES

This is a safety and tolerability, and proof-of-concept clinical trial of amobarbital as a treatment to prevent PTOA in fractured ankle joints. The study is a double blind, prospective, randomized, placebo-controlled, stepwise trial. Amobarbital will be delivered to ankle joints in solution with cross-linked hyaluronate (HA) as a vehicle. Amobarbital/HA injections (active dose) will be compared to HA alone (placebo dose). Our primary goal is to confirm safety and tolerability, but we will also preliminarily assess efficacy. In the proposed research, we will test the following hypotheses:

Hypothesis 1: Intra-articular injections of amobarbital will not lead to local or systemic side effects.

Hypothesis 2: The cellular effects of amobarbital injections will reduce the incidence and severity of PTOA.

Hypothesis 3: Variations in injury severity and joint incongruity will affect the response of cartilage to amobarbital.

3. SPECIFIC AIMS

This Phase I /IIa clinical trial will be conducted at the University of Iowa and at Indiana University. High energy ankle fractures involving the articular surface of the weight-bearing surface of the distal tibia are the ideal clinical model in that ankle PTOA develops swiftly (≤ 2 years) and with high incidence. After completing initial Phase I investigations, we will continue the study in a Phase IIa randomized design, in which we will compare the outcomes for amobarbital-treated patients with patients receiving placebo injections. We will pursue three Specific Aims to: 1) confirm the safety of intra-articular injections of amobarbital; 2) determine if amobarbital injections decrease the incidence and severity of PTOA; and 3) explore how variations in acute fracture severity and chronic elevated contact stress affect the response of cartilage to amobarbital.

Specific Aim 1: (a) Quantify changes in liver, kidney, and hematologic function (b) Determine if there are clinical signs of adverse reactions to joint injection (injection site erythema, joint pain, swelling).

Specific Aim 2: (a) Quantify changes in weight bearing plain radiographs of ankles. (b) Quantify preservation of joint space by measuring its volume on sequential weight bearing computed tomography (CT) scans. (c) Assess clinical outcomes using validated patient-reported outcomes measures.

Specific Aim 3: (a) Analyze computed tomography images to quantify injury severity by measuring inter-fragmentary surface area (comminution). (b) Analyze computed tomography images to quantify post-reduction contact stress (residual incongruity) using computational stress analysis. (c) assess how the cartilage response to amobarbital varies across the range of injury severity and post-reduction contact stress exposure.

4. STUDY OBJECTIVES

Primary Objective: Confirm systemic and local safety and tolerability of intra-articular injection of amobarbital (Aim 1).

Secondary Objectives:

- Determine clinical efficacy extending to 24 months after treatment (Aim 2).
- Assess the effects of age, injury severity, and chronic elevated contact stress on the efficacy of amobarbital treatment (Aim 3).

5. STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the protocol, Good Clinical Practice, and the applicable Food and Drug Administration and other Department of Health and Human Services regulatory requirements. All key personnel (all individuals responsible for the design and

conduct of this study) have completed Human Subjects Protection and Good Clinical Practice training.

IRB Oversight:

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6. LOCATION OF STUDY PROCEDURES

University of Iowa Hospitals and Clinics
200 Hawkins Drive
Iowa City, Iowa 52242

Indiana University Health Methodist Hospital
1701 N Senate Ave
Indianapolis, IN 46202

7. TRIAL DESIGN

We propose a prospective, randomized, placebo-controlled, stepwise clinical trial of amobarbital injections compared to placebo injections into the ankle joints of patients with IAFs of the distal tibia (pilon, posterior malleolar, or supination/adduction injuries). We estimate that 48 patients will undergo single-stage open reduction and internal fixation (ORIF) surgery and that 12 additional patients will be treated under a 2-stage protocol consisting of an initial external fixation surgery, followed by ORIF.

