A Prospective, Multi-Center, Randomized, Triple-Blinded, Placebo-Controlled study of IL-1RA Treatment in Patients with Acute ACL Tear and Painful Effusions

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Short title: EASI ACL

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LIST OF ABBREVIATIONS

ACL	Anterior cruciate ligament (ACL)
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CIOMS	Council for International Organizations of Medical Science
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CMS	Center for Medicare and Medicaid Services
COMP	Cartilage oligomeric protein
CSQ	Coping Strategies Questionnaire
CTXII	Collagen Type II
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Finance Report
GAG	glycomsaminoglycan
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
ICMJE	International Committee of Medical Journal Editors
IKDC	International Knee Documentation Committee
IL-1ra	Interleukin 1 receptor antagonist
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISO	International Organization for Standardization
KOOS	Knee Injury and Osteoarthritis Outcome Score
LSMEANS	Least-squares means
MedDRA	Medical Dictionary for Regulatory Activities
MOON	Multicenter, Orthopaedic Outcome Network
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
MSDS	Material Safety Data Sheet
NIH	National Institutes of Health
NIH IC	NIH Institute and Center
NtxI	Collagen Type I

OA	Osteoarthritis
OHRP	Office for Human Research Protections
PI	Principal Investigator
PTOA	Post-traumatic osteoarthritis
QA	Quality Assurance
QC	Quality Control
RA	Rheumatoid Arthritis
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
XO	Xanthine Oxidase

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following (use applicable regulations depending on study location and sponsor requirements; examples follow): • United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812) • ICH E6 All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

Sponsor: _____Austin Stone, MD,PhD_____
Print/Type Name

Signed: _____ Date: _____
Signature

INVESTIGATOR SIGNATURE

I, the undersigned Investigator, have read the following protocol and agree to conduct the study in compliance with the protocol, International Conference on Harmonization (ICH) Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and all applicable regulatory requirements.

I, the undersigned Investigator, agree:

- To assume responsibility for the proper conduct of the study and not to deviate from the procedures described in the protocol.
- I am thoroughly familiar with the investigational product(s) as described in the protocol, Investigator's Brochure and any other relevant information sources provided to me.
- I am aware of and will conduct the study in compliance with the protocol, ICH GCP guidelines and all applicable regulatory requirements.
- I agree to permit monitoring and auditing of the study by the Sponsor and its designates, and inspection by appropriate regulatory authority (ies).
- I agree to ensure that all persons assisting with the conduct of the study are properly qualified for their function.

Investigator Name & Signature:

Principal Investigator: Cale Jacobs, PhD
Print/Type Name

Signed: _____ Date: _____
Signature

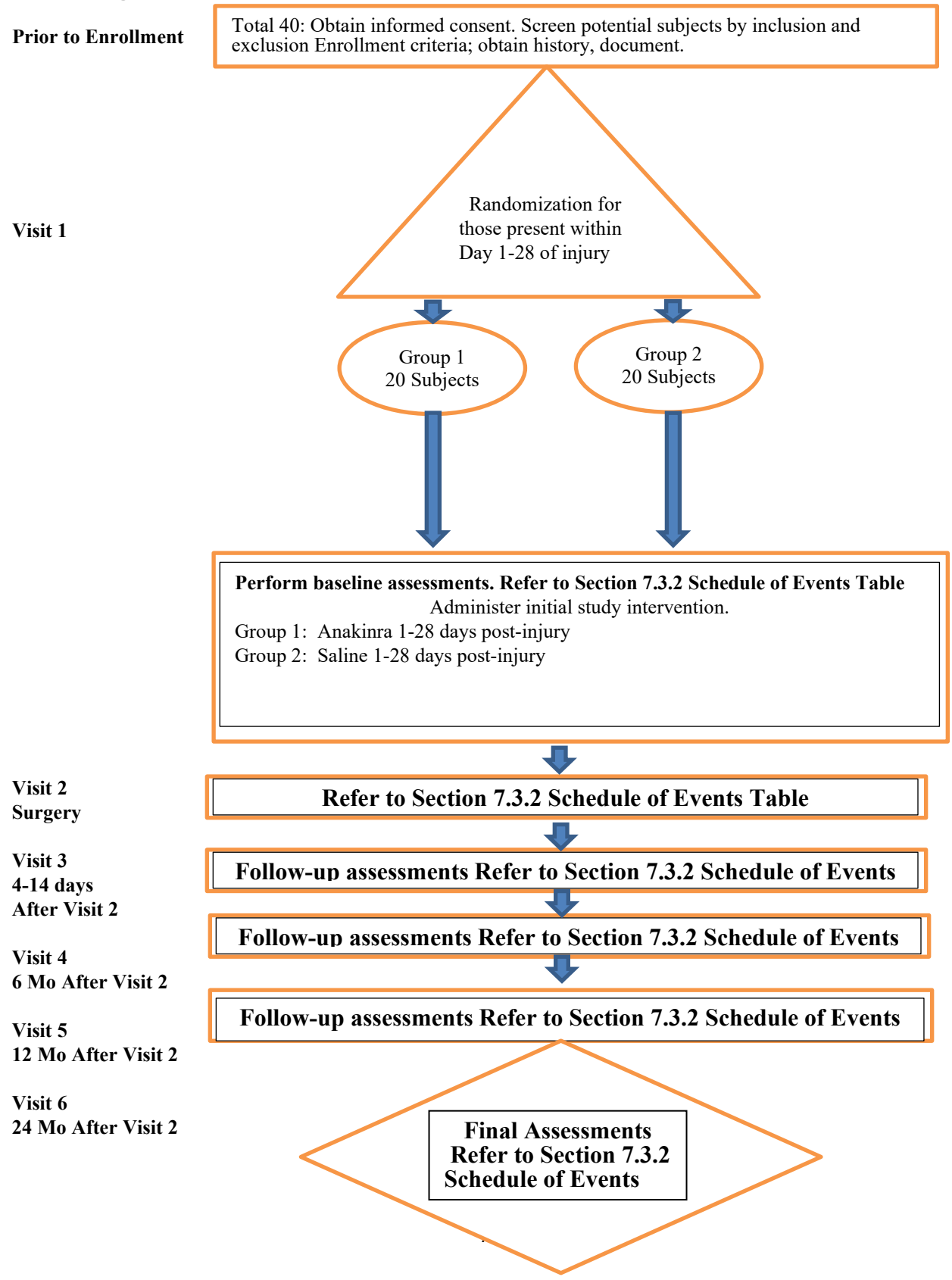
PROTOCOL SUMMARY

Title:	A Prospective, Multicenter, Randomized, Triple-Blinded, Placebo-Controlled study of IL-1RA Treatment in Patients with Acute ACL Tear and Painful Effusions
Précis:	This study aims to establish efficacy and timing of administration, to reduce post-injury pain, decrease inflammatory biomarkers in ACL injured patients undergoing ACL reconstruction and correlate function, patient reported outcomes and MRI findings at 1 years after ACL injury. The study population will include 40 subjects, ages 14-40 who will be randomized into 2 groups (20 subjects per group).
Objectives:	1. Test the efficacy of a treatment protocol using IL-1ra and knee arthrocentesis to alleviate inflammation related knee pain and improve patient-reported outcomes (PRO) following ACL rupture. 2. Determine if intra-articular IL-1ra therapy improves levels of a panel of inflammatory, meniscus and cartilage metabolism, and oxidative stress biomarkers as measured in synovial fluid, serum and urine from patients immediately after ACL injury (baseline, 2 weeks after injury and at 1 and 2 years after surgery). 3. Answer the question if MRI T1rho changes correlate with synovial biomarker levels and show predictive value for the overall subject patient outcome at 2 years.
Endpoint	**Primary endpoint :** Change in Patient reported outcomes as measured by KOOS-Symptoms **Secondary endpoints**: KOOS QOL, KOOS-pain, KOOS-Sports/Rec, Biomarker levels in synovial fluid, serum and urine at time of surgery MRI T1rho changes at 2 years **<u>Study Stopping Criteria</u>** • Local intolerance of the administered Anakinra (Kineret®) or any sign of allergic response. • Development of signs and symptoms before the second time point preventing the second administration of the study drug. • Any patient reported and drug related SAE after the first or second administration of the study drug. • Diagnosis with any condition as outlined in the exclusion criteria during the course of the initial 2 weeks of study enrollment.
Population:	The study population will include up to 40 subjects within 28 days following of injury. The study is open to male and female participants who are between the ages of 14 and 40, who have ACL tears. Participants will be enrolled at the University of Kentucky.

Phase:	2
Number of Sites enrolling participants:	1 (University of Kentucky)
Description of Study Agent :	IL-1ra (Kineret®, anakinra), supplied by Sobi Pharmaceuticals, is a recombinant, nonglycosylated form of human interleukin-1 receptor antagonist (IL-1Ra). Kineret® consists of 153 amino acids and has a molecular weight of 17.3 kilodaltons. It is produced by recombinant DNA technology using an *E coli* bacterial expression system. It is available as pre-filled syringes (100 mg in 0.67 mls) and is FDA approved for daily subcutaneous injection (100 mg/day) for treatment of rheumatoid arthritis (age > 10). A total of 1.5 ml (150 mg) will be injected intra-articularly after synovial fluid aspiration 'to dryness' once between 1 and 28 days after ACL injury.
Study Duration:	24-36 months
Participant Duration:	2 years

SCHEMATIC OF STUDY DESIGN

Flow diagram



1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

A majority of individuals with partial or complete rupture of the anterior cruciate ligament (ACL) develop post-traumatic osteoarthritis (PTOA) 5-15 years after the initial injury. The long-term consequences of PTOA include arthrofibrosis, pain, limited motion, and recurrent instability. Because ACL injuries occur most often in younger individuals (average age 14-29 years), pain and other debilitating symptoms occur most often during patients' most productive years costing society upwards of 3.06 billion dollars annually [1]. Current surgical and non-surgical treatment options for ACL injury, while relatively successful in restoring function and stability in the short term, do little or nothing to reduce or prevent the development of PTOA later in life.