- **Phase I: Open label.** Three patients will be treated with single dose of 0.8 mg amobarbital at the time of the initial surgery, and safety measures will be assessed. If the dose is well-tolerated, an additional 3 patients will be treated.
- **Phase IIa: Placebo controlled.** Once initial safety and tolerability is confirmed, we estimate that 48 single stage patients will be treated once with active or placebo doses (1:1 randomization) at the time ORIF (within 72 hours post injury). Twelve 2-stage patients will receive treatment at the time of external fixation (within 72 hours post injury) and a second treatment at the time of ORIF. Patients and attending surgeons will be blinded to the identity of the treatment.

8. MAIN SCREENING CRITERIA

We will recruit men and non-pregnant/non-lactating women of any ethnic background between the age ≥ 18 and ≤ 60 years that have sustained fractures of the distal tibia, often called pilon fractures. In addition, we will recruit patients with posterior malleolar, and supination/adduction injuries, which are vertical fractures that pass through the weight-bearing surface of the distal tibia.

9. SCREENING/RECRUITMENT PROCEDURES

Eligible study participants will be identified in the Iowa or Indiana emergency departments. The study team will be notified when a potential subject is seen in the emergency department. Study team will review potential subject's chart for possible inclusion/exclusion criteria. This pre-screening is done so that potential subjects and their families are not approached until the study team reviews and verifies that it would appear that the subject qualifies.

The study team will approach the potential subject and/or their Legally Authorized Representative (LAR) and tell them that they may qualify for a study and ask if they are interested in hearing about the study. If the potential subject and/or their LAR are interested, the study will be explained to them in detail. They will be encouraged to ask questions and talk it over with the treating physician and family members.

If the potential subject and/or their LAR would like the potential subject to participate, one of the members of the study team will go over the consent form with them, answering any questions they may have as the study team goes through the consent form. Once they have reviewed the entire consent form, the study team will ask them once again if they have any questions. Once any questions are answered, they can choose to sign the consent form if they choose to participate or the LAR chooses to have the potential subject participate. Consent will be obtained prior to induction of anesthesia.

To avoid any coercion, all subjects are offered the opportunity to talk with the treating physician or family prior to signing a consent form. Study participation has no influence on the standard of care they would otherwise receive for their disease process.

Inclusion Criteria:

- Age ≥ 18 and ≤ 60 years
- Acute closed or type 1 open ankle fractures (classified as OTA/AO 43 B 1-3 and 43 C 1-3 or classified as 42 B and C fractures with 25% talar displacement and one of the following; syndesmosis injury or medial malleolar fracture at or above the shoulder) (Marsh et al., 2007) without operative ipsilateral extremity trauma
- Posterior malleolar and supination adduction rotational fractures that have an articular fracture line across the articular surface of the distal tibia. Posterior malleolar fractures should affect 25% of the articular surface or greater.
- Fractures must have initial treatment within 72 hours of injury including initial injection of amobarbital or placebo.

Exclusion Criteria:

- Diabetes

- Pregnant or nursing mothers and individuals with child-bearing potential that are not using birth control methods with >99% efficacy.
- Allergy to poultry products or cinnamon
- Previous injuries to the ankle
- Pre-existing immunologic or hematologic diseases
- High grade open wounds
- Pre-existing immunologic or hematologic diseases
- Pre-existing ankle arthritis
- Ipsilateral fractures
- Associated injuries that preclude standard rehabilitation
- Pre-existing dysfunction of the kidneys, liver, blood, immune system, endocrine system (excluding diabetes)
- Serum creatinine ≥ 1.4 mg/dl; BUN > 30 mg/dl; ALT ≥ 60 IU/L in males and ≥ 50 IU/L in females; AST ≥ 45 IU/L in males and > 40 IU/L in females; bilirubin > 1.3 mg/dL; platelets $\leq 50,000/\mu\text{l}$; glucose > 200 mg/dL

10. STUDY INTERVENTIONS

- 10.1 Surgical treatment protocol:** Patients will be treated with standard of care (SOC) techniques chosen by their surgeon. At an initial operation, either definitive open reduction with internal fixation (ORIF), or a joint-spanning external fixator will be placed on patients within 72 hours of injury.

In patients treated with initial spanning external fixation, a second operation performed 3 to 21 days after the initial operation will replace the initial external fixator with internal fixation.

- 10.2 Study intervention with amobarbital:** Amobarbital for injection (AMYTAL Sodium ®CII) is a dry powder formulation in 0.5 g containers. It is manufactured by Alcam Corp. and marketed by Bausch Health as somnolent/hypnotic. Gel-One® is a 1% (10 mg/mL) solution of cross-linked HA. It is manufactured by Seikagaku Inc. and marketed by Zimmer Biomet for intra-articular injection to treat osteoarthritis pain.

- 10.3 Compounding and Administration:** Individual active and placebo doses will be compounded aseptically in hospital pharmacies using as-marketed amobarbital and HA according the [Pharmacy Manual](#). Doses will be delivered to ORs for administration within 90 minutes of compounding. The sterile serum empty vials to be used for compounding are purchased from Stallergenes Greer and will be furnished to both study sites.

Post-administration, all doses will be tested for sterility and the presence of endotoxin. Amobarbital content will be measured in the 6 doses prepared for patients in phase 1. For phase 2, all active doses given in first 3 months of recruitment will be analyzed (estimated 18). In addition, the first 10 placebo doses given during this period will be analyzed to confirm the absence of amobarbital.

The compounding procedure is as follows (all syringes are sterile and latex-free)

- Amobarbital reconstitution: Form a 100 mg/mL solution by adding 5 mL sterile water (drawn from a pre-filled 50 mL vial) to as-marketed vials containing 0.5 g of lyophilized amobarbital sodium powder.
- Check for clarity: The solution must be clear to proceed and will be discarded if particulates are visible at 1 minute after reconstitution (as directed in the package insert).
- Dilution 1: Reconstituted amobarbital is diluted to 10 mg/mL by transferring the contents of the amobarbital vial (5 mL) back to the 50 mL sterile water vial.
- Dilution 2: 0.3 mL of the 10 mg/mL solution is diluted with 1.7 mL normal saline (drawn from a 5 mL plastic vial with a 3 mL syringe) to form a 1.5 mg/mL solution.
- For placebo doses, 0.3 mL sterile water is substituted for the 10 mg/mL amobarbital solution.
- Final mixture: Mix 2.0 mL of the 1.5 mg/mL solution with 3.0 mL HA to form a mixture containing 0.6 mg/mL amobarbital, and 6 mg/mL HA. Inject the mixture into a 30 mL sterile glass vial for transport to the OR.

- The vial containing the dose will be labeled with a bar code linked to a study-specific Investigational Agent Accountability Record that documents the nature of the dose (active or placebo), the prescriber and dispenser, lot number and expiration date of the drug, dispensing date, patient identifiers, and the quantities of drug dispensed and returned to the pharmacy.
- Attending surgeon will inject 1.3 mL of an active dose containing 0.8 mg amobarbital (0.6 mg/mL) and 7.5 mg HA (6.0 mg/mL), or 1.3 mL of a placebo dose without amobarbital (7.5 mg HA only).

10.4 Post-administration Testing

After administration, doses will be returned to pharmacies for distribution to a CRO laboratory for sterility and endotoxin testing, and to the UI College of Pharmacy for amobarbital analysis. All 6 doses prepared for patients in phase 1 will be analyzed. For phase 2, the first 18 active doses and first 10 placebo doses will be submitted for analysis.

10.5 Second Amobarbital Dose:

If the safety of a single dose is confirmed in Phase I and initial Phase IIa studies, additional Phase IIa patients will receive a second amobarbital dose or placebo injection at the second operation.

11. CONCOMITANT INTERVENTIONS

N/A

12. OUTCOMES

Safety (Table 1): Investigators will conduct clinical exams and Patient Reported Outcome Measurement Information Systems (PROMIS) scoring to monitor the health and function of ankle joints. Serum and urine will be collected to evaluate major organ function *via* Complete Metabolic Panel, CBC, and standard urinalysis. In addition, amobarbital levels in blood and urine will be quantified by UV-HPLC. Considering the short plasma half-life of amobarbital observed in non-clinical studies (3-4 hours), samples will be collected prior to treatment, and at 30 minutes, 1 hour, 2 hours, 4 hours, and 8 hours post-injection.