ACL rupture, with or without accompanying damage to nearby cartilage and bone, initiates a persistent cascade of inflammation and catabolic enzyme activity leading to OA of the knee joint. We propose to disrupt the inflammation-driven cascade with recombinant Interleukin 1 receptor antagonist (IL-1ra).

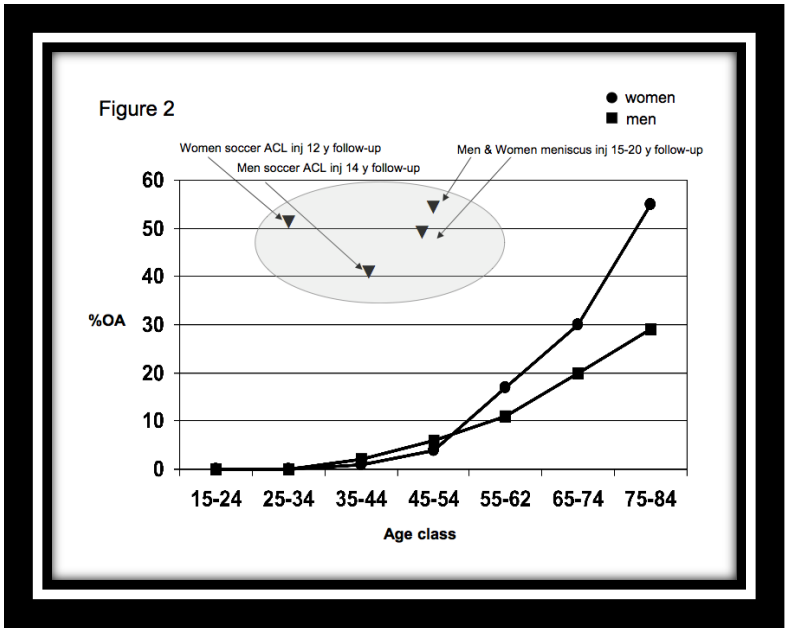
IL-1ra was chosen because it is safe, well tolerated, and has been used to treat RA, juvenile inflammatory arthritis, and post-surgical persistent knee effusions as well as knee stiffness.[2-4.] Our own preliminary data suggests that IL-1ra reduces pain and improves function in patients with acute knee injury and arthrofibrosis. IL-1ra binds to the Interleukin 1 receptor and nonproductively blocks the proinflammatory and cartilage catabolic activities of Interleukin 1 alpha and beta. IL-1ra levels in synovial fluid are initially higher following acute ACL injury but vanish rather quickly. Subsequently, IL-1β levels rise slower in joint fluid after ACL rupture and remain high for longer periods of time. IL-1ra has had mixed results in the treatment of established OA, possibly due to its short half-life in vivo. However, if given shortly after the initial inciting event potentially initiating the development of OA, the effectiveness may be significantly higher.

Unlike primary OA, which typically strikes older individuals and develops silently over the course of many years, post-traumatic OA is thought to begin at the moment of ACL injury in many individuals. Capitalizing on the expertise and experience of our collaborative team of investigators, we have chosen to seize this window of opportunity and provide the field with valuable new data regarding the importance of reduction of the initial inflammatory response to injury in acute ACL tears.

It is estimated 250,000 injuries to the anterior cruciate ligament (ACL) occur each year (1). These injuries predominantly occur at young age (14-18 years) The incidence of these injuries in Kentucky alone is 108/100.000 (2), a number that is on par with the incidence of prostate cancer in men in the US (125/100.000) and higher than lung cancer in the US (79/100.000)(2) A high-school soccer player has a knee injury risk of 1/50 player hours. In light of the fact that regardless of whether or not the ligament is reconstructed, at least 50% of these patients will develop post-traumatic osteoarthritis (PTOA) 10-20 years after injury. (3,4) The consequences of PTOA are debilitating, often resulting in participation restrictions and activity limitations that affect thousands of individuals each year. Currently the identification of treatment interventions, prevention strategies, or changes to current treatment algorithms to delay or deter the development of post-traumatic structural damage and subsequent PTOA development is limited as the precise mechanism and risk factors for the development of PTOA following ACL injury is unknown.

Figure 1: Onset of early posttraumatic OA in a 24-year old patient 2 years after ACL reconstruction and partial medial meniscectomy. medial joint space narrowing and beginning subchondral sclerosis as well as mild squaring of the lateral femoral condyle can be seen in comparison to the opposite, uninjured side. This patient ended up receiving a meniscal allograft transplant.

Early inflammation: Early inflammation leads to swelling, pain and pain related muscle shut down affecting recovery. Hemarthrosis and intra-articular pathogenic processes (e.g., down-regulation of proteoglycan synthesis and up-regulation of matrix metalloproteinases) initiate inflammation at the time of injury [5-7]. It is widely accepted that the balance between IL-1 and IL-1Ra are critical in the regulation of the pathological processes involved in joint tissue breakdown [8, 9]. IL-1 levels are elevated in patients with ACL rupture and correlate with severity of chondral damage in a study by Marks [10]. Additionally, chondroprotective cytokine levels are decreased with increasing severity of chondral damage [10]. The superficial cartilage layers have been shown to be more susceptible to IL-1 induced damage than deeper layers, in an *in vitro* study [11]. Moreover, synovial fluid levels of IL-1Ra are reported to decrease significantly after ACL injury, resulting in relatively unopposed activity of IL-1 [12]. This overall up-regulated catabolic state and inflammation leads to recurrent and painful effusions which significantly hinder early rehabilitation after injury but also post-operatively. Post-injury pain and intra-articular effusion are a major contributing factor to extensor mechanism shut down (quadriceps femoris) after injury or surgery which in itself is a significant risk factor for delayed recovery and posttraumatic OA development.



Development of posttraumatic OA: It is increasingly recognized that biochemical abnormalities of the joint precede radiographic abnormalities of primary or secondary OA by as much as decades. A growing body of evidence strongly suggests that the progression from ACL injury to osteoarthritis is multifactorial, involving the interplay between biomechanical disturbances and biochemical homeostasis of articular cartilage. However, unlike primary OA, post-traumatic secondary OA is initiated by intra-articular pathogenic processes with a known date of onset, namely the date of joint injury. This makes it much more amenable to early intervention than primary OA whose onset is not clearly definable at this time. For many years, however, it was believed that the biomechanically and anatomically correct reconstruction of the ACL would restore near normal joint kinematics. Biomechanically the knee joint can be restored to near normal knee kinematics using the latest

anatomic ACL reconstruction techniques. However, the considerable variation in response to surgical treatment even in well-controlled studies (2, 13-16) and recent systematic reviews has suggested that reconstructive surgery of the ACL or meniscus fails to protect against OA development (2,4). When abnormal joint stressors and abnormal joint physiology occur together, the outcome is potentially even more severe (Figure 2, from Lohmander et al (2)). This is illustrated by the fact that severe meniscal damage to the knee is more likely to cause eventual knee OA in patients with hand OA (evidence for a genetic predisposition to OA) compared with patients without hand OA (17).

ACL Injury and Damage to Articular Cartilage: Chondrocytes (matrix secreting cells) are pressure sensitive, and cannot survive compressive forces above a certain threshold, even if the cartilage matrix itself is not injured directly. This threshold may often be exceeded during ACL injuries. Without a sufficient number of chondrocytes to maintain a scaffold, the matrix eventually breaks down over time, causing pain and potential disability to the affected knee joint, often many years after ACL rupture. While the injury mechanism may lead to direct cartilage damage, resulting biomechanical changes of knee function such as increased rotational instability, postsurgical changes or subtle loss of range of motion may lead to progressive breakdown of articular cartilage in the medial or lateral compartment and may appear as early as 2 years after injury (18,19) as illustrated in Figure 1. Indeed, Neuman et al. (20) reported knee cartilage glycosoaminoglycans changes within 2 years after ACL injury. These changes are indicative of posttraumatic OA.

Previous Clinical Research: The IL-1ra drug we are planning to use has been previously utilized in this patient population. The use of this drug is advantageous as it constitutes a meaningful repurposing of an already existing drug that has been shown to be safe in this patient population.

Study 1: *IL-1Ra decreased pain in an open label trial of arthrofibrosis:* Dr. Virginia Kraus, Co-Investigator of this study, conducted two small clinical trials to test the beneficial effect of IL-1ra, an inhibitor of IL-1 biological activity, in vivo. Six patients who failed to meet entry criteria for a separate randomized trial because of age or time from injury to presentation, were entered into a small open label study in which they received 150 mg IL-1ra (anakinra, Amgen) intra-articularly for the treatment of acute ACL injury (n=2), or chronic, recurrent, arthrofibrosis following surgical lysis of adhesions (n=4). All 4 arthrofibrosis patients reported improvement in knee pain, swelling, and joint motion. Examination by the treating physician and by physician global assessment confirmed these results. **Five out of six patients reported clinically significant pain relief after the treatment. There were no other complaints related to the drug and no evidence of significant adverse events in any patient.** These results corroborate findings from animal studies and imply that adequate exposure to IL-1Ra early in the course of disease may potentially reduce pain and stiffness (21).