Efficacy (Table 2): Radiographs will be both for standard of care (SOC) and some budgeted for the study. SOC radiographs will be 3 views of the ankle and a CT scan at the time of injury to assess fracture severity. Additional 3 view radiographs will be obtained at all follow up visits and once the fracture is healed these will be weight bearing. 24-month radiographs will be budgeted to the study and will be used to assess PTOA on the Kellgren-Lawrence scale.¹⁶ Weight bearing CTs will be used for contact stress analysis and for sequential volumetric assessment of cartilage, an important dependent variable. In addition, PROMIS scores for pain interference, physical function, and global health, the American Orthopedic Foot and Ankle Society Score (AOFAS), and the Foot and Ankle Disability Index (FADI) will be recorded.

Table 1. Safety Outcomes

Single Dose n = 6 (Phase I)	Baseline < 48 hr	Internal Fixation^a	30 min, 1, 2, 4, and 8 hr P-O	Day 1, 2, and 7 P-O	All P-O Clinical Follow-ups	Additional Limb Check^b
CMP ^c	X	X		X		X
CBC	X	X		X		X
Urinalysis		X		X		
Amobarbital (blood and urine)			X			
Joint Monitoring					X	

Single Dose n=48 (Phase II)	Baseline < 48 hr	Internal Fixation^a	30 min, 1, 2, 4, and 8 hr P-O	Day 1, 2, and 7 P-O	All P-O Clinical Follow-ups
CMP ^c	X	X		X	
CBC	X	X		X	
Urinalysis		X		X	
Amobarbital (blood and urine)			X		
Joint Monitoring					X

Double Dose n = 12 (Phase II)	Baseline < 48 hr	External Fixation^a	30 min, 1, 2, 4, and 8 hr P-O	Day 1, 2, and 7 P-O	All P-O Clinical Follow- ups	Pre-op^d	Internal Fixation^a	30 min, 1, 2, 4, and 8 hr P-O	Day 1, 2, and 7 P-O	All P-O Clinical Follow- ups
CMP ^c	X	X		X		X	X		X	
CBC	X	X		X		X	X		X	
Urinalysis		X		X			X		X	
Amobarbital (blood and urine)			X					X		
Joint Monitoring					X		X			X

^a To be done immediately following surgery.

^b Additional Limb Check as needed per SOC.

^c The Comprehensive Metabolic Panel (CMP) includes the following tests:

- | | |
|---|---|
| 1. Glucose | 8. Blood urea nitrogen (BUN) |
| 2. Calcium | 9. Creatinine |
| 3. Albumin | 10. Alkaline phosphatase (ALP) |
| 4. Total Protein | 11. Alanine amino transferase (ALT, SGPT) |
| 5. Electrolytes (sodium, potassium) | 12. Aspartate amino transferase (AST, SGOT) |
| 6. Bicarbonate (Total CO ₂) | 13. Bilirubin |
| 7. Chloride | |

^d Observe joints for local adverse reactions

Table 2: Efficacy Outcomes

Phase I/IIa	Day of injury	3 months	6 months	12 months	24 months
Injury CT	X				
Radiographs	X	X	X	X	X
Physical Exam		X	X	X	X
PROMIS Pain		X	X	X	X
PROMIS Function		X	X	X	X
PROMIS Global Outcomes			X	X	X
AOFAS Outcome Score			X	X	X
FADI Outcome Score			X	X	X
Standing CT			X	X	X

13. ADHERENCE ASSESSMENT

Eligible patients will be logged. Compliance in completion of surgical procedure, infusion of amobarbital or placebo, and completion of exams and visits will be monitored.

14. ADVERSE EVENT REPORTING:

Based on GLP toxicology findings reviewed in the [Investigator Brochure](#), Adverse Events related to intra-articular injection (joint pain, swelling, erythema) are expected in the proposed clinical investigation.

14.1 Routine Adverse Event Reporting

For non-serious Adverse Events, documentation must begin from the time the subject signs the informed consent document and continue through the 3 month follow-up period after the last dose of study drug.

Collected information will be recorded. A description of the event, its severity or toxicity grade according to [Common Toxicity Criteria for Adverse Events \(CTCAE\)](#), onset and resolved dates (if applicable), and the relationship to the study drug will be included. The principal investigators have the responsibility of determining the attribution of the event as it is related to the study drug.

14.2 Serious Adverse Event Reporting

For any experience or condition that meets the definition of a serious adverse event (SAE), recording of the event must begin after signing of the informed consent and continue through the 3-month follow-up period after treatment is discontinued.

Investigators must report to the DSMB any SAE, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). SAEs must be reported to the IND Sponsor within 24 hours of learning of the event. An adverse event is considered **serious** if it results in ANY of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalization for ≥ 24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, [21 CFR 312.32](#); [ICH E2A](#) and [ICH E6](#)).

14.3 FDA Reporting Requirements (for Sponsor-Investigators)

It is the responsibility of the IND sponsor-investigator to comply with IND safety reporting as set forth in the Code of Federal Regulations, [Section 312.32](#). This responsibility includes providing an annual IND report to the FDA and completing IND safety reports (if necessary). [Table 3](#) provides the reporting criteria.

Table 3. AE Reporting Criteria

Entity	SAE that is NOT Fatal/Life-Threatening			SAE that is Fatal/Life-Threatening				Follow-up Info
	NOT Related	Related		NOT Related		Related		
	Expected or Unexpected	Expected	Unexpected	Expected	Unexpected	Expected	Unexpected	
Sponsor	Per protocol	Per protocol	Per protocol	Per protocol	Per protocol	Per protocol	Per protocol	Per protocol
IRB	Per IRB	Per IRB	Per IRB	Per IRB	Per IRB	Per IRB	Per IRB	Per IRB
FDA*	Annual Report (unless otherwise instructed)	Annual Report (unless otherwise instructed)	15 calendar days (written report)	Annual Report (unless otherwise instructed)	Annual Report (unless otherwise instructed)	Annual Report (unless otherwise instructed)	7 calendar days (fax or phone)	ASAP / 15 calendar days

14.4 Receipt of new information

During the course of a study, researchers may become aware of new information that would impact a subject's decision to participate, or continue participating in the research study. For example, interim analyses of data may identify a trend that impacts the safety of subjects, or may identify early efficacy (benefit) of one of the interventions under study. In addition, results from other research studies or changes in standards of practice or care may affect the conduct of a study and would need to be communicated to research subjects.

14.5 Noncompliance

Noncompliance is a failure to follow the federal regulations with respect to protection of human subjects in research or failure to follow the determinations of the IRB with respect to conduct of the research as approved by the IRB. Once per year, the IRB is required to review and approve all non-exempt research projects at intervals appropriate to the degree of risk, but not less than once a year. This is called "continuing review" Continuing review for non-exempt research is required to occur as long as the research remains active for long-term follow-up of the research subject, even when the research is permanently closed to the enrollment of new subjects and all subjects have completed all research-related interventions and to occur when the remaining research activities are limited to collection of private identifiable information.

15. ADVERSE EVENT MANAGEMENT

Adverse events will be managed by standard-of-care medical or surgical interventions.

16. ADVERSE EVENT MONITORING AND STUDY TERMINATION**Adverse Event Monitoring**

- The frequency of adverse experiences and the time to event will be compared using Z-tests with the Poisson distribution if rare but if the frequency is greater than 5% a Binomial distribution will be used. Time to event will be done with Kaplan-Meier estimation and logrank comparisons.
- The frequency and severity of adverse events will be compared overall and at the study time intervals using similar Poisson and Binomial methods. Severity comparison will be done using Chi-Square contingency analysis using the categorization of a Serious Adverse Event including death or hospitalization.
- The Data and Safety Monitoring Board (DSMB) will be immediately notified. After thorough evaluation, the DSMB will make decisions on the magnitude of the clinical symptoms and on proceeding with the current dose, changing doses, or terminating the study.