Study 2: *IL-1Ra decreased pain, and improved function, and lowered IL-1α levels in synovial fluid in a randomized pilot trial of ACL rupture:* In a second randomized trial, 11 patients with isolated ACL tears scheduled for later ACL surgery were treated with 150 mg (anakinra, Amgen; 6 patients) or equal volume saline placebo (5 patients) intra-articularly within the first 30 days of acute knee injury. Mean age of participants was 24 $\pm$ 4 (SD) years, (6 male, 5 female). Enrollment was limited to patients under 40 years of age to try to insure the lack of underlying pre-existing arthropathy. Patients were recruited a mean 15 $\pm$ 7 (SD) days from injury (range 6-27 days). Self-report of pain on a Likert scale 0-10 was recorded at baseline (0), 4 days, 14 days, and 28 days after treatment as well as on the day of surgery. The standardized KOOS and IKDC questionnaires were also obtained.

Using the non-parametric Wilcoxon signed-rank sum test, significant differences ($p<0.05$) were observed in the anakinra treated group only: Pain was reduced in the anakinra treated group of patients by 23% after 4 days, improvement in Activities of Daily Living by 46%, and improvement in total KOOS by 24%; in contrast the placebo group improved by only 4%, 6% and 7% for these outcomes, respectively. This suggests a significan early effect in this patient population that is beneficial for the early rehab process prior to surgery(22).
Synovial fluid IL-1α decreased over 1 month in response to a single IL-1Ra intra-articular injection in our pilot trial. The change with treatment (Day 0 to Day 28) in synovial fluid IL-1α constituted the most intriguing and promising cytokine result from this pilot trial. IL-1α increased in all the placebo treated patients (n=4 pairs of samples available); whereas IL-1α decreased in 4 or 5 of the anakinra treated patients (n=5 pairs of samples available)(21).

Study 3: In order to prove that we have the ability to perform a high level (GCP) randomized clinical trial our group has performed a randomized clinical trial involving ACL patients in the same study design featuring identical enrollment (inclusion/exclusion) criteria as the one proposed in this contract (MOON-AAA Clinicaltrials.gov identifier: NCT01692756). In this multicenter early intervention trial testing the effect of Triamcinolone administration patients underwent knee joint aspirations, blood draws and urine collections at three time points prior to ACL surgery. PRO's and clinical exams were performed at all time points prior to surgery and at 4 time points post-surgery up to 6 months post-surgery. We have successfully enrolled 3-4 patients per month in the last 12 months actually exceeding the goal of 2 patients / month as initially estimated. The average time from injury to enrollment is 4.2 days with an average patient age of 18.6 years. No patients have been lost to follow-up. This trial has just completed enrollment and is in the process of data analysis (see table 1). This trial has been extraordinarily successful and was able to show that the initial chondrodegradatory response, as measured in synovial fluid can be modified intraarticularly with the administration of triamcinolone acetonide.

Table 1: Enrollment data MOON-AAA trial

	range	mean
AGE	13-33	18.58
M:F	Male: 25	Female: 18
BMI	19.1-31.6	24.25
t visit 1	1-8	4.17
t visit 2	7-20	11.53
t surgery	10-51	28.94

Pediatric dosing - Kineret®, was studied in a single randomized, blinded multi-center trial in 86 patients with polyarticular course Juvenile Rheumatoid Arthritis (JRA; ages 2-17 years) receiving a dose of 1 mg/kg subcutaneously daily, up to a maximum dose of 100 mg. The 50 patients who achieved a clinical response after a 12-week open-label run-in were randomized to Kineret® (25 patients) or placebo (25 patients), administered daily for an additional 16 weeks.

A subset of these patients continued open-label treatment with Kineret® for up to 1 year in a companion extension study. An adverse event profile similar to that seen in adult RA patients was observed in these studies. Pediatric use of Kineret® was not recommended due to difficulty dosing the medication in smaller children requiring less than 100mg per dose and because efficacy could not be demonstrated due to low trial enrollment. No safety issues were seen. The dosing problem will not come to bear in our patient population as we only enroll children 14 years and older.

Trial Registration Number: NCT00339157. First prospective placebo controlled trial to evaluate the effectiveness of IL-1Ra for SJIA. Enrolled a total of 24 patients: mean (SD) age of 9.5 (5) years; female subjects entering the study were prepubescent, sexually inactive or required to use

effective contraception; 12 received 2 mg/kg sc daily for 1 year; 10 received placebo for one month then 2 mg/kg sc daily for 11 months. Max dose 100 mg qd. Anakinra treatment was effective in SJIA. They noted in the Discussion that: " PK data suggested that low-weight children might have benefited from a higher anakinra dosage."

Safety: Between month 1 and month 12, six patients stopped treatment owing to an adverse event (n=2), lack of efficacy (n=2) or a disease flare (n=2). "One patient was diagnosed with inflammatory bowel disease, a complication reported in some patients initially diagnosed with JIA and treated with anti-TNF agents. An additional patient experienced a sudden rise in serum transaminases." Of note, patients were on concurrent corticosteroid therapy.[39]
An open-label retrospective observational trial of 46 SJIA patients (median age 7.6 years) who received anakinra alone (n=10) or in combination with corticosteroids or remittive agents including methotrexate or cyclosporine.

Dose: "The median starting dose of anakinra was 1.5 mg/kg/day (IQR 1.1–2.0 mg/kg/day; minimum 0.93 mg/kg/day, maximum 11.2 mg/kg/day), and subsequent dose escalation was required in 24% of patients. Patients requiring dose escalation received a median initial dose of 1.3 mg/kg/day, compared with a median initial dose of 1.6 mg/kg/day in patients who did not (*P* not significant). Among 19 patients who started with a dose <1.5 mg/kg/day, 8 (42%) ultimately required a higher dose. One patient received anakinra twice daily. In most patients, therapy with anakinra could not be interrupted during the term of observation, but recurrent dose escalation (tachyphylaxis) was not observed. At the last follow-up visit, anakinra had been discontinued for inefficacy in 1 patient, for intolerance in 2, and because of remission in 3; 7 patients could be weaned to dosing every other day, although symptoms returned if the interval exceeded 48 hours." Of note, the patient who received the highest dose of anakinra monotherapy (11.2 mg/kg/day intravenously administered as 5.6 mg/kg every 12 hours) was being treated in intensive care for macrophage activation syndrome, a potentially life-threatening complication of SJIA. On this treatment: "She improved dramatically, but her response remained partial, and at the last follow-up visit, she was requiring anakinra at 2.3 mg/kg/day, abatacept, and monthly pulse corticosteroids."

They concluded that "in general, improvement was rapid and striking." series, children older than 7 years were more likely to exhibit a complete response to anakinra-based therapy than those ages 7 years and younger (75% versus 41%; $P < 0.05$).

Safety: Five patients developed macrophage activating syndrome while on anakinra but this was believed to be due to SJIA and not anakinra. They stated: "the role of anakinra as a trigger for these 5 cases cannot be determined, in no case was permanent discontinuation necessary. Further, dose escalation often seemed to help control macrophage activation syndrome." Injection site reactions occurred in 44% of the 45 patients with evaluable data, leading to permanent discontinuation of drug in 1 patient and transient discontinuation in several others.

There were 3 cases of serious infection. A 4-year-old child treated with anakinra at 1 mg/kg/day and 6 weeks of corticosteroids developed pneumococcal bacteremia at 2 months in the setting of parainfluenza infection. Anakinra was withheld for 1 week but was restarted because of rising levels of markers of inflammation. The patient remained well during an additional 6 months of observation. An 11-year-old child treated with anakinra (1.2 mg/kg/day) and high-dose oral corticosteroids developed an infection at a healed gastric feeding tube site. Anakinra was withheld for 2 weeks but was ultimately escalated to 1.9 mg/kg/day without further complication during 21 additional months of observation. A 3-yearold child receiving anakinra at 1.7 mg/kg/day was admitted to the hospital for several days with pneumonia. No organism was cultured. Anakinra was restarted after discharge."

Other events included two episodes of bronchitis, 1 episode of eosinophilic hepatitis requiring discontinuation of anakinra, elevation of liver enzymes in 2 patients but anakinra could be continued, and mild neutropenia resolved on alternate day anakinra.[40]

2.2 RATIONALE

We hypothesize that IL-1Ra administered intra-articularly during the early phase of acute ACL injury will provide symptomatic pain relief and decrease synovial fluid inflammatory and cartilage degradation markers. ACL injury produces high levels of inflammatory mediators, including as IL-1, which mediate knee pain, perpetuate synovitis, and decrease functional recovery post injury and post-surgically. We have designed a clinical trial that specifically tests the ability of IL-1ra to alleviate pain following acute ACL injury and potentially changes patient reported outcomes at 2 years.

A total of 1 mL (150 mg) will be injected intra-articularly after synovial fluid aspiration 'to dryness' once at study entry 1-28 days after injury depending on time of presentation).

Rational for including pediatric population

The specific reason for enrolling the age group of children aged 14-18 is that these children have a musculoskeletal physiology like adults.