Study Termination

The study will be automatically paused for DSMB review of adverse events (AEs) after the first 6 patients are treated, and again after treatment of 16, 32, and 42 patients. All AEs will be graded

on the 1-5 CTCAE scale ([Table 4](#)). Grade 3-5 AEs reported to DSMB at any time will be cause for immediately stopping the study.

Table 4. CTCAE Grading of Adverse Events

Adverse Event	Grade				
	1	2	3	4	5
Joint-effusion	Asymptomatic, clinical or radiographic findings only	Symptomatic; interfering with function but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	Death
Joint-function	Stiffness interfering with athletic activity; 25% loss of range of motion (ROM)	Stiffness interfering with function but not interfering with ADL; >25 – 50% decrease in ROM	Stiffness interfering with ADL; >50 – 75% decrease in ROM	Fixed or non-functional joint (arthrodesis); >75% decrease in ROM	–
Gait/walking	Limp evident only to trained observer and able to walk ³ 1 kilometer; cane indicated for walking	Noticeable limp, or limitation of limb function, but able to walk ³ 0.1 kilometer (1 city block); quad cane indicated for walking	Severe limp with stride ambulation limited to walker; crutches indicated	Unable to walk	–
Pain – Joint	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	–
Allergic/hypersensitivity reaction	Transient flushing or rash; drug fever <38°C	Rash; flushing; urticaria; dyspnea; drug fever; >38°C	Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension	Anaphylaxis	Death

17. ENDPOINTS

Primary Endpoints

- The local tolerability of amobarbital will be assessed by clinical observation
- Hematologic and urine assessment of systemic tolerability according to the schedule given in [Table 1](#).

Secondary Endpoints

- The radiographic progression of PTOA will be assessed at 3, 6, 12, and 24 months post-fracture. Standing (weight-bearing) CTs will be performed at 6, 12, and 24 months.
- PROMIS, AOFAS, and FADI assessments will be performed according to the schedule shown in [Table 2](#).

18. DATA MANAGEMENT

The following people/agencies may have access to subject data/records:

- Study team
- Federal government regulatory agencies
- Auditing departments of the University of Iowa
- University of Iowa IRB
- Indiana University IRB

To protect confidentiality, we will assign each subject a study ID. All records will be in a locked cabinet in a locked office or password protected computer system. Data and records will be managed as follows:

- Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.). Whenever possible, subject identifying information will be blacked out on all paper or hard copy records and replaced with the subject's unique study identifier. Paper records will be stored in a locked file cabinet in the study team's locked office.
- Electronic data related to the patient's clinical care is stored in the patient's electronic medical record (Epic). This data will be accessible only to individuals who are members of both the clinical and research team. Access to the electronic medical record is password protected, and subject to audit. Additional data files associated with the study will be entered into the PI research server that is provided by UIHC Health Care Information Systems (HCIS), which provides and supports information and communication systems and technology that facilitate quality patient care, progressive medical education, and innovative research. All electronic files are stored on password protected computers that are connected to a secured shared drive and maintained by UIHC HCIS. All members of the team have an individual log-on and secure password. Databases will be password encrypted and housed behind the UIHC firewall and server security will be maintained by HCIS at UIHC. Electronic data will only be transported or transferred to other password protected computers within the UIHC network. Any information transmitted to the DSMB will be coded with subject IDs (if necessary to transmit specific participant data).
- After the completion of the study, all identifiable information will be destroyed as soon as possible according to the University of Iowa's and Indiana University's IRB standards.
- The study will use REDCap, a metadata driven application for data management in clinical studies. The functionality allows data entry system using most web browsers to access an internet-connected database server. This will allow for convenient data entry for both clinical sites. Data will initially be collected on Case Report Forms (CRFs) designed specifically for the study.