- Their major growth plates are closed.
- These patients may be treated surgically like adult patients with exactly the same surgical preparation, technique and rehabilitation as adults.
- Children, particularly girls, between the ages of 14 and 18 have the highest statistical risk in any population of tearing their Anterior Cruciate Ligament. [25,45]
- Children in this age group are particularly vulnerable to the later development of osteoarthritis. Lohmander et al showed that young female soccer players in this age group developed symptomatic knee Osteoarthritis secondary to the ACL injury within 12 years of the injury.[24] One could therefore argue that particularly children between completed puberty and 18 years of age have to be the patients requiring most of our concern regarding development of OA.
- *Furman et al.* showed that early post-injury administration of IL-1Ra did not alter bone morphology or fracture healing after intra-articular fracture but significantly reduced cartilage degradation and inflammation.[(46)]
- The value of intraarticular IL-1 blockade when given immediately after intraarticular injury has been demonstrated repeatedly. In a recent publication of *Genemaras et al.* the early administration of IL-1Ra could be shown to significantly down-regulate pro-inflammatory Il1-β, TNF-α) and chondrodegredatory markers (MMP-3,ADAMTS-4,ADAMTS-5) immediately after non-destructive chondral impact injury to porcine articular cartilage[(45)].
- *Chevalier et al.* showed in 2005 that intra-articular injection of anakinra in patients with knee OA is safe [(47)]. It is important to realize that patients who have suffered an ACL injury are a substantial risk for chronic inflammatory changes leading to OA and, in fact, display these changes as early as 4 weeks after injury lasting for up to 5 years after ACL injury. [(48,49)]
- A study performed by Brown et al. enrolled 8 patients with arthrofibrosis who were treated with intra-articular administration of 150mg of anakinra. Five of the eight enrolled patients were under the age of 24, 3 under the age of 21, and one patient aged 15. None of these patients have shown any adverse events and all of them improved in their clinical outcomes. In addition the same group completed a study enrolling young patients (mean age 22$\pm$ 3) who suffered ACL injuries. This trial did also not show any

significant adverse events regarding safety of the drug administration. In contrary, they show improved post-injury pain scores.[52]

In summary: We would like to argue that skeletally mature adolescents of ages 14-18 are musculoskeletally no different than their 19 year old and older counterparts and therefore deserve the same potential benefit that early treatment with IL-1Ra may offer older patients. We find of no data to indicate that intra-articular use of IL-1Ra would have a different effect in the 14-18 year old over the 19-40 year old. There is no in-vitro or in-vivo data indicating a detrimental effect of Il-1Ra to articular cartilage or the knee joint as a whole in the dosages we are intending to use. We believe that the potential benefits of this trial for this patient population outweighs by far the uncertainty of unknown risks that may be associated specifically with being 14-18 year old.

The Federal Drug Administration has approved daily subcutaneous injections (100 mg) to patients with rheumatoid arthritis. Our goal is to sustain therapeutic levels of IL-1Ra in the joint during the early phase of acute joint injury. We hypothesize that the post injury intra-articular administration of 150 mg IL-1Ra (Kineret®) will be well tolerated.

By slowing the initial inflammatory process after injury and preventing the significant quadriceps muscle shutdown early, we aim to work towards this goal in this trial. We therefore conclude that given the limited risk of local side effects and the potential large benefit that this study would provide to the scientific community and its potential to significantly improve and alter patient care of young individuals suffering ACL injuries it is not reasonable to withhold pediatric patients aged 14-18 from this important trial.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Safety Precautions: Participants will be followed at regular intervals by their study doctor. During each visit participants will be evaluated for adverse events according to GCP guidelines and reported accordingly to IRB and FDA regulations.

Risks associated with Kineret®:

Reproductive Risks: It is not known whether receiving Kineret® is harmful to an unborn child. Pregnancy after administration of Kineret® may involve risks to the mother, and her embryo or fetus. Therefore, females who are capable of reproducing, must use effective contraceptive methods. These methods must be used from the time of screening until Visit 2 during which any remaining medication will be washed out of the joint during the surgery. If the participant does not elect to have surgery (visit 2) contraception methods will be documented until the end of the study. Examples of effective birth control are: hormonal contraceptives (the pill, implant, transdermal patch, or injection), barrier methods (condom with spermicide, diaphragm with spermicide), IUD, or a male partner had a vasectomy.

Lactating women: It is not known whether this drug or its metabolites are excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Kineret® is administered to a nursing woman.

Limitations, potential problems, and alternatives: This study will allow for safety and efficacy assessment in patients with acute effusive knee injury.

- Dose: Based on our work and the work of others, we believe 150 mg dosage is appropriate. The goal of the study is to sustain levels of IL-1Ra long enough and a high enough concentrations during the critical, early time period to be effective. For this reason, we have increased our dosage from a single injection (carried out in the preliminary study) to two injections.
- Patient attrition: Close and regular communication, particularly in the intensive first few months, will be provided to all subject participants. Patients will be fully informed of the time commitments and need for a total of three arthrocenteses in order to recruit study subjects willing to participate in the full study. Because arthrocentesis alone can be expected to benefit subjects, education regarding direct and indirect benefits of all aspects of the trial will be provided. Subjects will also receive appropriate compensation for the time and travel.

2.3.2 KNOWN POTENTIAL BENEFITS

IL-1Ra, while potent, has had mixed results in the treatment of established OA, possibly due to its short half-life in vivo. In this trial, we expect that an early intervention with IL-1Ra will effectively block the deleterious cascade of joint degradation events after joint injury. We expect a significant effect of IL-1Ra on post-injury pain and concomitantly an increased improvement in early function.

3 OBJECTIVES AND PURPOSE

Primary Objective: To assess the efficacy of using IL-1ra and knee arthrocentesis to alleviate inflammation related knee pain and improve patient-reported outcomes (PRO) following ACL rupture.

Secondary Objectives:

1. To determine if intra-articular IL-1ra therapy improves levels of a panel of inflammatory, meniscus and cartilage metabolism, and oxidative stress biomarkers as measured in synovial fluid, serum and urine from patients immediately after ACL injury (baseline, 2 weeks after injury and at 1 and 2 years after surgery).

2. To assess if MRI T1rho changes correlate with synovial biomarker levels and show predictive value for the overall subject patient outcome at 2 years

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This is a phase 2, prospective, randomized, triple-blinded, placebo-controlled study of IL-1RA treatment in patients with acute ACL tear and painful effusions. The study population will include up to 40 participants within 28 days of injury. The study is open to male and female participants who are between the ages of 14 and 40, who have ACL tears. Once enrolled will be randomized into 2 groups (20 subjects per group).

- **Group 1** will receive intra-articular injection of Anakinra (Kineret®, IL-1ra; 150mg) 1-28 days after ACL injury.
- **Group 2** will receive intra-articular injection of saline placebo 1-28 days after ACL injury.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

Patient reported outcomes as measured by KOOS-Symptoms

4.2.2 SECONDARY ENDPOINTS

KOOS QOL,
KOOS-pain,
KOOS-Sports/Rec,
Biomarker levels in synovial fluid, serum and urine at time of surgery
MRI T1rho changes at 2 years

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Isolated Acute ACL Tear (1-28 days post injury) and painful effusion, with no more than a clinical grade 2 MCL injury.
- Provision of signed and dated combined informed consent/HIPAA form and if a minor, a signed assent.
- Stated willingness to comply with all study procedures and availability for the duration of
- the study
- Male or female, aged 14-40 years
- For females of reproductive potential: use of highly effective contraception.
- Currently participating in a sporting activity
- Documentation of closed growth plates as noted on the screening x-ray

5.2 PARTICIPANT EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in the study:

- History of underlying inflammatory disease (i.e. Rheumatoid Arthritis, Psoriatic Arthritis etc.)
- History of having been diagnosed with hepatitis B or tuberculosis
- Currently have an infections, including infection of the skin, or have signs and symptoms of an infection, including fever.
- History any abnormalities in their white blood cell counts
- History of having a disease that weakens your immune system such as diabetes, cancer, HIV or AIDs
- History of other major medical condition requiring treatment with immunosuppressant or modulating drugs.

- A history of chronic use of non-steroidal anti-inflammatory drugs
- Currently taking immunosuppressant medication, including oral and parenteral corticosteroids (topical and stable dose inhaled corticosteroids are acceptable)
- Females who are pregnant or breastfeeding
- Received a "live" vaccine (smallpox, MMR (measles, mumps and rubella), flu, polio, typhoid, chicken pox, yellow fever, herpes zoster) 1 week prior to screening or are scheduled to receive a "live" vaccine within 1 week after study injection.
- History of bleeding disorders or are taking any blood thinning medications, aspirin or other medications affecting blood clotting.
- Previous exposure or allergic reaction to anakinra
- Allergy to latex or tape
- Allergy to Kineret or have had a reaction to any local or general anesthesia
- Prior ipsilateral knee surgery
- Complete ligament tear other than the ACL
- Received any investigational drug with 4 weeks of study Visit 1
- Does not have the cognitive ability to provide informed consent.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Participants in this trial will be recruited within 28 days from injury following a clinical appointment with one of the study's participating orthopaedic surgeons.

Participants in this trial will be recruited within 28 days from injury from the Emergency Department or walk-in clinics for acute injuries.

No prisoners will be enrolled into the study.

We will enroll children between the ages of 14-17. Parental consent will be needed in order to enroll children into the study.

Participants will receive $150.00 for taking part in this study. The participant will receive $50.00 for each MRI they complete (Visit 1, Visit 5, and Visit 6). Payment will be in the form of a check which will be mailed approximately four to six weeks following the study visit. If the participant is a minor the compensation will be mailed to the minor.

Advertising may be used to promote this study to the public. All advertisements and public marketing will be approved by the IRB prior to use.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Subjects are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

A subject may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the Investigator for due to safety or scientific reasons. Reasons for discontinuation from the study at any time point will be collected. If subject discontinues the study for any reason prior to 3-month visit, then the collected data will be evaluated but not included in the analysis and a replacement subject will be enrolled in the study.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include the above mentioned stopping criteria as well as:

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

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

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Unblinded study drug will be shipped from the manufacturer Swedish Orphan Biovitrum (Sobi) directly to the site research pharmacist, after all required regulatory and legal documents have been received by the Sponsor.

Replacement Procedures For Investigational Medicinal Product: Resupply shipments or replacement of study drug will be provided by Sobi after consultation with Dr. Stone.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Formulation, Packaging and Labeling: Kineret® is a recombinant, nonglycosylated form of human interleukin-1 receptor antagonist (IL-1Ra). Kineret® consists of 153 amino acids and has

a molecular weight of 17.3 kilodaltons. It is produced by recombinant DNA technology using an *E coli* bacterial expression system.

Kineret is supplied in single use prefilled glass syringes with 29 gauge needles as a sterile, clear, colorless-to-white, preservative free solution for daily subcutaneous (SC) administration. The solution may contain trace amounts of small, translucent-to-white amorphous proteinaceous particles. Each prefilled glass syringe contains: 0.67 mL (100 mg) of anakinra in a solution (pH 6.5) containing anhydrous citric acid (1.29 mg), disodium EDTA (0.12 mg), polysorbate 80 (0.70 mg), and sodium chloride (5.48 mg) in Water for Injection, USP.

The prefilled syringe contains an outer rigid plastic needle shield attached to an inner needle cover. The syringe or needle shield components are not made with natural rubber latex.

Each syringe will have a label that will contain, at minimum, the protocol number, contents (written as "Kineret®"), applicable regulatory cautions, storage conditions, route of administration, and a space to write in the dispensing date, subject identification number and subject initials.

6.1.3 PRODUCT STORAGE AND STABILITY

The prepared syringes of Kineret or placebo will be stored in a separate, temperature controlled refrigerator at the investigative site.
Kineret® should be stored in the refrigerator at 2° - 8° C (36° - 46°F). **DO NOT FREEZE OR SHAKE.** Protect from light. Kineret® should not be used after the expiration date unless otherwise notified by the Principal Investigator.

The study drug will be sent to the designated research staff at the University of Kentucky.

Once a subject is identified and consented, the study coordinator will utilize the syringe corresponding to the assigned patient ID to administer the study medication. It has been shown by HPLC analyses, that Kineret is stable for at least 4 hours at ambient temperature and in light in 0.9%NaCl and in a plastic culture vial.[38]

The refrigerator temperature must be monitored by site personnel on a regular basis and must be recorded on a temperature log sheet or recorded electronically. In the event that the refrigerator's temperature goes outside the 2° - 8° C (36° - 46°F) range, the Sponsor or designee should be contacted immediately at the telephone number provided in the protocol. Only temperature excursions outside of the acceptable range, per the Sponsor's procedures, are reported as protocol deviations.

6.1.4 PREPARATION

The study drug will be kept in a temperature controlled environment according to the manufacturer's recommendations

Under aseptic clean conditions, a total of 200 mg (1.34 mL) of Kineret® (concentration 100mg/0.67ml) will be injected from 2 sterile original prefilled 100 mg syringes of Kineret into a sterile vial. A sterile graduated 5 mL syringe will then be used to draw 150 mg (1 mL) of Kineret for intraarticular injection into the knee. The medication must be transferred from the original prefilled Kineret syringes as the needles are intended for subcutaneous delivery and are not adequate for intra-articular knee injection. No dilution of the medication will be performed.

For the placebo we will utilize 1 mL of 0.9%NACL sterile saline solution drawn up in the same type of syringe as for the Kineret administration in order to maintain double blinding of the study investigator and patients.
The number of study syringes prepared by the designated research personnel or research pharmacist, per day, will depend on the number of potential enrollees or return patients requiring Kineret® injections that day.

6.1.5 DOSING AND ADMINISTRATION

Study treatment must only be dispensed by a pharmacist or other medically qualified staff. Study treatment is to be dispensed only to subjects enrolled in this study.

Once a subject is identified and consented, the investigator will utilize the syringe corresponding to the assigned patient ID to administer the study medication. It has been shown by HPLC analyses, that Kineret is stable for at least 4 hours at ambient temperature and in light in 0.9%NaCl and in a plastic culture vial.[38]

- **Group 1** will receive intra-articular injection of Anakinra (Kineret®, IL-1ra; 150mg) 1-28 days after ACL injury.
- **Group 2** will receive intra-articular injection of saline placebo 1-28 days after ACL injury.

6.1.6 ROUTE OF ADMINISTRATION

Intra-articular injection

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

The starting dose of Kineret is 150mg. There is no planned dose escalation.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

No dose adjustments or modifications are allowed in this study.

6.1.9 DURATION OF THERAPY

Enrolled participants will receive, based on their randomization, either Kineret or a matching saline placebo within 1-28 days after suffering an ACL injury.

If a participant discontinues the study for any reason prior to 3-month visit, then the collected data will be evaluated but not included in the analysis and a replacement participant will be enrolled in the study.

6.1.10 TRACKING OF DOSE

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

It is the responsibility of the Sponsor/Investigator to supervise accurate monitoring of the receipt, storage, dispensing, and accounting of all study drug according to accepted medical and pharmaceutical practice, and should also comply with state and local regulations. The originals of all invoices of study drug shipments must be retained. Accurate, original site records of study

drug inventory and dispensing must be maintained using the forms provided by the Sponsor/Investigator. All disposition records must be made available for inspection by the FDA, DSMB, monitor or designee upon request.
Forms will be provided for the accounting of study drug and for the accounting of study drug for each subject. A reason(s) must be given for any study drug that is not accounted for. Site must keep all used and unused study drug in their original box until the monitor either arranges return to the distribution center or gives instruction on their disposal. If any unused study drug remain at the end of the study, they will be accounted for at the end of the study in the presence of the monitor who will provide instructions on their disposal

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY RELATED

Demographic information: Demographic data to be collected at Visit 1 will include height, weight, date of birth, age, gender and race.

Medical and Medication History A detailed past medical history and a list of current medication, to include prescription and over-the-counter medications, will be collected at Visit 1. Assessment of eligibility should include a review of permitted and prohibited medications. Both the medical and medication history will be reviewed with the subject throughout the study for any changes in baseline history.

Physical examination including vital signs, height, weight, BMI. All subjects must have a clinical exam that is consistent with an ACL tear at Visit 1. All subjects will have range of motion (ROM) collected at all visits.

***Knee Aspiration*:** All subjects undergo knee aspiration to dryness at initial encounter Visit 1 (1-28 days after ACL injury), Visit 2 (the time of surgery) and again at Visit 3 (4-14 days post-op). Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will receive treatment according to this protocol but, aspiration at time of surgery will be omitted.

Assessment of Pain and Function –Subject Reported Outcomes – The study will use the standardized subject self-report Knee Injury and Osteoarthritis Outcome Score (KOOS) instrument to assess pain and function in response to the intervention for acute ACL injury. The KOOS is a 100 item instrument that consists of 5 subscales (pain, other symptoms, function in daily living, function in sport and recreation, and knee-related quality of life). The 5 separate subscales provide a complete picture of patients' perceptions of their knee injury and consequences to their daily activities, etc.. KOOS includes the WOMAC osteoarthritis index, which allows for potential comparison with other earlier studies. The KOOS has high test-retest reproducibility (ICC > 0.75). The KOOS subscales for function in sport and recreation and knee-related quality of life have been shown to be the most sensitive subscales pre-operatively and change the most post-operatively (27). The study will supplement the KOOS with self-reported function score assessments from the International Knee Documentation Committee (IKDC) form for evaluation of knee ligament injuries (28) as well as the. Veterans Rand 12-Item (VR-12) health survey and the Patient Acceptable Symptoms State (PASS) question. The questionnaires will be assessed either via an internet based collection software that allows secure data collection

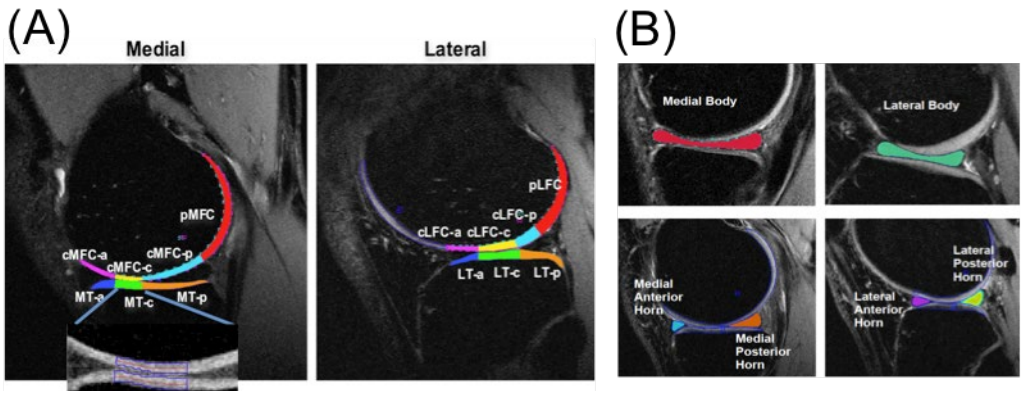
through a password coded entry portal or during routine clinical visits using an IPad or paper forms. Paper forms may be used during routine clinical visits or directly mailed to patients.