19. SAMPLE SIZE AND ACCRUAL

Phase I: An open label study of 3 patients will be done. If no dose limiting toxic (DLT) side effects occur, then an additional 3 patients will be done. If no DLT events occur, the study will proceed to Phase II. However, if side effects occur, then 3 additional patients will be studied at a reduced dose, and if safe, followed by an additional 3 patients. There will be 90% probability of detecting an adverse event that occurs in 2 patients.

Phase IIa: The study will randomize eligible patients in a 1:1 ratio of amobarbital to placebo. In order to conduct a safety assessment where an adverse event occurs at 5% or greater proportion with a 64% probability of observing an event, 20 treated patients will be needed. With 30 patients, the detection of an event rate of 5% will have a probability of 79%. If the true relative risk of adverse events for experimental subjects relative to controls is 3, with a study 30 experimental subjects and 30 control subjects we will be able to reject the null hypothesis if the baseline rate is 20% with probability (power) 0.9. The Type I error probability associated with this test of this null hypothesis is 0.05.

20. DATA ANALYSES

We will use the Intent-to-Treat population for all comparisons. However, for safety we will use the Per Protocol population (patients with at least 1 dose of amobarbital or placebo). Comparison of demographic, prognostic and safety variables at baseline will use T-Tests and Fisher's Exact Tests between treatments and control groups.

For Specific Aims 1a and 1b, comparison of Phase IIa will utilize repeated measures GLM ANOVA between single dose amobarbital and placebo for continuous distributions, e.g. renal and liver tests. A planned comparison will be the time post-infusion. Proportions of clinical adverse events and the proportion of abnormal lab tests with 95% confidence intervals and will be compared by a Fisher's Exact Test. To proceed to the two-dose application of amobarbital significance will assessed with a one-sided test ($\alpha=0.05$) where amobarbital can be no worse than placebo. If safe, then the two dose groups (single and two) will be compared to control by GLM ANOVA or Chi-Square contingency table analysis overall and with the planned comparison of the amobarbital groups combined. Adequate power exists to detect adverse events at the 5% incidence and to determine if it exceeds 3-fold increase if the baseline rate is 20%.

For Specific Aims 1a-1d and Aim 2, the two dose groups (single and two) will be compared to control by GLM ANOVA or Chi-Square contingency table analysis overall and with a planned comparison of the amobarbital groups combined. Analysis will be done across time using mixed model analysis for continuous variables and log-linear models for the categorical variables (KL grade). A two-sided test will be used with $\alpha=0.025$. We will also examine the trajectory of clinical outcomes over time and compare curves between the treatment groups using regression models (linear or ordinal GEE logistic models). If the detectable difference is 2-3 fold then the power for detection is greater than 90%.

For the subgroup analysis of CT assessment of injury severity and contact stress for Specific Aim 3, the two dose groups (single and two) will be compared to control by GLM ANOVA or Mantel-Haenszel Chi-Square contingency table analysis overall and with a comparison of the amobarbital groups combined. If the data are not normally distributed, a log transformation will be used and if that is not normal, the Kruskal-Wallis test will be used. We will also conduct a

stratified analysis using injury severity categories and quartiles of contact stress. A two-sided test will have expected low power so this will be exploratory and to examine levels of response.

21. PAYMENT FOR PARTICIPATION:

All study-related medication and study procedures will be paid for by the study. Subjects will be paid \$75.00 after the completion of the month 24 visit.

22. SUBJECT SAFETY:

- To minimize risks all subjects are carefully pre-screened to identify any factors that could contribute to increased risk.
- All testing is completed at University of Iowa Hospitals and Clinics and Indiana University by a very experienced and well-trained staff and monitored by the Principal Investigator.
- All confidential information is kept in locked offices and password protected computers available only to study team members.

23. DATA SAFETY MONITORING BOARD (DSMB): The DSMB Charter is included for reference ([DSMB final.doc](#))

24. REFERENCES

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