Specific Pain assessments - Pain coping strategies are important in understanding improvements in pain and psychological disability [46-48]. This study will use a simple Likert pain scale as a pain assessment tools (Figure 3). Pain catastrophizing has been identified as one of the strongest predictors of pain and has been defined as "an individual's tendency to focus on and exaggerate the threat value of painful stimuli and negatively evaluate one's own ability to deal with pain." [49] Not only do individuals who catastrophize, experience more experimental pain (29-31) but also, catastrophizing has been shown to account for 7% to 31% of the variance in pain ratings in varied populations of patients having persistent pain.

MRI analysis*:* All subjects will undergo MRI examination following Visit 1 and subsequent MRIs will be performed at Visits 5 and 6. Sagittal and axial MRI images will be acquired using a 3.0-T MRI scanner and an 1Tx/15Rx phased array knee coil. Sagittal and axial T1-weighted spin echo images (FSE) will be acquired to allow semi-quantitative analysis at the conclusion of the study. These sequences will be followed by a sagittal high-resolution 3D dual echo steady state (DESS) sequence for cartilage segmentation and a sagittal combined T1ρ/T2 mapping for the quantification of cartilage composition. The major parameters for the DESS sequences are: FOV = 140 mm, matrix = 384×307×176, resolution = 0.36×0.45×0.7 mm^3, time of repetition (TR)/time of echo (TE) = 17.6ms/6.0ms. The T1ρ/T2 mapping will be acquired using a 3D MAPSS sequence with major parameters: FOV = 140 mm, image matrix = 320×160×24, resolution = 0.44×0.44×4 mm^3, time of recovery = 1.5 seconds, bandwidth = 400 Hz/Pixel, TR/TE = 7ms/3ms, time of spin lock (TSLs) = 0, 10, 40, 80 ms for T1ρ mapping and preparation TEs = 0, 20, 40, 60 ms for T2 mapping. The scan time will be approximately 12 minutes for the combined T1ρ/T2 mapping. Images will obtained with the knee in full extension.

Images collected at the University of Kentucky will then be transferred digitally to the University of California, San Francisco to be analyzed by Dr. Majumdar and her laboratory. Based on the high resolution DESS images, articular cartilage will be segmented using a spline-based, semiautomatic technique. The T1ρ map will be created on a voxel-by-voxel basis using established fitting routines. The segmented masks will then be overlaid on the T1ρ maps after registration between DESS and T1ρ images, and mean T1ρ values will be calculated for the entire tibiofemoral cartilage, medial and lateral tibial plateau, medial and lateral femoral condyles, and the patellofemoral compartment will be calculated.

Figure 3. (A) Cartilage subcompartment definition in medial (left) and lateral (right) sides. (B) Meniscal region definition in medial (left) and lateral (right) sides. [109]



7.1.2 STANDARD OF CARE STUDY PROCEDURES

Radiographic Analysis:
Plain x-ray analysis: All subjects would have undergone a routine clinical x-ray analysis prior to Visit 1. This includes bilateral x-ray views of the knee joint using a standing a/p, synaflexer standardized flexion weight bearing p/a (Synarc®), Merchant views and weight bearing lateral views. Radiographs will be assessed to determine study eligibility as open growth plates are an exclusion criteria. Plain radiographs will be reviewed for radiographic progression of joint space narrowing or osteophyte formation using the Kellgren-Lawrence classification system as well as the Iwano classification for the patello-femoral joint.

Previous studies have shown that that joint space narrowing measured on a standardized synaflexor platform has a high reproducibility and accuracy. At 2 years koint space narrowing has been identified in an ACL injured cohort using this system (32).

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Clinical Laboratory Evaluations: none

Pregnancy test: Female participants of child bearing potential will have a urine pregnancy test at Visit 1. Pregnancy testing results must be negative in order for enrollment into the study.

Procedure for minors: A female minor is identified as a potential candidate for the study. The investigator or approved study staff approach the patient and accompanying parent/guardian to inform them that the patient may qualify for a study. Both parties are informed of the study. If interested in participating in the study, each research team will perform a pregnancy test for inclusion/exclusion in correspondence with their locally approved minor pregnancy testing protocol.

Non-Clinical Laboratory Evaluations:

Sample collection including joint arthrocentesis. Synovial fluid will be aspirated and spun at 3500 RPM for 10 minutes and the supernatant will be frozen at -70°C. Joint aspiration will be performed aseptically with local anesthesia through a superolateral suprapatellar approach. The aspiration syringe will be gently removed and the study drug/placebo-containing syringe will be attached, followed by injection. The supernatants will be stored, pending analysis, in the UK Center for Clinical and Translational Science Bioanalysis Lab. All biomarker analyses in synovial fluid, serum, and urine will be performed by co-Investigator Dr. Virginia Kraus at Duke University.

IL-1ß, IL-1α, IL-6 and IL-1Ra analyses - These cytokines will be measured in the synovial fluid using the high-sensitivity Quantikine (sandwich) Immunoassays (R&D). As described previously (39), the standard curve will be extended in the low range for the IL-1α assay to further improve the specificity as we have found the standard curve of the assay to be reproducible and linear below the lower standard described in the procedural literature accompanying the reagents.

Glycosaminoglycan (GAG) - The S-GAG content, a marker of proteoglycan degradation, will be measured in synovial fluid with the dimethyl methylene blue dye (DMMB) assay as noted previously (40). Chondroitin sulfate from shark cartilage will be used as a standard between 5 and 50 mg/ml.

Type II collagen (cartilage) degradation assay - CTX-II is derived from the C-terminal crosslinked telopeptide of type II collagen. Following degradation of cartilage it is released into the synovial fluid, the circulation, and subsequently secreted into urine. CTX-II correlates with the degree of joint destruction and increases significantly within one month after ACL tear (P=0.012)(41). CTX-II will be measured in synovial fluid by ELISA (IDS, Herlev, Denmark (42).

Type I collagen (meniscus) degradation assay – NTX-I is derived from the N-telopeptide of type I collagen and was significantly elevated in synovial fluid after acute ACL tear (P=0.008) in our pilot study (42) We will measure NTX-I in synovial fluid by ELISA(42) In serum it is considered to be indicative of bone resorption. In synovial fluid in the setting of acute joint injury we believe it to be indicative of meniscal injury and metabolism due to the fact that meniscus is primarily a type I collagen containing tissue.

Cartilage Oligomeric Matrix Protein (COMP) - COMP is a pentameric, anionic, noncollagenous glycoprotein and member of a thrombospondin family of extracellular proteins that was initially isolated from cartilage (43) Although other joint tissues express COMP (44), it is most abundant in articular cartilage. Serum COMP levels are representative of cartilage catabolism (45,46) and we have demonstrated that serum COMP is associated with the presence and severity of radiographic OA and progression of OA [60]. COMP will be measured by sandwich ELISA (Biovendor) with mAbs 17C10 and 16F12 a recombinant COMP standard with an assay range of 0.1-32 U/L and the CV is <5%.

Xanthine Oxidase (XO) - generates superoxide, a powerful reactive oxygen species. Synovial fluid XO is indicative of oxidative stress and increases in the first month after joint injury (Kraus unpublished data). It is measured by a multistep enzymatic reaction available from Cayman Chemical (Ann Arbor, Michigan) whose end product resorufin, is a highly fluorescent compound that can be easily analyzed using an excitation wavelength of 520-550 nm and an emission wavelength of 585-595 nm.

7.2.2 OTHER ASSAYS OR PROCEDURES

None

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Refer to the study lab processing manual

7.2.4 SPECIMEN SHIPMENT

Refer to the study lab processing manual

7.3 STUDY SCHEDULE

7.3.1 SCREENING/ENROLLMENT

Visit 1 Screening 1-28 days following injury)

- Obtain informed consent and assent, if necessary, of potential subjects
- Review medical history to determine eligibility based on inclusion/exclusion criteria.
- Review medication history to determine eligibility based on inclusion/exclusion criteria.
- Perform medical examinations and collect vital signs as needed to determine eligibility based on inclusion/exclusion criteria. All subjects must have a clinical exam that is consistent with an ACL tear.
- Collection of urine and blood for laboratory testing. Women of child bearing potential will be given a urine pregnancy test. Test must be negative in order to enroll into the study.
- Subjects will have the following assessments: range of motion, knee instability (Lachman's test) and standardized MTP-2 weight bearing x-rays.
- Standard questionnaires including the KOOS, IKDC, VR-12 and a Likert pain scale.
- Research knee MRI

Following the review of above assessment results and a review of inclusion/eclusion criteria, subjects will be randomized into 1 of 3 treatment groups. Following randomizing, subjects will undergo a knee aspiration and will receive their first dose study medication.

Visit 2 (day of surgery or 4-6 weeks post injury)

- Knee aspiration (in the operating room under anesthesia)
- Patient reported outcomes (PROs) administered prior to surgery
- KOOS, IKDC, VR-12 and a Likert pain scale administered prior to surgery
- Biomarkers (urine and blood) pre-operatively
- Review for AE's and SAE's
- ROM will be performed prior to surgery

Visit 3 (4-14 days After Visit 2)

- Knee aspiration (if clinically indicated)
- Biomarkers (urine and blood)
- Review for AE's and SAE's
- ROM will be performed

Visit 4 (6 month After Visit 2+/- 2 weeks)

- Patient reported outcomes (PROs) administered
- KOOS, IKDC, VR-12 and a Likert pain scale administered
- Review for AE's and SAE's

- ROM will be performed

Visit 5 (12 monthsAfter Visit 2+/- 2 weeks)
- MRI
- Patient reported outcomes (PROs) administered
- KOOS, IKDC, VR-12 and a Likert pain scale administered
- Biomarkers (serum and urine only)
- Review for AE's and SAE's
- ROM will be performed

Visit 6 (24 month After Visit 2+/- 2 weeks)
- MRI
- Patient reported outcomes (PROs) administered
- KOOS, IKDC, VR-12 and a Likert pain scale administered
- Biomarkers (serum and urine only)
- Review for AE's and SAE's
- ROM will be performed

7.3.2 SCHEDULE OF EVENTS TABLE

This section should capture the procedures that will be accomplished at each study visit and correspond to the descriptions in the above sections.

Procedures	Screening (Visit 1)	Visit 2[e]	Visit 3	Visit 4	Visit 5	End of Study Visit 6
Time point	1-28 days post injury	Surgery day or 4-6 weeks post injury	4-14 After Visit 2	6 months After Visit 2 (+/- 2 weeks)	12 After Visit 2 (+/- 2 weeks)	24 months After Visit 2 (+/- 4 weeks)
Informed Consent	X					
Demographics	X					
Medical history	X	X	X	X	X	X
Randomization	X					
Administer Investigational Product	X					
Concurrent meds	X	X	X	X	X	X
Physical Exam	X					
Range of motion	X	X	X	X	X	X
Vital signs	X					
Height	X					
Weight	X					
Questionnaires	X	X		X	X	X
Knee Aspiration	X	X[a]	X[h]			
Biomarkers	X	X	X		X[d]	X[d]
Safety Lab Tests[b]	X					

Procedures	Screening (Visit 1)	Visit 2[e]	Visit 3	Visit 4	Visit 5	End of Study Visit 6
Urine pregnancy tests[c]	X					
Adverse event evaluation	X	X	X	X	X	X
X-Ray[f]	X					
MRI[g]	X				X	X

a: Should a subject be enrolled into the study and choose not to undergo ACL reconstruction aspiration at time of surgery will be omitted.
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
c: Urine pregnancy test (women of childbearing potential).
d: Serum and urine only
e. Should a subject be enrolled into the study and choose not to undergo ACL reconstruction, subject will received treatment according to this protocol. For subjects that do not have ACL reconstruction, the time windows will be relative to Visit 2. For example, Visit 3 will be 4-14 days after Visit 2
f. All subjects must have standardized MTP-2 weight bearing x-ray. Documentation of closed growth plates at screening will be noted in the routine SOC x-ray
g. All subjects enrolled will have an MRI performed regardless if surgery is to be scheduled or not. However, randomization can be performed prior to MRI as the MRI examination is not necessary or required to diagnose the ACL tear.
h. We will only perform the aspiration at Visit 3 if participants had elected to have surgery performed, and if they have fluid on their knee.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Not Applicable.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

All concomitant prescription medications taken during study participation will be recorded on the case report forms (CRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the CRF are concomitant prescription medications, over-the-counter medications and non-prescription medications including herbal supplements.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

Treatment with (list specific drugs) will not be permitted unless discussed with and approved by the PI.

- Immunosuppressant including oral and parenteral corticosteroids (topical and stable dose inhaled corticosteroids are acceptable)or modulating drugs.
- Non-steroidal anti-inflammatory drugs
- Any blood thinning medications, aspirin or other medications affecting blood clotting.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Not Applicable

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Not applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

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

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

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

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

OHRP considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

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

This study will use the OHRP definition of UP.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

All AEs will be assessed by the clinician using a protocol defined grading system. The protocol defined grading system to be used is:

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

8.2.2 RELATIONSHIP TO STUDY AGENT

All AEs will have their relationship to study agent or study participation assessed with a level of specificity appropriate to the study design. The clinician's assessment of an AE's relationship to study agent (drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study agent assessed. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study agent, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established. OR For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

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

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

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

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

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

8.2.3 EXPECTEDNESS

Expected adverse reactions are AEs that are common and known to occur for the study agent being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction.

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the protocol or the USPI, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

The Principal Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

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

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization as well as seriousness of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.
Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

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

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

The Principal Investigator will assess the occurrence of AEs throughout the subjects' participation in the study. Subjects will be followed for toxicity for 30 days after treatment has been discontinued or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject, which occur after the subject has signed the informed consent, are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- seriousness of the event
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

The Principal Investigator will report adverse events to the IRB according to their policies and procedures in reporting adverse events.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

Investigator Reporting to the FDA: Adverse reactions will be reported promptly to the Food and Drug Administration (FDA) via Medwatch by the Sponsor if the type of event is serious, unlisted/unexpected and possibly related to the study drug. A clear description of the suspected reaction will be provided along with an assessment as to whether the event is drug or disease related. The Sponsor will call the FDA as soon as he/she is aware (within ten (10) working days) that an adverse reaction has occurred. The phone number for the FDA is 301-594-5778.

The study sponsor will be responsible for notifying FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

The Principal Investigator will complete an Internal Prompt Reporting Form within the following timelines:

EVENT	TIMELINE
An Unanticipated problem involving risks to subjects or others 1. ***Unexpected*** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; 2. ***Related or possibly related*** to participation in the research; and 3. ***Suggests that the research places subjects or others at a greater risk of harm*** (including physical, psychological, economic, or social harm) than was previously known or recognized.	If the event is life-threatening, report within **7 calendar days** of receipt of the information. Follow-up reports for these events should be Submitted within 14 calendar days of receipt of information. All other events/problems must be reported within **14 calendar days** of the investigator's receipt of the information
All Research-Related Deaths (anticipated/unanticipated)	Immediately (i.e. within 48 hours) upon receipt of information
Other event that in the PI's judgment, warrants reporting or is in the best interest of the subject(s) (e.g., because it may affect the safety and/or welfare of subjects; it changes the risk level of the study; or the frequency of the same event significantly increases)	If life-threatening, report within 7 calendar days of receipt of the information. All other events/problems must be reported within 14 calendar days of the investigator's receipt of the information.
Other unanticipated problems that impact the conduct or integrity of the study (e.g. FDA Clinical hold or recall, published literature or data and safety monitoring board report impacting risk-benefit ratio, FDA Form 483 or warning letter, investigator medical license restriction or suspension, participant is incarcerated)	If life-threatening, report within 7 calendar days of receipt of the information. All other events/problems must be reported within 14 calendar days of the investigator's receipt of the information.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable.

Sponsor Safety Reporting contact information:
Name, degree, title: Austin Stone, MD, PhD
Institution Name: University of Kentucky
Address: 760 S Limestone, Suite K401
Lexington, KY 40536-0284
Phone Number: 859-218-3065
Email: austin.stone@uky.edu
FAX: (859)-323-2412

Name: Caitlin Conley, PhD
Address: 740 S Limestone, Suite K401

Phone #: (859) 257-1939
Email: caitlin.whale@uky.edu
Fax: (859) 323-2412

The reporting of serious adverse events to the study sponsor does not relieve the investigator from other regulatory reporting responsibilities.

Investigator reporting to SOBI: The Sponsor must inform Swedish Orphan Biovitrum (SOBI) in writing using a SOBI SAE or a MEDWATCH 3500A form of any SAE, independent of causality. The written report must be completed and supplied to SOBI by e-mail or facsimile within 24 hours/1 business day of first awareness. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report.

A final report to document resolution of the SAE is required. A copy of the email transmission of the SAE report to SOBI (drugsafety@sobi.com) should be attached to the SAE and retained with the patient records.

SOBI Safety Reporting contact information:
Drug Safety Swedish Orphan Biovitrum SE-112 76 Stockholm, Sweden Fax: 011-46 8 697 32 30
Phone: 011-46 8 697 20 00 (switchboard) e-mail: drugsafety@sobi.com
The reporting of serious adverse events to SOBi does not relieve the sponsor from other regulatory reporting responsibilities.
SOBi Safety Reporting contact information:
Fax: 011-46 8 697 32 30
e-mail: drugsafety@sobi.com

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the DCC/study sponsor. The UP report will include the following information:

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

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

- UPs that are SAEs will be reported to the IRB and to the DCC/study sponsor within of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within of the investigator becoming aware of the problem.

• All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within (insert timeline in accordancy with policy) of the IRB's receipt of the report of the problem from the investigator.

8.4.4 EVENTS OF SPECIAL INTEREST

Not applicable.

8.4.5 REPORTING OF PREGNANCY

Pregnancy is neither an AE nor SAE. All subject pregnancy must be reported to their study doctor or study staff immediately. Once confirmed, study medication will be stopped and the study site will request permission from the woman to follow her for pregnancy outcomes. All confirmed pregnancies will be reported to the study sponsor, FDA, SOBI and IRB and followed until resolution (i.e., termination [voluntary or spontaneous] or birth) to assess for pregnancy-associated AEs or SAEs such as congential anomaly.

8.5 STUDY HALTING RULES

Administration of study agent will be halted when three grade 3 AEs determined to be "probably related" are reported to the FDA/IRB and enrollment screens will stop accepting new study participants. The study sponsor will inform the DSMB members within 24 hours of this occurrence and will provide the DSMB with AE listing reports. The DSMB will convene an ad hoc meeting by teleconference or in writing as soon as possible. The DSMB will provide recommendations for proceeding with the study to the study sponsor. The study sponsor will inform the FDA of the temporary halt and the disposition of the study.

Study Stopping Criteria

- Local intolerance of the administered Kineret or any sign of allergic response.
- Development of signs and symptoms before the second time point preventing the second administration of the study drug.
- Any patient reported drug related SAE after the first or second administration of the study drug.
- Diagnosis with any condition as outlined in the exclusion criteria during the course of the initial 2 weeks of study enrollment.

8.6 SAFETY OVERSIGHT

Monitoring for adverse events will be conducted in real-time by the study investigators and study coordinators. Risks involved with this study are considered greater than minimal risk. For this reason, we will utilize the standing independent Data Safety Monitoring Board (DSMB) as chartered by the Center for Clinical and Translational Science (CCTS). The DSMB will meet semiannually or as needed, and will review subject recruitment, AE's, side effects, laboratory results, dropouts, protocol violations, and inclusion/exclusion criteria. More frequent meetings will take place if side effects or other problems are prevalent.

9 CLINICAL MONITORING

Clinical site monitoring will be performed by Bluegrass Research Consultants Inc. The monitor will conduct monitoring to verify that a) the rights and well-being of human subjects are protected; b) the reported trial data are accurate, complete and verifiable from source documents; c) the conduct of the trial is in compliance with the currently approved protocol/amendment(s), Food and Drug Administration (FDA) regulations and ICH guidelines d) Provide practical guidelines for Site Monitors, Study Monitors and Medical Monitors; e) Provide consistent monitoring procedures across all clinical sites; f) Ensure the quality and integrity of clinical study data; g) Evaluate the progress of the study and ensure completion of the clinical study in a timely manner. A monitoring visit report will be provided to the Sponsor within 21 business days following the completion of a visit.

The first monitoring visit will be conducted within two weeks following enrollment of the first subjects. Subsequent monitoring visits will be completed based on enrollment and are expected to occur every four to six weeks.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

10.1.1 GENERAL APPROACH

Sample Size and Power Analysis - The primary outcome aim of the study is to determine if treatment with the study medication results in greater improvements in patient-reported outcomes one year following surgery. We will use the validated Knee Osteoarthritis and injury Outcome Score (KOOS),[27] which is a self-administered, knee-specific instrument that evaluates the course of knee injury and treatment outcomes. To reduce the risk of an underpowered RCT, an a priori sample size estimation was calculated from data collected from recent studies of ACL-injured and –reconstructed patients.[28-32] The previously published one-year KOOS Quality of Life Scores are 60.2 ± 24.2 [31] and the minimal detectable change of this instrument is 7.2 [32]. Assuming that the standard deviation of the change scores between the day of surgery and one year postoperative visits will be 20% of the standard deviation of the standard deviation of the one-year KOOS Quality of Life Scores (.2 x 24.2 = 4.8), a sample size of 16 patients per group (32total) would be at least 80% powered to detect a difference of 7.2 points between the treatment group and the control group (One-way ANOVA model with alpha = 0.05, Effect size = 7.2/4.8 = 1.5, G*Power 3.0.10). This sample size would adequately power the study for the primary outcome variable, but would also adequately power the study for the second aim of the study. The second aim of the study is to compare chondrodegenerative biomarkers between groups at the 1 year follow up. We have previously observed large effect sizes between groups in preliminary studies (ES = 1.34), and a sample size of 32 patients (16 per group) would be at least 80% powered to detect effect sizes of this magnitude. However, to protect against an underpowered study due to patient attrition, we will monitor compliance and potentially enroll up to 20 patients per group in order to adjust for attrition. Patient costs will not occur until sample or MRI analysis.

The power calculations include several caveats. First, for purposes of power calculation, we did not assume a full repeated measures analysis, but instead analyzed at a single time point (one-year follow-up). Second, we note that in trials such as this, the power of a non-parametric analysis is never much lower than its parametric analog (the “asymptotic relative efficiency” of

the Wilcoxon test is at least 0.95), leading us to conclude that our parametrically based power calculations should apply with equal force to a non-parametric analysis.

Statistical analyses - Results from a sample of this size may not be normally distributed. Therefore, non-parametric statistical measures may be used. Primary outcome measures evaluating for change in KOOS will be assessed at multiple points and will be analyzed for change between groups using Friedman two-way ANOVA for repeated measures. Synovial fluid analyses comparing the three groups at five time points will be analyzed using the Wilcoxon Signed Rank Sum Test with Hochberg corrections for multiple time points. Alternatively, if results are normally distributed or can be transformed (e.g., by log transformation) to meet the criteria for normality for parametric analysis, then repeated measures ANOVA will be utilized comparing treatment groups. P-values of less than or equal to 0.05 will be considered statistically significant.

Predicted Results: IL-1ra, while potent, has had mixed results in the treatment of established OA, possibly due to it's short half-life in vivo. In this trial, we expect that an early intervention with IL-1ra will effectively block the deleterious cascade of joint degradation events after joint injury. We expect a significant effect of IL-1ra on post-injury pain and concomitantly an increase in patient-reported outcomes due to the improved early function

Minimization of bias*:* Due to the randomized study design and the blinding of the investigator and patient to the drug used, we hope to eliminate any investigator or subject bias. Using broad and previously established enrollment criteria we hope to reduce selection bias to a minimum while protecting potentially vulnerable individuals through the exclusion criteria. Procedural bias and measurement bias will be reduced by the multicenter design and the blinded data analysis.

10.1.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

A safety review of treatment Groups 1 and 2will be performed. Safety endpoints will be monitored at each study visit and at each monitoring visit.

Study Stopping Criteria

- local intolerance of the administered Kineret or any sign of allergic response.
- development of signs and symptoms before the second time point preventing the second administration of the study drug.
- Any patient reported SAE after the first or second administration of the study drug.
- Diagnosis with any condition as outlined in the exclusion criteria during the course of the initial 2 weeks of study enrollment.

10.1.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Not applicable

10.2 MEASURES TO MINIMIZE BIAS

10.2.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Randomization: Following informed consent and after successfully being screened into the study, subjects will be randomized to one of three treatment groups.

- **Group 1** will receive intra-articular injection of Anakinra (Kineret®, IL-1ra; 150mg) 1-28 days after ACL injury.
- **Group 2** will receive intra-articular injection of saline placebo 1-28 days after ACL injury.

Blinding: To ensure triple-blindness, an uninvolved third party will generate the allocation sequence of the intervention and provide this to the principal investigator. Allocation of drug or placebo will be by permutated random block size, ensuring that approximately equal numbers of subjects will be treated with drug or placebo in the unlikely event that the study will need to be terminated prematurely.

If subject discontinues the study for any reason prior to 3-month visit, then the collected data will be evaluated but not included in the analysis and a replacement subject will be enrolled in the study.

10.2.2 EVALUATION OF SUCCESS OF BLINDING

Not applicable.

10.2.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

Breaking the randomization code will be performed by the Principal Investigator and will occur if it is necessary for the care of the subjects. For example, if an AE or SAE occurs with the a subject and knowledge of the drug randomization would be important for clinical care or to determine appropriate treatment, then the principal investigator will break the code and the subject will be withdrawn from the study.

Unblinding of a subject(s) dosing will be documented and will include an explanation of why the study medication was unblinded.

Following data lock, subject dosing information can be unblinded and the unblinded information can be sent to the study participants at the discretion of the principal investigator.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The principal investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. The principal investigator will permit authorized representatives of the FDA, Institutional Review Board, Center for Clinical and Translational Science, Bluegrass Research Consultants, Inc. and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participant's memory aids or evalulation checklists, pharmacy dispensing records, recorded audio tapes of counseling sessions, 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, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

It is **not** acceptable for the CRF to be the only record of a patient's participation in the study. This is to ensure that anyone who would access the patient medical record has adequate knowledge that the patient is participating in a clinical trial.

12 QUALITY ASSURANCE AND QUALITY CONTROL

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

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

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

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6. If the study is conducted at international sites, the statement could be as above and/or could reference compliance with the Declaration of Helsinki, Council for International Organizations of Medical Science (CIOMS), International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002), or another country's ethical policy statement, whichever provides the **most** protection to human subjects.

13.2 INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

Prior to the start of the trial, the investigator should have the IRB's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the participants.

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

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

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

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

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

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by research staff

will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the the University of Kentucky.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

All samples/specimens or data collect will be used for the purpose of this research study only. No future use of the samples/specimens or data will be allowed.

13.5 FUTURE USE OF STORED SPECIMENS

N/A

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

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

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the CRF (CRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the CRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered onto paper CRFs or a eCRF 21 CFR Part 11-compliant data capture system provided by the Sponsor. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 STUDY RECORDS RETENTION

Records should not be destroyed without the sponsor's agreement. Study documents should be retained for a minimum of 6 years in keeping with local IRB record retension policy.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

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

It is the responsibility of the site to use continuous vigilance to identify and report deviations within working days of identification of the protocol deviation, or within working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to Program Official and protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

14.4 PUBLICATION AND DATA SHARING POLICY

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events.

The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.

NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The study leadership will be comprise of the sponsor and site principal investigators.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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18 APPENDIXES

Figure 3 – Pain VAS – Initial Visit